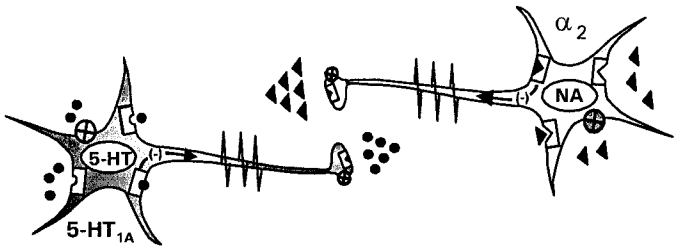
Interactions between serotonergic and noradrenergic systems: their involvement in antidepressant treatment of anxiety and affective disorders



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I dedicate this thesis to my parents, Steven T. Szabo Sr. and Kasija Szabo, whom raised me in a loving and nurturing environment and burdened themselve financially to provide me with means to achieve academically. My aunt Olga, who looked after me, treated me as her own son, and made my transition and return to Montréal enjoyable. My fraternal twin brother, David T. Szabo, with who numerous scientific and insiprational conversations stemmed, underscoring our unique sibling bond and time together in graduate school. All of my colleagues, friends, and the McGill Wrestling Team whom made it possible for me to have an enjoyable life outside of my academics pursuits. This group provided me with the encouragement, support, and motivation to aggressively pursue my research endeavours. This thesis is not only a compilation of my scientific work, but it must also be recognized as a reflection and testament of the support of my close family and friends which ultimately made this document and its contents possible.

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STATEMENT OF CONTRIBUTIONS

This thesis is comprised of seven co-authored original research papers. I have personally composed these manuscripts with only the guidance and thoughtful criticisms of Pierre Blier, M.D., Ph.D. All experimental work presented herein has been carried out first-hand by myself. It should be noted that in the appendix, I have enclosed two manuscripts where I am second author. Nasser Hadjerri, Ph.D. performed a greater proportion of the microiontophoretic experiments in the published manuscript. This collaboration was instrumental in the teaching of the microiontophoretic technique and was later implemented for my experiments with reboxetine (see Chapter VIII). Furthermore included in the appendix are two review-articles; article one (where my name appears first) highlight the importance of the research findings presented herein and in relation to previous work on the mechanism of action of antidepressants. I composed this article with Dr. Blier mentoring my progress. The second review manusript, on which I am third author, underscores the importance of other neurotransmitter systems, in addition to the serotonergic system, in mediating the antidepressant effect. A commentary is also included in the appendix suggesting that caution should be used when making generalizations relating to the attenuation on locus coeruleus activity being common to all antidepressants, as mirtazapine (Remeron®) does not follow this trend. This endeavour was initiated by myself, and after editorial and many thoughtful critisms being offered by Dr. Blier, the commentary was submitted for publication.

ACKNOWLEDGEMENTS

I take this opportunity to express my sincere gratitude and thankfulness to my mentor and friend Pierre Blier M.D., Ph.D., Professor at McGill University and the University of Florida, for accepting me as a Ph.D. student and supervising my research with great competence. Prior to my graduate training, as a former chemical addicition counselor for heroin addicts at a methadone clinic, I had to opportunity to observe first hand the high prevalence of anxiety and affective disorders in this patient population. Dr. Blier acknowledged my keen interest in the field of addiction and geared my training towards examining an area of the brain, not only implicated in anxiety and affective disorders, but also opiate withdrawal pertubation. I applaude Dr. Blier for his efforts and accomplishments in the translational style of research he conducts on affective disorders with his bench to bedside approach. Because of his aspirations and contributions to science and medicine, I experienced a unique and rewarding graduate training that extends far beyond the acquisistion of sound experimental technique. Dr. Blier has donated to me a great deal of his time to attend to my countless questions or concerns I have at the immediate moment, while energetically taking the opportunity to teach me about medical practice, the pharmaceutical industry, and the design/implementation of clinical trials. His eargerness to allow me to have an active role in reviewing of manuscripts submitted to journals where Dr. Blier is on the editorial board provided me with an invaluable opportunity, for now I have a good grasp on manuscript composition and evaluation of scientific work.

I am thankful to Claude de Montigny M.D., Ph.D., Director of the Neurobiological Psychiatry Unit, and Dr. Guy Debonnel M.D., Professor at McGill University, whom both agreed to accept me into the unit where I was readily befriended. I had the opportunity to benefit from a stimulating scientific environment that Drs. Blier and Debonnel generated by their infectious enthusiasm towards elucidation of neurophysiological processes during my training in Montréal. I am also fortunate to have experienced and completed the

remainder of my studies at the University of Florida that provided me with access to an additional diverse group of psychiatric research scientists and physicians whom contributed a different perspective to my research and broadened me as a researcher. Most notably is Mark S. Gold, M.D., Distinguished Professor and Chief of Addiction Medicine, entertained many scientific discussions related to drugs of abuse and lead to the series of experiments I am currently conducting with the relationship of MDMA (Ecstacy) relating to anxiogenesis. Mark Lewis, Ph.D. who allowed me free access to his lab and equipment while aiding in the acqusition of MDMA. John Pettito, M.D., and Nathan Shapira, M.D., Ph.D., who through scientific and friendly conversations made me feel at home, ensuring that my transition into the Department of Psychiatry at the University of Florida would be a pleasurable one. Wayne Goodman, M.D., Chairman of Psychiatry, by displaying interest in my current and future research endeavours, including me in scientific departmental meetings, and providing me with additional salary support. I would also like to acknowledge all the research students, fellows, technicians, and secretaries whose help and friendship made being a graduate student at the Neurobiological Psychiatry Research Unit and the University of Florida Brain Institute a memorable experience.

Lastly, I would like to thank the members of the Thesis Commitee for contributing their time and effort to the evaluation of this lengthy document, and I leave you with this quote:

"I have learned the novice can often see things that the expert overlooks. All that is necessary is not to be afraid of making mistakes, or of appearing naïve"

Abraham Maslow (1908 - 1970)
US psychologist, philosopher "Eupsychian Management."

ABBREVIATIONS

ACh: Acetylcholine
cAMP: adenosine 3',5' monophosphate
4-AP: 4-Aminopyridine
CA_{1-4}: *Cornu ammonis* (Ammon's horns of the hippocampus)
Ca^{2+}: Calcium ion
Cl^-: Chloride ion
cDNA: Complementary deoxyribonucleic acid
CNS: Central nervous system
DA: Dopamine
DOI : 2,5-dimethoxy-4-iodoamphetamine
5,7-DHT: 5,7-Dihydroxytryptamine
DSP-4: *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine
EAA: Excitatory amino acid
EEDQ: N-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline
GABA: γ-aminobutyric acid
GDP: Guanosine diphosphate
GDPγS: Guanosine 5'-O-(2-thiodiphosphate)
GTP: Guanosine triphosphate
GTPγS: Guanosine 5'-O-(3-thiotriphosphate)
HRP: Horseradish peroxidase
5-HT: 5-Hydroxytryptamine, serotonin
i.p.: Intraperitoneal
IP_3:1,4,5 Inositol triphosphate
i.v.: Intravenous
K^+: Potassium ion
LSD: *Lyserg Säure Diäthylamid* (Lysergic diethylamide acid)
MAO: Monoamine oxidase
MAOI: Monoamine oxidase inhibitor
mRNA: Messenger ribonucleic acid
Na^+: Sodium ion

NA: Noradrenaline
NRI: Noradrenergic Reuptake Inhibitor
6-OHDA: 6-Hydroxydopamine
8-OH-DPAT: 8-Hydroxy-2(di-N-propylamino)tetralin
PCA: Parachloroamphetamine
PCPA: Parachlorophenylalanine
Pgi: (Nucleus) Paragigantocellularis
PI: Phosphatidyl inositol
PKA: Protein kinase A
PKC: Protein kinase C
PLC: Phospholipase C
PrH: (Nucleus) Prepositus hypoglossi
s.c.: Subcutaneous
SSRI: Selective Serotonin Reuptake Inhibitors
TCA: Tricyclic Antidepressant
TTX: Tetrodotoxin

PRÉFACE

The studies presented in this thesis focus on the serotonergic and noradrenergic systems, their interactions, and implications in the mechanism of action of antidepressant treatments with respect to anxiety and affective disorders.

As per the "Guidelines for Thesis Preparation" required by the McGill University Faculty of Medicine, under Manuscript-Based Thesis:

"Candidates have the option of including, as part of the thesis, the text of one or more papers submitted, or to be submitted, for publication, or the clearly-duplicated text (not the reprints) of one or more published papers. These texts must conform to the Guidelines for Thesis Preparation with respect to font size, line spacing and margin sizes and must be bound together as an integral part of the thesis. (Reprints of published papers can be included in the appendices at the end of the thesis.)

The thesis must be more than a collection of manuscripts. All components must be integrated into a cohesive unit with a logical progression from one chapter to the next. In order to ensue that the thesis has continuity, connecting text that provide logical bridges between the different papers are mandatory.

The thesis must conform to all other requirements of the "Guidelines for Thesis Preparation" in addition to the manuscript.

The thesis must include the following:

(a) a table of contents;
(b) an abstract in English and French;
(c) an introduction which clearly states the rational and objective of the research;

(d) a comprehensive review of the literature (in addition to that covered in the introduction to each paper);

(e) a final conclusion and summary;

As manuscripts for publication are frequently very concise documents, where appropriate, additional material must be provided (e.g., in appendices) in sufficient detail to allow a clear and precise judgement to be made of the importance and originality of the research reported in the thesis.

In general, when co-authored papers are included in a thesis the candidate must have made a substantial contribution to all papers included in the thesis. In addition, the candidate is required to make an explicit statement in the thesis as to who contributed to such work and to what extent. This statement should appear in a single section entitled "Contributions to Authors" as a preface to the thesis. The supervisor must attest to the accuracy of this statement at the doctoral oral defense. Since the task of the examiners is made more difficult in these cases, it is in the candidate's interest to clearly specify the responsibilities of all the authors of the co-authored papers.

When previously published copyright material is presented in a thesis, the candidate must obtain, if necessary, signed waivers from the co-authors and publishers and submit these to the Thesis Office with the final deposistion.

Irrespective of the internal and external examiners reports, if the oral defence committee feels that the thesis has major omissions with regard to the above mentioned guidelines, the candidate may be required to resubmit an amended version of the thesis. See the Guidelines for Doctoral Oral Examinations," which can be obtained from the web (http://www.mcgill.ca/fgsr), Graduate Secretaries of departments or from the Thesis Office, James Administration Building, Room 400, (514) 398-3990.

In no case can an author of any component of such a thesis serve as an external examiner for that thesis.

Examiners are asked to make a recommendation for Dean's Honour List, based on the quality of the thesis. Only if the thesis is outstanding, i.e., with a rating of "excellent" in all, or almost all, aspects, should the examiner recommend that the student be considered for the Dean's Honour List. This is expected to be the top 10% of the students' theses you have read. The examiner should justify the recommendation in writing in the comments.

Please note that the examiners must base their decision on the thesis alone; a recommendation cannot be changed retroactively as a result of finding out what the other examiners recommended."

The experimental parts of this thesis (Chapters II to VIII) consist of seven original articles that have been already published, accepted for publication, or submitted for publication, and the overall summary and discussion of the different studies of this thesis are made in chapter IX. Manuscripts with original work where I am second author, review articles, and commentary are located in the appendix.

Financial support throughout my graduate career that pertains to the body of this thesis (i.e., experiments carried out solely by myself) is indicated in the acknowledgement sections of Chapters II through VIII.

ABSTRACT

Pertubations in serotonergic (5-HT) and noradrenergic (NA) function are implicated in the pathophysiology of anxiety and affective disorders. This is strengthened by all antidepressants regardless of targeting these monoamines produce specific alterations in one or both of these systems after a prolonged administration. These alterations are congruent to their delayed onset of action in anxiety and affective disorders and may be of relevance. Using *in vivo* electrophysiological paradigms in the rat, the present research endeavor was undertaken to investigate whether antidepressant drugs inhibiting one monoaminergic reuptake transporter can induce an alteration in the other system. More specifically, impact of 5-HT and adrenergic receptors on the regulation of monoaminergic and hippocampal activity after acute and sustained antidepressant treatments was assessed.

Long-term, but not subacute administrations of selective 5-HT reuptake inhibitors (SSRIs) attenuate the spontaneous firing activity of locus coeruleus (LC) NA neurons. On the other hand, subacute and sustained treatment regimens with NA reuptake inhibitors (NRIs) induce a robust and sustained decrease on NA firing without altering that of 5-HT. Interestingly, sustained SSRI and NRI treatments both abolished 5-HT_{1A} receptor augmentations of LC firing, but left inhibitory 5-HT_{2A} receptor responses normal or slightly desensitized. The SSRI induced dampening on LC firing is reversed by 5-HT_{2A} receptors blockade. Thus, an overactivation of 5-HT_{2A} receptors during chronic SSRI administration results from desensitization of 5-HT_{1A} receptors in the presence of 5-HT transporter reuptake inhibition.

Antagonism of 5-HT_{1A} receptors attenuates LC NA firing, but is completely reversed by 5-HT_{2A} receptors blockade. 5,7-DHT experiments indicate that these receptors in the LC are postsynaptic to 5-HT neurons, but the 5-HT_{1A} effects are dependent on intact 5-HT neurons. This served as the impetus to a proposed neuronal circuitry detailing the mechanism by which these 5-HT receptors, and SSRI induce adaptations thereof, alter the NA system. This complex circuitry implicates other neurotransmitters being supported further by iontophoretic data demonstrating 5-HT_{1A}

receptor effects involve alterations in glutamate and 5-HT to mediate 5-HT_{2A} receptor activation and regulate GABA release in the LC.

Given the abovementioned results, it was striking that a subacute treatment with YM992 (SSRI and 5-HT_{2A} antagonist) attenuated NA firing to a similar extent as reported with NRIs. This was concluded to be due to overactivation of presynaptic α_2-adrenoceptors. In contrast to NRIs, a 21-day treatment with YM992 desensitized this receptor subtype and is responsible for normalization of LC firing.

Reboxetine produces similar effects on 5-HT and NA neuron firing and reuptake blockade on CA_3 pyramidal neurons in the hippocampus as the TCA desipramine. Unlike desipramine, reboxetine is able to alter 5-HT reuptake function and 5-HT_{2A} receptors mediated responses by DOI after a prolonged administration and did not induce a sensitization of hippocampal 5-HT_{1A} receptors. Thus, for the first time, experimental evidence supports that this latter effect is due to TCA structure and not NA reuptake blockade.

These results are extrapolated to the beneficial and side effects produced by antidepressants with hopes of expanding upon the former while reducing the latter in the treatment of anxiety and affective disorders.

RÉSUMÉ

Certaines perturbations du système sérotoninergique et noradrénergique sont impliquées dans la pathophysiologie des troubles anxieux et affectifs. Ceci est supporté par le fait que tous les antidépresseurs administrés de façon prolongée, qu'ils agissent sur l'un ou l'autre système, produisent de tels changements. Ces modifications sont tout à fait congruentes aux délais d'action de ces médicaments dans les troubles anxieux et affectifs. A l'aide de paradigmes électrophysiologiques in vivo chez le rat, les études présentes ont été enterprises pour étudier si les antidépresseurs inhibant la recapture d'une monoamine pouvaient produire des changements dans l'autre système neuronal. Plus spécifiquement, l'effet de modifications potentielles des récepteurs 5-HT et adrénergiques a été étudié sur la modulation de ces systèmes monoaminergiques et l'activité hippocampique suite à l'administration ponctuelle et soutenue de médicaments antidépresseurs.

L'administration à long-terme d'inhibiteurs sélectifs de la recapture de la 5-HT (ISRS) diminue le taux de décharge spontané des neurones NA du locus coeruleus, alors que des traitements subaïgus sont inefficaces. D'autre part, des régimes subaïgus et prolongés induisent une diminution marquée et soutenue du taux de décharge des neurones NA sans affecter celui des neurones 5-HT. Il est intéressant de souligner le fait que des traitements prolongés avec des inhibiteurs de la recapture de la 5-HT et de la NE abolissent l'augmentation du taux de décharge des neurones du LC normalement produite par l'activation de récepteurs 5-HT_{1A}, tout en laissant intact, ou en diminuant légèrement, l'effet inhibiteur de l'activation de récepteurs 5-HT_{2A}. L'inhibition du taux de décharge des neurones du locus coernleus est renversé par le bloc des récepteurs 5-HT_{2A}. Donc, une suractivation des récepteurs 5-HT_{2A} durant l'administration chronique d'ISRS résulterait de la désensibilisation des récepteurs 5-HT_{1A} en présence de l'inhibition du transporteur 5-HT. Le bloc des récepteurs 5-HT_{1A} diminue le taux de décharge des neurones NA du LC, mais est entièrement renversé par l'antagonisme des récepteurs 5-HT_{2A}. Des lésions des neurones 5-HT indiquent que ces récepteurs sont localisés sur des neurones postsynaptiques, mais que les effets des ligands 5-HT

$_{1A}$ dépendent de l'intégrité des neurones 5-HT. Ces observations nous ont mené à proposer un circuit neuronal détaillant le mécanisme par lequel ces récepteurs 5-HT, et en consequence les ISRS, modifient le système NA. Ce circuit complexe, comporte des interactions impliquant d'autres neurotransmetteurs. Ceci est étayé par des données iontophorétiques démontrant que les effets des ligands 5-HT_{1A} implique le glutamate, ceci menant au changement de libération de GABA dans le LC.

A la lumière de ces données, il est surprenant de constater qu'un traitement subaïgu avec le YM992 (un ISRS qui est aussi un antagoniste 5-HT_{2A}) diminue le taux de décharge des neurones du LC au même degré que les bloqueurs de la recapture NA. Cet effet serait attributable à la suractivation des récepteurs α_2-adrénergiques présynaptiques. Contrairement aux bloqueurs de la recapture NA, un traitement de 21 jours au YM992 désensibilise ce sous-type de récepteurs et résulte en une récupération à la normale du taux de décharge des neurones du LC.

La réboxetine produit des effets similaires à la désipramine, tous deux des bloqueurs de la recapture de la NA, sur le taux de décharge des neurones 5-HT et NA et sur l'activité des neurones pyramidaux CA_3 de l'hypocampe. Contrairement à la désipramine, la réboxetine a la capacité d'altérer le processus de la recapture de la 5-HT ainsi que les réponses de type 5-HT_{2A} produites par la DOI suite à un traitement à long-terme, sans induire une sensibilisation des récepteurs 5-HT_{1A} de l'hippocampe. Donc, pour la première fois, une évidence expérimentale a été fournie au fait que la structure tricyclique et non le bloc de la recapture NA produit cet effet.

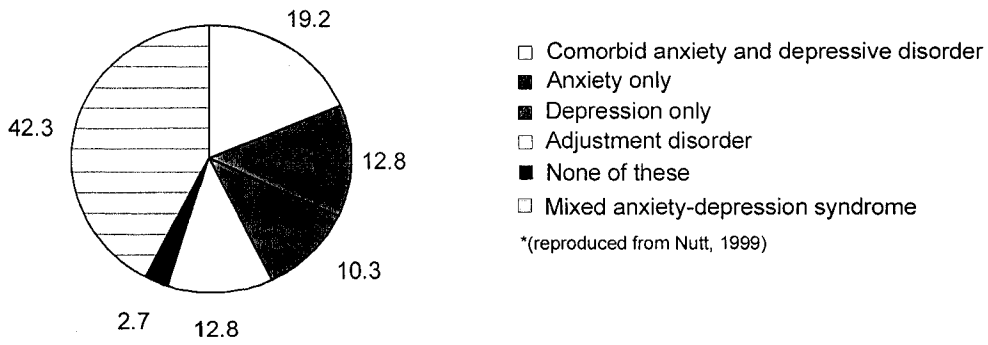
Ces données sont extrapolées aux effets bénifiques et secondaires des antidépresseurs dans la perspective d'améliorer anticipation de développer leurs effets thérapeutiques tout en diminuant leurs incomforts dans les traitements des troubles anxieux et affectifs.

CHAPTER 1: REVIEW OF THE LITERATURE

1. Introduction

Depression is a disabling disease and currently represents one of the most common psychiatric disorders in the world (Kessler et al., 1994; Kessler et al., 1998; Lepine, 2001). This disorder has a major effect on economic productivity, individual well-being, and social functioning around the globe, not merely in developed countries. Recently, the World Health Organization (WHO) has predicted that major depression will carry, in industrialized nations, the greatest burden of any other disorder or disease by the year 2020, surpassing ischemic heart disease. In the Diagnostic and Statistical Manual (DSM) IV of the American Psychiatric Association (1994), anxiety and affective disorders are listed as separate entries with distinct diagnostic criteria and unique symptomology. In society, however, anxiety frequently coexists with depression (Zimmerman et al., 2000), either as a comorbid anxiety disorder, or as anxiety symptoms accompanying a primary depressive disorder (Figure 1; Gorman and Coupland, 1996; Lecrubier, 2000).

Figure 1. Distribution of Depressive and Anxiety Symptoms in a Primary Care Population*



A primary diagnosis of anxiety is often followed by depression, and this sequence occurs more commonly than a diagnosis of depression followed by anxiety (Kendell, 1974; Angst and Vollrath, 1991; Wittchen et al., 1991). Patients with a comorbidity of

major depression and anxiety report increased severity of symptoms and poorer treatment outcome (Tylee, 1999; Tylee et al., 1999). Furthermore, they are likely to have low mood and panic attacks, possibly contributing to the greater disruption to social, work, and family life reported in this population (Lecrubier, 2000; Nutt, 2000; Wittchen et al., 2000). Anxiety is also a significant predictor of suicide in patients with major depression, and attempted suicide is more common among patients with mixed anxiety-depressive disorders (up to 30% versus 10% as with depression alone). Effective treatment of those suffering from depression and anxiety is paramount as these individuals make up the vast proportion of the psychiatric population, are more mentally ill, and are extremely psychosocially affected-afflictive. A brief clinical description of these diagnostically different, but potentially related pathophysiologic disorders will be presented below based on the DSM-IV and their prevalence.

Major depression is characterized by a depressed mood or a loss of interest or pleasure in nearly all activities for a period of at least two weeks. Depressed individuals must also present at least four of the following symptoms: changes in appetite or weight, sleep, and psychomotor activity (i.e. either an increase or a decrease in activity), fatigue or decreased energy, feeling of worthlessness or guilt, decreased ability to think, concentrate or make decisions, and recurrent thoughts of death or suicidal ideation. There is an atypical form of depression in which appetite and sleep are increased. Although this presentation is much more rare, it is noteworthy because patients with this form of depression respond well to monoamine oxidase inhibitors (MAOI) and selective serotonin (5-HT) reuptake inhibitors (SSRIs), but less so to the tricyclic antidepressant (TCA) imipramine (Liebowitz et al., 1988). A less severe form of depression is now recognized, frequently referred to as dysthymia. This form requires that fewer symptoms be present, in addition to a depressed mood, and that the latter not necessarily be present everyday, but rather for a two-year duration. The incidence of major depression in the general population is around 5%, with a female to male ratio of affected individuals of about 2 to 1, and a lifetime prevalence of 15-20%. All antidepressant drugs require an administration of at least two weeks before exerting a detectable therapeutic effect.

In 1980, panic disorder was recognized as a distinct diagnostic entity in the DSM-III (American Psychiatric Association, 1980). The essential features of panic disorder are the presence of recurrent, unexpected panic attacks followed by at least 1 month of persistent concern about having another attack, worry about the possible implications or consequences of the panic attacks, and a significant behavioural change related to the attacks. An unexpected, spontaneous, and uncued panic attack is one that is not associated with a situational trigger, and at least two such attacks are required for the diagnosis. These attacks can cause extreme anxiety and may mimic a life threatening illness. The patients may also feel as if they are "going crazy" or losing control. The lifetime prevalence of panic disorder is estimated to be between 1.5% and 3.5% of the general population, based on epidemiological studies carried out worldwide. Among patients with general anxiety disorder (GAD), it has been reported that the majority have depressive symptoms and the lifetime prevalence of major depression is over 50%. In turn, depression with anxiety symptoms and a variety of anxiety disorders including panic disorder, social phobia, obsessive compulsive disorder, and GAD have been treated with antidepressants. Early discontinuation of antidepressants is likely to result in relapse in about 50% of the cases.

Due to the high common occurrence of anxiety disorders with major depression, I, as others, have postulated on common/shared biological bases in the treatment of these illnesses. For example, the nosological model of the anxiety-depression continuum, as described by Frances et al., (1992) is one such postulate. This continuum is divided into three sections; a discrete model, continuum model, and a shared feature model (figure 2). The discrete model takes into consideration that anxiety and depression are separate entities, but that they may predispose an individual to other psychiatric disorders. The continuum model proposes that there is a progression of disease state from one disorder to encompass the other. The shared features model places emphasis on these disorders being highly interrelated, but at the same time possess distinct features. It is this shared model of anxiety-depression that is becoming widely accepted. This model may be pertinent to the NA and 5-HT system in relation to the antidepressant effects of anxiety and affective disorders.

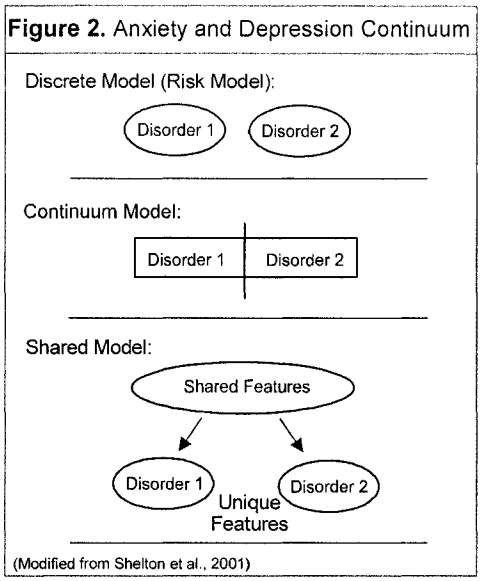


Figure 2: *Discrete model*: anxiety and affective disorders are distinct entities. *Continuum model*: a common progression of disease state to encompass both anxiety and depression. *Shared Model*: underlying diathesis which presents some unique symptomology and allows classification as a separate disorders.

Although the etiology of anxiety and affective disorders are not fully understood, the development of antidepressant drugs during the last five decades has been based primarily on monoaminergic deficiencies of the catecholamine (NA) and/or the indolamine (5-HT) systems. In the 1960's, it was discovered that MAOIs and TCAs were clinically effective in patients with major depression and subsequently became indicated for the treatment of various anxiety disorders. The former class of agents is still regarded as the "gold standard" for GAD treatment. It was later demonstrated that the TCA imipramine and amitryptyline inhibit the reuptake of NA (Glowinski and Axelrod 1964; Ross and Renyi, 1967) and 5-HT (Carlsson, 1970). This was important as other antidepressants are also able to augment the availability of these monoamines, while agents that deplete monoamines or impair catecholamine synthesis induce depression and anxiety like symptoms, respectively, (Bunney and Davis, 1965; McCann et al., 1995; Miller et al., 1996). It soon became clear that the 5-HT and NA systems are critical in the therapeutic efficacy of these drugs, which lead to the development of the monoamine theory of anxiety and depression (Schildkraut, 1965; Bunney and Davis, 1965). Furthermore, the 5-HT system appears to be more indicative of affective disorders, whereas anxiety is more reflective of NA perturbations (Thase and Howland,

1995). For more information on the relationship between the NA system and anxiety see Szabo and Blier et al., 2001 (located in the appendix). Much interest has also been directed at linking alterations in the hypothalamic-pituitary-adrenocortical axis, neuropeptides, and hormonal fluctuations in relation to the etiology and pharmacotherapy of these disorders. Agents developed at these targets have yet to become approved for the treatment of these psychiatric disorders and will not be mentioned further.

Notwithstanding the efficacy of the MAOIs and TCAs in anxiety and affective disorder treatment, a considerable drawback to these agents is that they are plagued with side effects that are both bothersome and even lethal to some patients. The clinical use of MAOIs is limited largely because hypertensive crises can occur when certain medications (tyramine or sympathomimetics) or foods (aged meats and cheeses containing tyramine) are injested while the patient is being treated with this antidepressant class. The poor TCA tolerability profile (mainly due to anticholinergic effects) has been shown to adversely effect compliance and results in a significant amount of patient withdrawal from treatment in comparison with that of newer antidepressants (Montgomery and Kasper, 1995; Srisurapanont,1998). At the other extreme, another problem with the TCAs is their potential for lethal overdose. Potential for overdose is an important factor in treatment as there is a higher risk of suicide in depressed patients with anxiety as compared with those solely afflicted with depression (figure 1; Fawcett, 1990; Wunderlich et al., 1998; Tylee et al., 1999). This potential for overdose, coupled with a lack of sufficient knowledge about this family of agents often results in many primary care physicians to be aggressive enough when it comes to dosing. This may lead to undermedicated and/or untreated patients. Thus, TCAs and MAOIs for these reasons are not ideal.

Despite the discovery of these first effective antidepressant agents being derived from serendipity and possessing an undesirable side-effect profile, the study of their mechanism of action has allowed for the development of more selective drugs aimed at specific targets. As a result, newer antidepressant agents have fewer and less-severe side effects than first generation drugs (TCA and non-selective/irreversible MAOIs) due

to their lack of affinity for amine and acetylcholine (ACh) receptors. The development and treatment of anxiety and affective disorders with SSRIs represents a successful, if not spectacular direct result of such a rational drug-development approach. Since their induction in the 1980's, SSRIs have become the most prescribed antidepressant drugs. It is currently the first line treatment approach for major depression, panic disorder, social phobia, post-traumatic stress disorder (PTSD), dysthymia, GAD, obsessive-compulsive disorder (OCD) and bulimia (Gorman and Kent, 1999). Notwithstanding this progression, these agents are still accompanied by a relatively high number of non-responders and a delayed onset of therapeutic action of at least two weeks. Indeed, these two major drawbacks represent the main challenge to which newer antidepressant strategies as well as my research is aimed at improving. Given the widespread usage of SSRIs for the treatment of numerous disorders, elucidation of its mechanism of action will be a major focus of this document.

A debate exists concerning whether SSRIs are as effective as TCAs in certain subsets of depressed patients. A meta-analysis of the literature indicates that TCAs are consistently more effective than the SSRIs in "severely depressed" or endogenous/melancholic depression (Perry, 1996). Table 1., represents data from two landmark studies that highlights this point. Many researchers have envisaged that the inhibition of NA reuptake may be an important and even necessary pharmacologic effect for reversing somatic symptoms in some patients (Schatzberg, 1998). This may account for findings where dual 5-HT/NA reuptake inhibitors display greater efficacy in the treatment of major depression as compared to agents selectively targeting only one of these monoaminergic systems (Einarson et al., 1999; Clerc et al., 1994; Danish University Antidepressant group, 1990; Danish University Antidepressant group, 1986; Shaeffer et al., 1998; Silverstone and Ravindran, 1999). Alterations on both monoaminergic systems may be more representative of a pathological state in major depression and account for why patients more often then not suffer with anxiety. Due to the unique therapeutic profile, but relative risks associated with TCA therapy, the antidepressant reboxetine was developed to be a selective NA transporter (NAT) reuptake blocker devoid of TCA moiety (Wong et al., 2000). In an antidepressant class

of it own, reboxetine possesses a good safety profile and efficacy in major depression (Tanum, 2000) and some anxiety disorders (Keller, 2001). Interestingly, it has also been demonstrated to be superior to SSRIs in the treatment of depression-related social anxiety and negative self-perception (Massana, 1998). More importantly for my research purposes, reboxetine when used as a pharmacological tool may be a major step towards further elucidation of the mechanisms of action of TCAs and other antidepressant agents in the treatment of depression and anxiety. Differences obtained experimentally between these antidepressant classes may shed light on their varying efficacy.

Table 1. Tricyclic Antidepressant Versus Serotonin Selective Reuptake Inhibitors in Severe Depression

	Study 1 (5 - week outcome)						Study 2 (4 - week outcome)					
	Citalopram % (N = 50)			Clomipramine % (N = 52)			Paroxetine % (N = 56)			Clomipramine % (N = 46)		
Patients	CR	PR	NR	CR	PR	NR	CR	PR	NR	CR	PR	NR
Endogenous depression	34	32	34	62	8	30	15	40	45	28	58	14
Nonendogenous depression	8	75	17	54	33	13	25	31	44	30	60	10
Total	28	42	30	60	15	25	18	37	45	28	59	13

*Complete response (CR) = 17-item Hamilton Rating Scale for Depression (HAM-D) < 7 score; partial response (PR) = HAM-D score 8-15; no response (NR) = HAM-D score > 16.
Study 1; date taken from Danish University Antidepressant Group (1986).
Study 2; data taken from Danish University Antidepressant Group (1990).
(Modified from Schatzberg, 1998)

With the success of SSRIs, the NA system has become the forgotten monoamine in depression. This is reflected by much research efforts during the past decade being focused on the mechanism by which antidepressants impact the 5-HT system. As a result, it has been demonstrated that all major classes of antidepressants induce a robust effect on the 5-HT system after a long-term administration (Blier and de Montigny, 1994). A net increase in 5-HT transmission to forebrain structures occurs during a prolonged antidepressant treatment, of which the mechanisms for this may vary according to drug class. Nonetheless, this commonality between all antidepressants during a prolonged administration has become accepted as being of major importance in the mechanism of action of these agents. This will be reviewed in Section 5 to a greater extent.

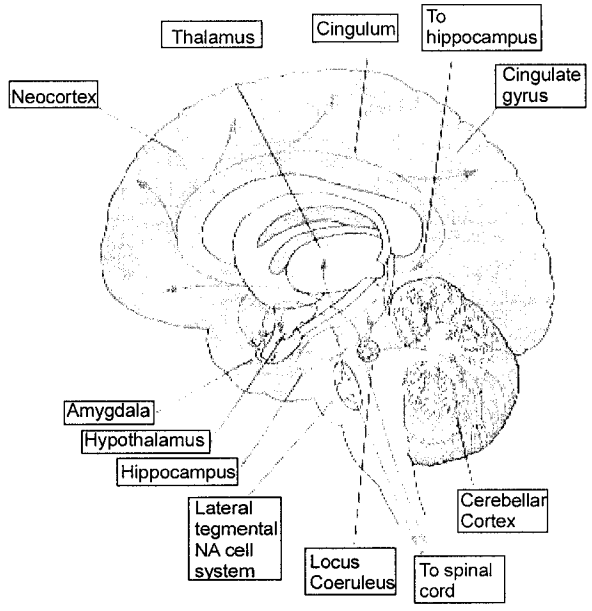
The 5-HT and NA neurons located in the brainstem send reciprocal innervation to each other (Haddjeri et al., 1997; Mongeau et al., 1997). The inter-modulation of the 5-HT and NA systems with respect to the mechanisms of antidepressant action and efficacy in anxiety and affective disorders during sustained antidepressant treatments is the main focus of this document.

This thesis is comprised of results derived from *in vivo* electrophysiological experiments carried out in rats and puts forth evidence of antidepressant agents targeting the 5-HT system do indeed alter the NA system. In turn, and with the advent of reboxetine, the ability of this agent to be able to alter parameters of the 5-HT system was assessed. The results obtained with reboxetine on the 5-HT and NA system contrasted to that obtained with the TCA and NAT reuptake inhibitor desipramine. This endeavor was important as it provides the ability to experimental evaluate the effects of TCAs being linked to increased efficacy in the treatment of depression is attributed to chemical moiety. A speculative neuronal circuitry is proposed to account for the alterations 5-HT ligands and SSRIs impart on LC NA neurons during a long-term treatment. Particular attention is placed on the time course and varying effects of these antidepressants on the 5-HT and NA systems. Extrapolation of these results to the beneficial and side-effect profile of antidepressants in anxiety and affective disorder treatment are made with hopes of identifying new strategies aimed at curtailing the delayed onset while enhancing antidepressant efficacy.

2. Noradrenergic System

Named *sympathine* because initially encountered as being released by sympathetic nerve terminals, it later adopted the name noradrenaline (NA) after meeting the criteria as a neurotransmitter in the CNS (see Cooper et al., 1996). Figure 3, illustrates the NA innervation of the brain and some of the behaviors thought to be regulated by NA. Alterations in behaviors regulated by the NA

Figure 3. The Noradrenergic System



(reproduced from Kandel et al., 1991)

Figure 3. Schematic of the NA innervation of the brain. Some of the behaviors thought to be regulated by NA are listed below.

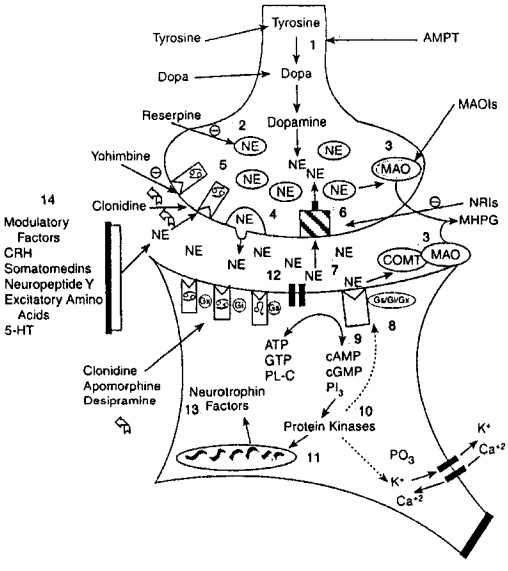
Noradrenergic Regulation
Alerting
Selective Attention
Learning
Memory
Movement
Initiation
Speed
Stamina
Regulatory
Energy
Drive
Motivation
Neurovegetative
Sleep-wake cycle
Feeding Behavior
Peripheral Sympathetic Regulation

system are highly representative of anxiety and affective disorder symptomology observed in patients (see Szabo and Blier, 2001, located in the appendix). NA is produced from the amino acid precursor L-tyrosine found in neurons in the brain, chromaffin cells, sympathetic nerves, and ganglia. The first step in synthesis of this catecholamine is hydroxylation of the precursor by the enzyme tyrosine hydroxylase (TH) that must be in the presence of Fe^{2+}, O^2 and a tetrahydroteridine cofactor. This rate limiting step and enzyme can be blocked by α-methyl-para-tyrosine, a drug used to

halt synthesis. The product formed is, 3,4-dihydroxyphenylalanine (DOPA) that then becomes decarboxylated by decarboxylase in the presence of vitamin B6, to form dopamine (DA). DA is then taken up from the cytoplasm into vesicles and hydroxylated by DA-β-hydroxylase in the presence of O^2 and vitamin C to form NA. The catabolism of NA is performed, in the presence of an aldehyde reductase, by MAO-A. This reaction then yields 3,4-dihydroxyphenolglycol (DOPEG). Once this product is excreted, catechol-O-methyl transferase (COMPT) in the presence of S-adenosylmethionine induces the formation of 3-methoxy-4-hydroxy-phenylglycol (MHPG; figure 4). The dietary depletion of tyrosine and α-methyl-para-tyrosine has been used in clinical studies to delineate the necessity of NA (and also DA) with respect to anxiety and affective disorders (McCann et al., 1995; Berman et al., 1999; Coupland et al., 2001). MHPG levels have been extensively evaluated in anxiety and affective disorder patients, however, the validity of this measure as representative of CNS function remains unknown.

Figure 4.

Role of NA in the Etiology of Depression and Mechanism of Action of Antidepressants*



(reproduced from Anand and Charney et al., 2000)

*Schematic model of central NA neurons indicating sites that may be involved in the etiology of depression and the mechanism of antidepressant action. **1.** *Enzymatic synthesis:* α-methylparatyrosine (AMPT) blocks tyrosine hydroxylase, the rate-limiting enzyme for NA synthesis. **2.** *Storage:* Reserpine interferes with the uptake-storage mechanism of amine granules, and chronic treatment causes depletion of catecholamines. **3.** Metabolism and turnover: NA is metabolized by MAO presynaptically and catechol-O-methyltransferease (COMT) in the synapse; 3-methoxy-4-hydroxyphenylglycol (MHPG) is the major metabolite. **4.** Release:Amphetamine increases net release of NA. **5.** Autoreceptors are α2 type. Clonidine has agonist activity and yohimbine, antagonistic activity; stimulation of autoreceptors leads to decrease in NA transmission. **6.** Reuptake site: NA has its action terminated by being taken up into the presynaptic terminal. Desipramine is a selective uptake inhibitor. **7.** Postsynaptic receptors are α1, α2, β types; clonidine, apomorphine, and desipramine are agonists at α2 receptor sites. β-Receptor down-regulation is one of the most consistent effects of long-term antidepressant treatment. **8.** G proteins: Coupling protein translate the effects of postsynaptic receptor stimulation into effects on second messanger, e.g., cyclic adenosine monophosphate (cAMP) system. **9.** Second messenger system: Consists of cAMP, cyclic guanosine monophosphate (cGMP), and the phosphatidylinositol (PI) system; production is stimulated or inhibited by G proteins and they in turn activate or inhibit protein kinases. **10.** Protein kinases: The third messanger system activates or inhbits phosphorylation of enzymes involved in protein synthesis i n the neurons and can affect synthesis and distribution of receptors. **11.** Genome: Protein kinases may also act by activating the synthesis of proteins and enzymes directly from the genetic code. **12.** Ion channels are ultimately responsible for neuronal firing; they can be directly activated via G protein or their modified by actions of protein kinases. **13.** Neurotrophic factors: Protein kinases may stimulate production of neurotrophic factors such as neurotrophin-3 (NT-3), which can increase NA transmission and increase the survival of NA neurons. **14.** Modulatory factors: A number of modulatory factors can affect NA transmission, including neuropeptides such as corticotropin-releasing hormone (CRH), somatomedin and neuropeptide Y, excitatory amino acids, e.g., Glutamate, aspartate, and serotonin (5-HT). Other abbreviations: ATP = adenosine triphosphate, GTP = guanosine triphosphate, MAOI = MAO inhibitor, NRI = noradrenergic reuptake inhibitor, PL-C = phospholipase C.

There are seven NA cell groups in the mammalian CNS, designated as A1 - A7. In the brain stem, these being the lateral tegmental neurons (A5 and A7) and the LC (A6; Paxinos et al., 1985). A5 and A7 neurons project to the spinal cord, the brain stem, thalamus, cerebellar and cerebral cortices (Paxinos, 1995). In general, the projections from A5-A7 are more restrictred to brainstem areas and do not interfere with that of the A6. It is this latter cell group that will be the most described due to 1) directly being implicated in anxiety and affective disorders and treatment thereof and 2) quite possibly represents the best chemically characterized nucleus in the rat brain. The name "locus coeruleus" was derived from the greek language because of its saddle shape and exhibiting a blueish color (*caeruleum*), it is located bilaterally in the mammalian brain. The LC is the most widely projecting CNS nucleus known (Foote, 1983), responsible for approximately 90% of the NA innervation of the forebrain and 70% of the total NA in the brain (Paxinos, 1995). For instance, the dorsal hippocampus receives a dense NA innervation exclusively from the LC in the rat (Jones et al., 1977; Menkes et al., 1983; Sutin and Minneman, 1985). These fibers reach the hippocampus formation by two pathways: the dorsal one travels via the fornix and the cingulum; the ventral one through the ventral and amygdaloid bundle (Loy 1980; Haring and Davis, 1985).

Under Nissl staining, the LC appears as a densely packed cluster of darkly stained cells in the rostral rhombencephalic tegmentum in which the nucleus is shaped as a tapered cylinder that extends approximately 1 mm in the rostrocaudal axis and 300 μm in diameter at its widest dorsoventral extent (Bezin et al., 1994). The LC traverses along the ventrolateral edge of the IV ventricle. Laterally and ventrally, the LC is bordered by the mesencephalic V (trigeminal) nerve that is often used as an anatomical landmark for *in vivo* electrophysiological recordings given the very small size of this nucleus (figure 5). The mesencephalic V nerve functions as a "monosynaptic jaw jerk reflex" whereby depressing the lower jaw of the rat while extracellularly recording in this nucleus action potentials are generated which can be viewed on the oscilloscope. Once this phenomenon is encountered, systematic movements of the electrode medially in this nucleus until the reflexive response is lost often results in LC NA neurons to be encountered at the same depth. Given that the LC nucleus is fairly homogenous,

composed almost exclusively of NA neurons (Paxinos, 1995) with a range of 1400 to 1800 of them, once in this nucleus it is most likely that an NA neuron is recorded. Furthermore, there is an electrophysiologic method to ensure further NA neuron specificity. Once a potential NA neuron is discriminated on-line and meets characteristic features thereof (see materials and method section of Chapter II), by pinching the contralateral paw, but not ipsilateral paw of the rat, a brief cessation in the spontaneous pacemaker firing activity of the NA neuron occurs for approximately 1 second, and then neuron the regains firing (Chiang and Aston-Jones, 1993a). This tonic pacemaker activity in LC NA neurons depends on endogenous adenosine 3',5'-cyclic monophosphate (cAMP) levels and involves the cAMP phosphorylation pathway. Endogenous cAMP appears to induce a persistent Ca^{2+}-independent/TTX-insensitive inward current that depolarizes the cell membrane (Alreja and Aghajanian, 1995). The specific substrate that may be phosphorylated by endogenous cAMP via protein kinase A to initiate and maintain tonic firing in LC neurons remains to be identified.

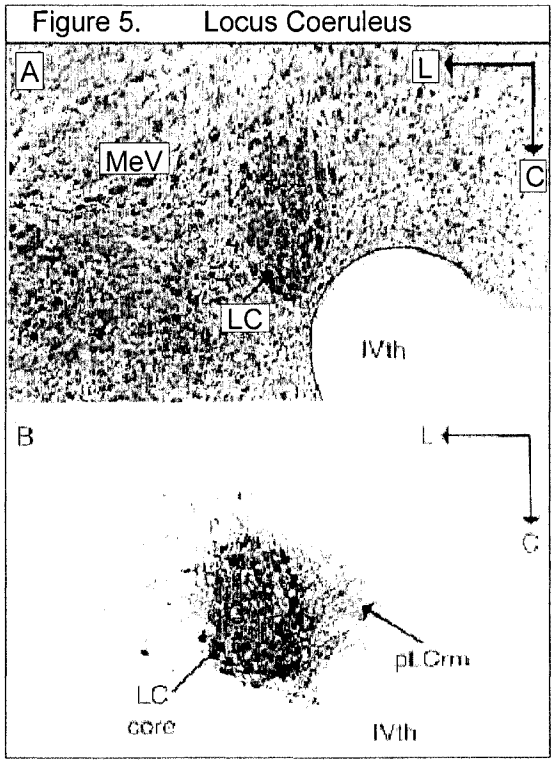


Figure 5. Brightfield photomicrographs of horizontal brain tissue sections processed for Nissel staining (A) and peroxidase labeling of the catecholamine synthesizing enzyme, TH (B) in the pontine nucleus LC. Panel A shows a distinct cluster of neurons lying between the mesencephalic nucleus of the trigeminal nerve (MeV) and the fourth ventricle (IVth). The clustering of NA somata is defined as the "core" area. The NA dendrites extend within the peri-LC (pLCrm) area primarily rostromedially (rm) in this place of section (pLCrm). Arrows point laterally (L) and caudally (C). Bar = 250 μm.

(reproduced from Van Bockstaele et al., 2001)

2.1. Noradrenergic Receptors

The α and β catecholamine receptors were first discovered more than 50 years ago (Ahlquist, 1948) and later subdivided further into α_1, α_2, and β_1, β_2, β_3 adrenoreceptors based on pharmacological and functional criteria (Langer, 1974). They are all members of the superfamily of seven transmembrane domain, G protein-coupled receptors. Through experiments using amino acid sequences, signal transduction, and radioligand binding, the adrenoceptors are now denoted as the $\alpha_{1A,B,D}$ subtypes being positively coupled to phospholipase C (PLC) and A_2, $\alpha_{2A/D,B,C,}$ subtypes which couple negatively to adenylate cyclase, and $\beta_{1,2,3}$ adrenoceptors which are all positively coupled to adenylate cyclase (Bylund et al., 1994). The α_{2A}-, α_{2B}-, and α_{2C}-adrenoceptors correspond to the human genes α_2-C10, α_2-C2 and α_2-C4, respectively (see Bylund et al., 1994; MacKinnon et al., 1994). The bovine, guinea-pig, rat, and mouse α_{2D}- is thought to be a species homologue or variant of the human α_{2A}-adrenoceptor (Bylund et al., 1994), and will be referred to as $\alpha_{2A/D}$ from herein.

Adrenoceptors are located throughout the brain and in the peripheral nervous system. For the purposes of this document, focus on adrenoceptors located in the raphe and LC brainstem nuclei, hippocampus, and frontal cortex, as the former two nuclei send monoaminergic inputs to the latter forebrain structures. Indeed, these forebrain structures are implicated in the malfunctioning of higher level processing being related to psychiatric disorder symptomology (see Szabo and Blier, 2001, located in the appendix). Only receptors that have been implicated in the antidepressant response will be discussed in detail.

2.1.1. α_1-adrenoceptors

Through the use of autoradiography and *in situ* hybridization techniques, reports have demonstrated that $\alpha_{1A,B,D}$ receptor subtypes are widely distributed in the rat CNS (Xiong and Sun, 1987). Autoradiographic studies reveal low levels of binding to α_1-

adrenoceptors binding in the hippocampus, moderate level in the raphe nuclei, and a high level in the LC (Unnerstall et al., 1985; Jones et al., 1985; Palacios et al., 1987; Chamba et al., 1991). A high level of mRNA corresponding to $\alpha_{1A/D}$- and α_{1B}-adrenoceptors are present in the hippocampus and raphe nuclei, respectively, whereas only very low levels of α_{1A}-adrenoceptors were detected in the LC (Pieribone et al., 1994; Nicholas et al., 1996). Given that α_1-adrenoceptors are mainly not located on NA cells but predominantly found in areas that become innervated by NA neurons, this receptor subtype acts predominantly as a heteroceptor (Nicholas et al., 1996). However, because trace amounts of binding to α_{1A}-adrenoceptors were present in the LC, this may correspond to heteroceptors located on a few of the non-NA cells in this nucleus. Studies using double labeling with a catecholamine marker would aid in ascertaining this prospectus. Comparing the NA innervation in the rat hippocampus demonstrates that the density of NA containing varicosities is much greater in areas where a lesser amount of α_1-adrenoceptors if found. This suggests a possible involvement of other adrenoceptors in NA mediated responses in the hippocampus (Zilles et al., 1991).

The α_1-adrenoceptors act primarily via the phospholipase C-protein kinase C (PLC-PKC) pathway to induce their physiologic actions (Summer and McMartin, 1993). As NA binds to its recognition site on the receptor complex, activation of the 1,4,5 inositol phosphate (PI) pathway is triggered. A cascade effect then results and the subsequent production of diacylglycerol and IP_3 with the release of Ca^{2+} from internal stores ensue. This release of Ca^{2+} produces activation of PKC and Ca^{2+}/calmodulin-dependent protein kinases to mediate phosphorylation/regulation of numerous types of channels and pumps. Also, the release of Ca^{2+} can directly influence the activation of K^+ channels.

2.1.2. α_2-adrenoceptors

As previously stated, α_2-adrenoceptors are composed of $\alpha_{2A,B,C,D}$ subtypes, which are located postsynaptically for the most part. Specifically, it has been estimated that approximately 80% of binding sites in the rat brain labeled with the α_2-adrenoceptor antagonist [^{3}H]idazoxan, is unaltered by the lesioning of NA neurons in the LC with the neurotoxin DSP-4 (Heal et al., 1991; Sakia et al., 1993). The α_{2A}- and α_{2D}-adrenoceptors are orthologous receptors (see Starke, 1987), meaning that these receptors vary only between species, and thus will be referred to as $\alpha_{2A/D}$-adrenoceptors from herein. Functionally, $\alpha_{2A/D}$-adrenoceptors inhibit adenylate cyclase activity through a G_i/G_o protein mechanism (Summers and McMartin, 1993), which produce in general an inhibitory action on the cell.

Given the lack of available pharmacologic ligands that discriminate between the different subtypes of α_2-adrenoceptors, the distribution of these receptors was extensively investigated with autoradiography (Boyajian and Leslie, 1987; Bruning et al., 1987; Hudson et al., 1992; King et al., 1995), immunohistochemical (Aoki et al., 1994; Rosin et al., 1996), and *in situ* hydridization techniques (McCune et al., 1993; Nicholas et al., 1993; Scheinin et al., 1994; Winzer-Serhan et al., 1997a,b). The $\alpha_{2A/D}$-adrenoceptors is proposed to predominate in the rat LC (Wamsley et al., 1992). This is in accord with binding studies in the LC showing a high amount of labeling with the α_2-adrenoceptor agonist oxytazoline (Alburges et al., 1993). This is also concordant with studies reporting mRNA labeling for the $\alpha_{2A/D}$-adrenoceptor in this structure, as well the DR nucleus, CA_3 pyramidal layer of the hippocampus, and cerebral cortex (Nicholas et al., 1993; Scheinin et al., 1994). Furthermore, labeling of α_{2C}-adrenoceptors with an antibody directed at this subtype is documented in the LC (Rosin et al., 1996). Also, observed in the LC were mRNA labeling for α_{2C}-adrenoceptors, however, the α_{2B}-adrenoceptor subtype was devoid of labeling in the brain except for the thalamus (Nicholas et al., 1993; Scheinin et al., 1994). With the advancements in molecular biology, utilization of probes have demonstrated that only the α_{2C}-adrenoceptor subtype

can be found at intracellular sites, thus providing another means to be able to discriminate between subtypes of α_2-adrenoceptors (Rosin et al., 1996). Recently $\alpha_{2A/D}$-, α_{2B}-, and α_{2C}-adrenoceptor-mutant mice were generated and confirm that $\alpha_{2A/D}$-adrenoceptors mainly make up the autoreceptors present on NA neurons with a minor proportion of α_{2C}-adrenoceptors being demonstrated (Altman et al., 1999). With these genetically altered mice, the conclusion was reached that 5-HT axons which possess α_2-adrenoceptors are of the same types and in the same proportion found on LC NA neurons as the α_{2C}- and $\alpha_{2A/D}$-adrenoceptor subtypes (Scheibner et al., 2001).

Yet, another drawback to experiments utilizing pharmacological ligands aimed at localizing α_2-adrenoceptors is the labeling of imidazoline (I) binding sites of which catecholamines do not have affinity to. Two classes of I binding sites are defined: I_1 as being labeled with [^{3}H]clonidine and [^{3}H]para-amino clonidine, of which the parent compound clonidine possess high affinity. Agmatine (decarboxylated arginine) has been proposed to be the endogenous ligand for I_1 (Li et al., 1994). The I_2 receptors are labeled by [^{3}H]idazoxan and its non-radioactive form has a high affinity for this receptor subtype (Miralles et al., 1993; Ernsberg et al., 1987; Molderings et al., 1993, 1994; Mackinnon et al., 1995). Autoradiographic studies with such compounds suggest the presence of both I_1 and I_2 in the DR and LC (Bousquet et al., 1992; Mackinnon et al., 1995). A recent study from Szabo et al., (1996) rules out the presence of a functional impact of I_1 and I_2 receptors on LC NA neuron activity. This is supported by a study demonstrating that agmatine, which also recognizes α_2-adrenoceptor binding sites is devoid of pharmacological activity at these receptors (Pinthong et al., 1995) and fails to modify the firing activity of LC NA neurons *in vitro* (Pineda et al., 1996). Also, the *in vivo* excitatory effect of I_1 ligands on the firing activity of LC NA neurons previously described by Pineda et al., (1993) appears to be due to an indirect effect. This has been proposed to be mediated by I receptors located in the medulla and is associated with the paragigantocellularis (PGi) nucleus and modulated by an inhibitory 5-HT mechanism (Ruiz-Ortega et al., 1995; Szabo et al., 1996). This later aspect may be incorporated into the speculative neuronal circuitry for the mechanisms of action of SSRIs on LC

activity that will be presented in Chapter IV. Indeed, there is a hypothesis regarding the pathophysiology of depression being related to the I system, of which antidepressant treatments alter (for review see Piletz and Halaris, 1995).

2.1.3. β-adrenocepetors

β-adrenoceptors are composed of three subtypes denoted as β_1, β_2, and β_3. All β-adrenoceptors have traditionally been demonstrated to activate adenylate cyclase through a stimulatory G-protein regardless of subtype (Bylund et al., 1994). Additional discussion of the β_3-adrenoceptors will be deferred here, as it is not present in the brain (Nicholas et al., 1996). β_1-receptors have a high affinity for both NA and adrenaline, whereas the β_2-adrenoceptors have preference for adrenaline (Bylund et al., 1994). Both β_1- and β_2-adrenoceptor mRNAs have distinct labeling patterns in the rat CNS and is similar to that observed in primates (Nicholas et al., 1993). *In situ* hybridization and radioligand binding studies have indicated that β-adrenoceptors do not label cells in the LC or raphe nuclei (Alexander et al., 1975; Sporn and Molinoff, 1976). β_1-adrenoceptors have a much more disperse labeling in the CNS than β_2-adrenoceptors which displayed the highest labeling in areas of the olfactory bulb, piriform cortex, hippocampal formation, thalamic interlaminar nuclei and cerebellar cortex (Nicholas et al., 1996). Regions of interest which demonstrating high labeling of β_1-adrenoceptors is the ventrolateral pontine and medullary reticular formations (Nicholas et al., 1996), as neurons in these areas exert a potent impact on NA activity in the LC (Aston-Jones et al., 1991).

Electrical stimulation of the LC exerts a brief suppression on CA_3 pyramidal neuron firing in the hippocampus that is followed by a period of excitation (Curet and deMontigny, 1988). It was pharmacologically elucidated that the inhibition and excitation on CA_3 pyramidal firing from LC stimulation is due to due to activation of α_1- and β-adrenocepetors, respectively (Curet and deMontigny, 1988). Antidepressants downregulate β-adrenoceptors in forebrain structures (Anand and Charney, 2000).

However, this phenomenon occurs with a time-course that often precedes the onset of action of antidepressants as well as occurring with non-antidepressant agents. It is daunting whether this effect contributes to treatment response (Blier and de Montigny, 1994).

2.2. Noradrenergic transporters

A characteristic feature of neurotransmitters that represent an important function is upon release in the synaptic cleft, they must have their action rapidly terminated by some process (see Cooper et al., 1996). The NA transporter (NAT) was the first of the monoamine transporters to be cloned in humans and transports NA from the synaptic cleft back into the neuron (Pacholczyk et al., 1991). This transporter was subsequently cloned in bovine (Lingen et al., 1994) and rat brains (Bruss et al., 1997). The NAT is comprised of twelve putative transmembrane domains and bears a large hydrophilic region (extracellular) between region 3 and 4. The rat transporter possesses a 93% and 91% homology of its amino acid sequence in human and bovine, respectively (Bruss et al., 1997; Lingen et al., 1994; Pacholczyk et al., 1991). Receptor autoradiography with various NA reuptake inhibitors has been used to determine the brain distribution of the NA transporters. Ligands such as [^{3}H]desipramine, [^{3}H]tomoxetine or [^{3}H]mazindol were demonstrated to be of limited use because the first agent listed possesses a high non-specific/heterogenous binding profile and the remaining two bind to other transporters. [^{3}H]nisoxetine has proven to be much more of a reliable ligand for this purpose (Tejani-Butt et al., 1990; Gehlert et al., 1995) but is not an antidepressant agent. As one would expect, a high level of NAT is found in the LC, with moderate to high levels found in the dentate gyrus, raphe nuclei, and hippocampus (Tejani-Butt, 1992). This pattern of expression is consistent with the NA innervation to these structures. [^{3}H]nisoxetine in the human brain has revealed an even distribution of NAT in the rostral-caudal axis of the LC and is similar to that found in the raphe (Ordway et al., 1997). The NAT is expressed mainly on NA terminals as demonstrated by a drastic reduction of labeling in most post-synaptic regions following NA destruction

with the neurotoxin 6-OH-DA or DSP-4 (Tejani-Butt et al., 1990; Tejani-Butt., 992; Cheetham et al., 1996). This is consistent with *in situ* hybridization studies showing that mRNA for NAT is detected in the LC of rats (Lorang et al., 1994) and humans (Eymin et al., 1995).

The NAT is dependent on extracellular NA^+ to mediate NA reuptake and the effectiveness of NA reuptake inhibitors in inhibiting NA reuptake (Bruss et al., 1997; Harder and Bonisch 1985; Friedrich and Bonisch 1986; Bonisch and Harder, 1986; Lingen et al., 1994). Through a Na+/co-transport process, energy for inward solute transfer is coupled to influx of NA down its concentration gradient. Furthermore, the influx of NA is sensitive to intracellular K+ (Harder and Bonisch, 1985). The uptake of NA is Cl^- dependent, meaning that the electrogenic process of NA transport is Na^+ and Cl^- driven (Lingen et al., 1994; Bruss et al., 1997; Harder and Bonish, 1985). In addition to the electrogenic process, the NAT demonstrates properties of a channel-like pore in that it would transport NA showing an infinite stoichiometry that can be blocked by cocaine and desipramine (Galli et al., 1995; Galli et al., 1996). Given this, as well as the commonality relying on similar ionic events, it was deemed that reuptake inhibitors bind to the same site as the NA recognition site. However, NA was demonstrated to be incapable of displacing [^{3}H]nisoxetine from the transporter (Tejani-Butt, 1992), which is in contrast to that of 5-HT for [^{3}H]cyanoimipramine (Kovachich et al., 1988). This suggested that all NA reuptake inhibitors may not overlap the binding site for NA. Eventhough NA is able to inhibit the binding of [^{3}H]desipramine, it does so less potently than the reuptake of [^{3}H]NA itself (Raisman et al., 1982). Previous studies based on chimeric proteins have suggested that transmembrane domains 5 to 8 of the NAT are involved in the high affinity binding of TCA drugs, and recent work by Roubert et al., (2001) involving 22 mutants of the human NAT have concluded that domains 6 and 7 may play an important role in the binding of TCA to the NAT, but domain 8 appears likely involved in the high affinity binding of TCA drugs to the NAT. Being that some NA reuptake inhibitors may inhibit the transport of NA by competing for the substrate cite, other agents which are not antidepressants but hallucinogens such as ketamine and PCP, as well as some sigma receptors, are able to non-competitively inhibit the

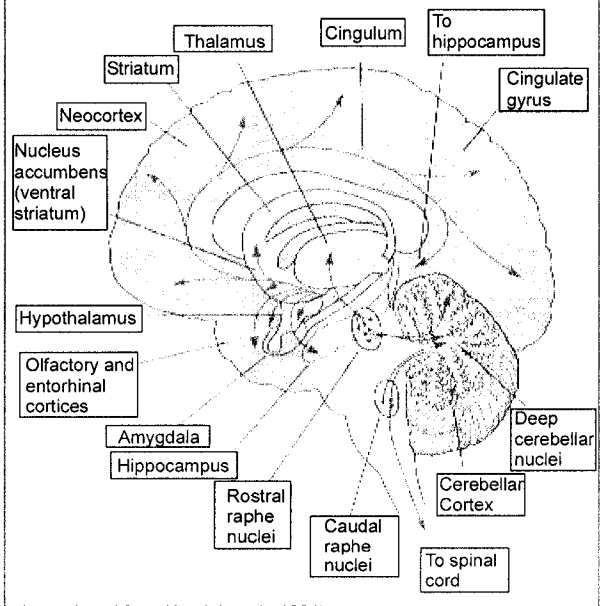
reuptake of NA (Baker and Blakely 1995). Thus, NA may allosterically regulate the binding of NA reuptake inhibitors, as well as reuptake inhibition of NA may modulate the transport of NA by acting non-competitively via a site different from the substrate.

A number of studies suggest that NAT can be regulated by diverse stimuli, neuronal activity, peptide hormones, as well as second messangers being elevated after receptor activation (Barker and Blakely, 1995; Kaye et al., 1997). Recently, cell surface radioligand binding studies demonstrate that activation of mAChR in human (h) blastoma expressing NAT acutely regulates cell-surface density of hNATs. In these cells, mAChR regulation of hNAT involves PKC and direct activation of PKC with phorbol esters influences surface hNAT density (Apparsundaram et al., 1998). Further studies by the group of Blakely using radioligand binding, surface biotynylation, and confocal imaging of immunolabeled transporters strengthen the claim of a major role for change in cell surface distribution as underlying the reductions in NA transport capacity observed after acute PKC activation (Apparsundaram et al., 1998). Recent studies reveal that all monoaminegic transporters (DAT, NAT, and 5HTT) are rapidly regulated by direct or receptor-mediated activation of cellular kinases, particularly PKC (Bauman et al., 2000). PKC activation results in an activity-dependent transporter phosphorylation and sequestration. Protein phosphatase 1/2A (PP_1/PP_{2A}) inhibitors, such as okadaic acid (OA) and calyculin A, also promote monoaminergic transporter phosphorylation and functional downregulation (Bauman et al., 2000). These phenomena that occur beyond the receptor level must be taken into account when considering the impact of antidepressants to alter the reuptake capability of these transporters. In turn, activation of receptors that alter PKC and other second messenger systems may be able to interfere with the transport reuptake process. These interactions should be kept in mind when attempting to develop an antidepressant using a rational approach.

3. Serotonergic System

Serotonin (5-HT) was given the name by Rapport et al., (1947) because of its activity as an endogenous vasoconstrictor in blood serum. This was later acknowledged as being the same molecule (secretine) found in the intestinal mucosa "secreted" by chromaffin cells (Brodie, 1900; Trifaro et al.,1984). Following this, 5-HT soon became characterized as being a neurotransmitter in the CNS (Bogdansky et al., 1956). 5-HT is found in platelets (8%), chromaffin cells of the intestine (90%), and in neurons (2%). Figure 6., illustrates that the 5-HT system innervates many aspect of the brain, as well as some of the behaviors linked to this neurotransmitter. Notice that the 5-HT system is linked to affective state. 5-HT neurons project to many similar structures as that of the NA and likely reflect their similarities in the regulation of behaviors (figure 3). The precursor to 5-HT is L-tryptophan, an amino acid that primarily comes from the

Figure 6. The Serotonergic System



(reproduced from Kandel et al., 1991)

Figure 6. Schematic of the 5-HT innervation of the brain. Some of the behaviors thought to be regulated by 5-HT are listed below.

Serotonergic Regulation
Affective State
Sleep-wake cycle
Feeding Behavior
Sexual Behavior
Automomic Regulation
Temperature Regulatory
Neuroendocrine Secretion
Circadian Rhythmicity
Hallucinogenesis
Pain
Motor Activity

diet and crosses the blood brain barrier through a non-specific carrier. However, due to competition with other amino acids for this carrier, only 4% of the circulating tryptophan contributes to 5-HT synthesis in the CNS. Tryptophan depletion via dietary restrictions has been used as a means to assess the role of the 5-HT system in many psychiatric illnesses (Young, 1993). Synthesis of 5-HT consists of hydroxylation of tryptophan by the enzyme tryptophan hydroxylase in the presence of 2 cofactors: O^2 and erythrotetrahydrobiopterin. The activity of this enzyme can be antagonized by parachlorophenylalanine (PCPA) and has been used as a means to deplete 5-HT (Sanders-Bush et al., 1974). This paradigm has been instrumental in assessing the effects, or rather the lack, of 5-HT on anxiety and affective disorders (Goodwin and Post, 1974; Carlson, 1976) as well as efficacy of antidepressant treatments (see Delgado, 1999). Reserpine (a 5-HT releaser that subsequently depletes 5-HT intracellular stores) has also been used to assess the impact of this monoamine in depression and antidepressant efficacy (Mendels and Frazer, 1974; Price et al., 1987). Because only a small amount of L-tryptophan is able to penetrate the brain, the amino acid constitutes as the rate-limiting step in 5-HT synthesis. Next, and in the presence of vitamin B6, decarboxylation of 5-hydroxytryptophan by the enzyme L-amino acid decarboxylase occurs to yield 5-HT. The catabolism of 5-HT is performed by MAO to produce 5-hydroxyindoleacetaldehyde that is further oxidized to 5-hydroxyindoleacetic acid (5-HIAA). Catecholamines and 5-HT can be catabolized by the two isoforms of MAO (MAO-A and MAO-B), at least under certain conditions (Johnston, 1968), for which selective inhibitors have been developed. The oxidative deamination of 5-HT as well as NA and epinephrine is preferentially carried-out by the A isoform (abundant in the LC), whereas the MAO-B form (abundant in the raphe) preferentially deaminates phenylethylamine and benzylamine. DA is deaminated by both forms (Westlund et al., 1985; Denney and Denney, 1985; Saura et al., 1992). Figure 7., provides and brief overview of the 5-HT neuron and its components.

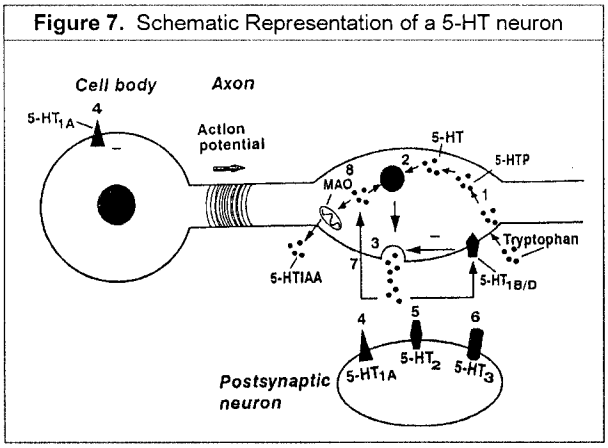


Figure 7. Schematic representation of 5-HT neurotansmission.The figure illustrates the site of action of prototypic drugs (see text). (1) PCPA; (2) reserpine; (3) fenfluramine; (4) the 5-HT_{1A} receptor anxiolytic-buspirone; (5) the $5\text{-HT}_{2A/2C}$ receptor antagonist ritanserin; (6) the 5-HT_3 receptor antagonist-odansetron; (7) the SSRI fluoxetine; and (8) the MAOI moclobemide. (reproduced from Graeff et al., 1997)

The first detailed anatomical map of the 5-HT system in a mammalian brain was provided by Dahlstrom and Fuxe, 1964, utilizing a method of being able to capture (freeze dry) 5-HT in tissue in combination to allowing its detection by fluorescence microscopy, known as the Falck-Hillarp method. This initial technique, and others over the years, have allowed the 5-HT in the brain to be localized to the central gray, in the surrounding reticular formation, and in cell clusters located in the center, thus adopting the name *raphe* from latin meaning midline. Due to the high proportion of 5-HT neurons located in the raphe nuclei, as well as most of the studies pertaining to the body of work presented in this document being reference to, this is the only 5-HT cell population that will be elaborated on.

The raphe nuclei consists of a dense cluster of 5-HT cells that are subdivided into nine groups (B1-B9), and its numerical order corresponds to their caudal to rostral orientation (Dahlstrom and Fuxes, 1964). The B1-B4 raphe nuclei are commonly referred to as the Caudal Linear Nucleus (CLN; Azmitia and Gannon, 1986; Tork, 1990) and extend along the rostral boundry of the superior cerebellar decussation. The depth of this structure is defined by ventrally bordering the interpeduncular nucleus and the dorsal limit is the dorsal raphe nucleus (DR; B6 and B7). The former limit of the CLN is sometimes viewed (Lorez et al., 1978) as an extension of the median raphe nucleus (MRN; B5 and B8). However, because of morphologic variations and these neurons projecting to different terminal fields, it is unlikely that they are. The DR is the largest brainstem 5-HT nuclei and contains approximately 50% of the total 5-HT neurons in the

mammalian CNS, whereas the MR comprises of 5% (Wiklund and Bjorklund, 1980; Descarries et al., 1982). The DR is not as homogenous of a structure for 5-HT neurons as that of NA neurons in the LC. The DR is primarily composed of 5-HT cells (70%) with the remaining being various peptidergic and non-peptidergic neurotransmitter containing neurons (Moss et al., 1983; Glazer et al., 1981; Beitz 1982; Descarries et al., 1986).

Electrophysiology on 5-HT neurons in both the MR and DR of anaesthetized rats has been performed (Aghajanian et al., 1968, 1970). These 5-HT neurons possess a regular discharge pattern resulting from a pacemaker cycle attributed to a Ca^{2+}-dependent K+ outward current. The depolarization is followed by a long afterhyperpolarization period, which diminishes slowly during the interspike interval. During the depolarization, extracellular Ca^{2+} enters the neuron via a voltage-dependant Ca^{2+} channel activating a K^{+} outward conductance leading to an AHP. Ca^{2+} is then sequestered/extruded and the afterhyperpolarization period diminishes slowly. When the membrane potential reaches the low-threshold Ca^{2+} conductance, a new action potential is triggered (Aghajanian and Lakoski, 1984; Burlhis and Aghajanian, 1987; Aghajanian et al., 1990). For a brief synopsis on methodology used to discriminate a DR 5-HT neurons *in vivo*, see the materials and methods section of Chapter VII.

3.1. 5-HT Receptors

In 1957, the existence of two separate 5-HT receptors were first proposed primarily due to the opposing phenomenon this neurotransmitter produces in reference to cholinergic mediation of smooth muscle contraction (Gaddum and Picarelli, 1957). Today, based on radioligand binding, signal transduction, and amino acid sequences, 5-HT effectors are currently comprised of seven distinct receptors ($5HT_{1\text{-}7}$). The following subtypes: $5\text{-}HT_{1A,\ B,\ D,\ E,\ F}$ are negatively coupled to adenylate cyclase, $5\text{-}HT_{2A,B,C}$ sybtypes are positively coupled to PLC, $5\text{-}HT_3$ receptors are the only fast mediated excitatory 5-HT receptor and is coupled to a ligand gated ion channel, and the $5\text{-}HT_{4,5,6,7}$

subtypes are positively coupled to adenylate cyclase (Humphrey et al., 1993). In relation to the present work, only the $5HT_1$ to 5-HT_2 receptors will be discussed in detail.

3.1.1. 5-HT_1 receptors

Shortly after identification of the first two receptor subtypes (Peroutka and Snyder; 1981), Pedigo et al., (1981) identified the 5-HT_{1A} receptors with the use of spiperone, a drug that possesses a high and low affinity for the 5-HT_{1A} and 5-HT_{1B} binding cites, respectively. Following this, synthesis of the tetraline derivative 8-OH-DPAT was characterized as the first selective 5-HT_{1A} receptor agonist (Gozlan et al., 1982; Hjorth et al., 1989), which now is acknowledged to possess affinity for the 5-HT_7 receptor as well (see Hoyer et al., 1994). Since then, many other tetraline derivatives have effectively labeled the 5-HT_{1A} receptors, however, most possess only partial agonistic properties in postsynaptic structures (Smith and Peroutka, 1986; Martin and Mason, 1987; Gartside et al., 1990; Yocca, 1990; Van der Hoof and Galvan; 1991; Blier and de Montigny, 1987). On the other hand, buspirone and ipsapirone are regarded as full agonists, but are plagued with sharing a common metabolite, 1-pyrimidinyl-piperazine (1-PP). This metabolite is a potent α_2-adrenoceptor antagonist. Recently, 3-OH-gepirone (a metabolite of gepirone) is indicated to act as a full agonist in some brain structures (Blier et al., 2000). More on 5-HT_{1A} receptors ligands relating to the antidepressant response will be presented in section 5.2.

Binding and autoradiography experiments indicate that 5-HT_{1A} receptors throughout the brain of various species possess a high density in many limbic structures including the hippocampus, septum, amygdala, entorhinal cortex, as well as 5-HT neurons of the dorsal and median raphe (Marcinkiewicz et al., 1984; Pazos and Palacios, 1985; Welner et al., 1989; Hall et al., 1985; Waeber et al., 1989). The highest labeling is found in the DR with lower densities observed in the remaining raphe nuclei (Pazos and Palacios, 1985; Weissmann-Nanopoulos et al., 1985; Verge et al., 1985; Verge et al.,1986; Hensler et al., 1991; Pompeiano et al., 1992; Li et al., 1997). In the abovementioned experiments, all 5-HT_{1A} receptors were associated with being at least

50% in the raphe nuclei. This was accomplished by demonstration that a selective degeneration of 5-HT neurons by intracerebral injection of the 5-HT neurotoxin 5,7-DHT is associated with a significant loss of 5-HT_{1A} binding only in the raphe nuclei. This is consistent with [^{3}H] 8-OH-DPAT labeling a high amount of mRNA coding for the 5-HT_{1A} receptor, and a hybridization signal for this receptor subtype in the DR becoming abolished following a 5,7-DHT lesion (Pompeiano et al., 1992). Given that in postsynaptic areas the labeling for 5-HT_{1A} receptors correlate well with the presence of a hybridization signal for mRNA suggests further that the location of the 5-HT_{1A} receptors are somatodedritic in most regions (Pompeiano et al., 1992). On the other hand, labeling for the 5-HT_{1A} receptor in the CA_3 region of the hippocampus is low and corresponds to most of this receptor subtype being found in dendrites of pyramidal neurons (Pompeiano et al., 1992).

Through the use of molecular biology techniques, the 5-HT_1 receptor subtype has been shown to be coupled to multiple G-proteins. The 5-HT_{1A} receptor subtype inhibits adenylate cyclase via pertusis toxin-sensitive G_i proteins of which it is preferentially coupled (de Vivo and Maayani, 1985; Okada et al., 1989). Consistent with this observation is in cultured transfected cells, 5-HT in the nM range, is able to inhibit the forskolin-stimulated adenylate cyclase activity (Fargin et al., 1989; Albert et al., 1990) and paradoxically can stimulate IP_3 and PKC production (Claustre et al., 1988; Raymond et al., 1989; Liu and Albert, 1991). It has been well demonstrated that the suppressant effect of 5-HT_{1A} receptor activation on DR and dorsal hippocampus firing activity is mediated by a pertussis toxin-sensitive mechanism that does not require a second messenger (Innis and Aghajanian, 1987; Innis et al., 1988; Andrade et al., 1986).

5-HT_{1D} receptors are virtually absent in the rodent but detected in guinea-pig and man (Bruinvels et al., 1993). It has been proposed that 5-HT_{1B} receptors are the rodent homologue of 5-HT_{1D} receptors (see Saxena et al., 1998). The 5-HT_{1D} subtype shares a modest homology of 74% with the 5-HT_{1B} receptors (Hamblin et al., 1992; Weinshank et al., 1992). In light of this, most pharmacological agents have not been able to differentiate between the two and possess a similar affinity for both subtypes. Also, the

distribution of the 5-HT_{1D} receptors in guinea pig and man are roughly equivalent to 5-HT_{1B} receptors in the rat (Bruinvels et al., 1993). This is concordant with electrophysiological data from our, and other laboratories, implicating that 5-HT_{1D} autoreceptors mediate a negative feedback influence on the release of 5-HT (Cerrito and Raiteri, 1979; Martin and Sanders-Bush, 1982; Gothert and Weinheimer, 1979). Similar to their 5-HT_{1A} somatodendritic autoreceptor counterparts, 5-HT_{1D} receptors are also negatively coupled to adenylate cyclase. Both the 5-HT_{1B} and 5-HT_{1D} receptors have been demonstrated in various models to inhibit the stimulation of forsklin-mediated cAMP (Hamblin et al., 1992; Zgombick et al., 1993; Hoyer et al., 1990; Weinshank et al., 1992; Schoeffter and Hoyer, 1989). Activation of 5-HT_{1B} and 5-HT_{1D} receptors stimulates PLC that then elevate intracellular Ca^{2+} (Zgombick et al., 1993).

The 5-HT_{1D} receptors are also located on 5-HT neurons in the rat DR and modulate the release of 5-HT in this nucleus (Hamblin et al., 1992; Piñeyro et al., 1995). This was experimentally deduced as the preferential 5-HT_{1B} agonist CP 93,129 in a concentration dependent manner inhibited the electrical evoked stimulation of [^{3}H]5-HT in preloaded hippocampus slices, was without effect on mesencephalic slices containing DR 5-HT neurons (Piñeyro et al., 1996). These results implied that the autoreceptor in the raphe was not of the 5-HT_{1B} subtype. Furthermore, in hippocampus slices prepared from 5-HT_{1B} knock out mice, CP 93,129 did not inhibit evoked 5-HT overflow, in contrast to its marked suppressant effect in wild type mice (Piñeyro et al., 1995). It has been also demonstrated that terminal 5-HT_{1B} autoreceptors are not coupled to G-proteins (Blier, 1991), but in the rat substantia nigra they do in fact inhibit forskolin stimulation of adenylate cyclase (Bouhelal et al., 1988; Schoeffter and Hoyer, 1989). Thus, different receptor coupling mechanisms may vary according to brain region.

3.1.2. 5-HT_2 receptors

There are three subtypes of 5-HT_2 receptors denoted as 5-$HT_{2A,B,C}$. The highest level of 5-HT_{2A} binding sites and mRNA for these receptors exist in the cortex, and are

implicated in the production of hallucinations with psychomimetic agents (for review see Aghajanian and Marek, 1999). In addition, 5-HT neuron lesions with 5,7-DHT did not reduce the 5-HT_2 receptor density reported in brain regions (Hoyer et al., 1986; Fischette et al., 1987; Conn et al., 1987; Hoffman and Mezey 1989; Pompeiano et al., 1994; Wright et al., 1995; Raghupathi et al., 1996). This indicates that these receptors are located postsynaptically. Recently, MDL 100,907 has been identified as a selective and potent 5-HT_{2A} receptor antagonist (Sorensen et al., 1993; Johnson et al., 1996; Kehne et al, 1996). Autoradiography with [^{3}H]MDL 100,907 has localized 5-HT_{2A} receptors to many similar brain regions in the rat and primate brain (Lopez-Gimenez et al., 1998). One of these brain regions is the LC (Lopez-Gimenez et al., 1999, 2001). Until recently, no selective ligands for the 5-HT_{2C} receptors (formerly denoted as 5-HT_{1C}) were available. Competitive studies with other radioligands (Yagaloff and Hartig, 1985; Sanders-Bush and Breeding 1988; Westphal and Sanders-Bush, 1994) and its mRNA distribution indicate 5-HT_{2C} receptors being considerably widespread through the CNS with the highest density in the choroid plexus (Hoffman and Mezey, 1989). 5-HT_{2C} receptors have been detected in both the DR and LC (Molineaux et al., 1989; Pompeiano et al., 1994; Wright et al., 1995; Abramowski et al., 1995), but nowhere in the brain is 5-HT_{2B} receptors detected (Pompeiano et al., 1994; Hoyer et al., 1994).

All of the 5-HT_2 receptor subtypes are linked to the phosphoinisitide (PI) signaling system and their activation produces inositol triphosphate (IP_3) and diacylglycerol, via PLC activation (Conn and Sanders-Bush, 1987; Conn et al., 1987; Launay et al., 1994). Several tritiated ligands such as spiperone, ketanserin, mianserin, metergoline or [^{125}I] LSD and [^{125}I] ketanserin, have been used to describe 5-HT_2 receptors, as well as agonists such as DOB and DOI (Titeler et al., 1987; McKenna and Peroutka, 1989). In addition to the display of these 5-HT_2 receptors, it has been demonstrated that agonist binding induces a rapid internalization (Willins et al., 1998). This would be equivalent to an antagonistic-like effect and represents an important issue to consider when evaluating the mechanism of action of antidepressants.

3.2. 5-HT Transporters

Termination of 5-HT in the synaptic cleft includes degradative metabolism of, but are not limited to enzymes, is the ability of 5-HT transporter (5-HTT) to remove 5-HT from the synaptic cleft by an ion dependent reuptake process (figure 7). 5-HT is taken up into the presynaptic terminals where it is metabolized by MAO or sequestered into secretory vesicles by the vesicular transporter. The cDNA for the brain 5-HT transporters (5-HTT) has been cloned from rat (Blakely et al., 1991; Hoffman et al., 1991), mouse (Chang et al., 1996) and humans (Lesch et al., 1993; Ramamoorthy et al., 1993). Cloning and sequencing of cDNA encoding 5-HTT revealed two related proteins with twelve transmembrane domains (similar to that of the NATT) containing the secondary structure required for the substrate translocation, ion, and antagonist binding (Blakely et al., 1991; Hoffman, 1994). 5-HTT are located outside of the CNS in the periphery, being produced by enteric 5-HT neurons (Wade et al., 1996) as well as non-neuronal cells, such as mast cells (Gripenberg, 1976), cript epithelial cells, and enterochromaffice cells (Wade et al., 1996). 5-HTT is also located in platelets (Rudnick, 1977; Quian et al., 1995), lung membranes (Quian et al., 1995) and maternal brush-border of syncytiotrophoblasts (Cool et al., 1990; Ramamoorthy et al., 1993).

In the brain, 5-HTTs have been radiolabled with [^{3}H] imipramine (Langer et al., 1980; Dawson and Wamsley, 1983; Hrdina et al., 1985) and more selectively with 5-HT uptake inhibitors such as [^{3}H]cyanoimipramine (Wolf et al., 1988; Kovachich et al., 1988; Soucy et al., 1994), [^{3}H]paroxetine (Habert et al., 1985, de Souza and Kuyatt, 1987; Langer et al., 1987; Marcusson et al., 1988) and [^{3}H] citalopram (D'Amato et al., 1987). [^{3}H] imipramine as compared with the [^{3}H]SSRIs (Hrdina et al., 1990; Duncan et al.,1992) appears to be similar, but regional differences in density were detected. The former yields a much higher density for binding in the forebrain areas such as cortex and hippocampus. This is presumably due to [^{3}H]imipramine being able to bind to two classes of sites on the 5-HTT, being the high and low affinity sites. However, only the high affinity sites seem to be related to 5-HT uptake (Moret and Briley, 1986; Marcusson et al., 1986; Hrdina 1987, 1988; D'Amato et al., 1987). Many SSRIs have been

radiolabeled, however, due to low specific to non-specific binding ratios, paroxetine (Arranz and Marcusson, 1994) and citalopram (Descarries et al., 1995) are regarded as optimal for *in vitro* studies.

Cellular localization of 5-HTT in the CNS has been accomplished by using site-specific antibodies (Lawrence et al., 1995a,b; Qian et al., 1995). Immunocytochemistry directed against sites on the second and third intracellular loops of the 5-HT carrier revealed both neuronal and glial staining in areas of the rat brain containing 5-HT somata and terminals (i.e., DR and hippocampus; Lawrence et al., 1995b). 5-HT uptake ability has been documented in primary astrocyte cultures (Katz and Kimelberg, 1985; Kimelberg and Katz, 1985) and accounts for 50 to 80% in the frontal cortex and periventricular region, respectively (Anderson et al., 1992). It is hypothesized that the first step in 5-HT transport involves the binding of 5-HT to the 5-HTT and then a co-transport with Na^+, while the second step involves the translocation of K^+ across the membrane to the outside of the cell. SSRIs bind to the same site on the transporter as 5-HT itself. The regional distribution of 5-HTT corresponds to discrete regions of rat brain known to contain cell bodies of 5-HT neurons and synaptic axon terminals (Backstrom et al., 1989; Hrdina et al., 1990; Mann and Hrdina, 1992).

4. Functional interactions of NA and 5-HT systems

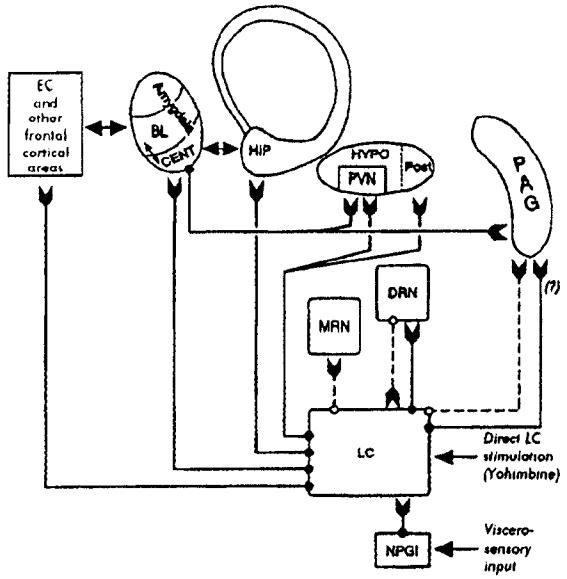
It has been well established that DR 5-HT neurons receive projections from the LC (Loizou, 1969; Anderson et al., 1977; Baraban and Aghajanian, 1981; Jones and Yang, 1985; Luppi et al., 1995; Haddjeri et al., 1997). Pharmacological studies suggest that the firing activity of 5-HT neurons in the DR is dependent on a tonic activation of NA being mediated by postsynaptic α_1-adrenoceptors on 5-HT neurons (Svensson et al., 1975; Baraban and Aghajanian, 1980; Clement et al., 1992; Marwaha and Aghajanian, 1982). The release of NA to the DR is also modulated presynaptically by α_2-adrenoceptors on LC NA neurons (Mongeau et al., 1993). Moreover, activation of α_2-adrenoceptors attenuate 5-HT synthesis in the DR and hippocampus (Yoshioka et al., 1992). In turn, several lines of evidence support the notion that the 5-HT system impacts upon the NA but considerably less attention has been placed on this inverse sequence of events. In contrast to that of NA on 5-HT, electrophysiological studies have revealed a tonic inhibitory role of 5-HT on the function of LC NA neurons (Haddjeri et al., 1997). Lesions of raphe nuclei or pretreatment with the 5-HT synthesis inhibitor PCPA increase both tyrosine hydroxylase activity and the neuronal firing rate of NA neurons in the LC (Crespi et al., 1980; McRae-Deguerce et al., 1981, 1982; Reader et al., 1986). The next two sections will review the literature on the functional impact of these two monoamines on each other.

4.1. NA innervation of the raphe nuclei

The NA input to the 5-HT system occurs almost exclusively from the LC (Sakai et al., 1977; Hebert and Saper, 1992). The LC and other NA nuclei, the lateral tegmentum, send efferents to the raphe nuclei (Pazos, 1995). The LC has been implicated in panic disorder (see Szabo and Blier, 2001; located in the appendix) and projects to many brain structures related to the possible manifestation of anxiety and depression (figure 8). One of these structures receiving an exclusive NA innervation is the hippocampus. Interestingly, upon a DSP-4 treatment, which selectively destroys

LC NA neurons while sparing other NA nuclei (Fritschy and Grzanna, 1989), it was demonstrated to markedly suppress the firing activity of 5-HT neurons in the DR (Svensson et al., 1975). This provide functional evidence that LC NA neurons are largely responsible

Figure 8. The NA system in Panic Disorder



(reproduced from Grove et al., 1997)

for the tonic activation of 5-HT neurons in the DR. Pharmacological studies also suggest that the firing activity of 5-HT neurons in the DR is dependent on a tonic activation of NA being mediated by postsynaptic α_1-adrenoceptors on these neurons and modulated by presynaptic α_2-adrenoceptors on LC NA neurons (Svensson et al., 1975; Baraban and Aghajanian, 1980; Clement et al., 1992; Marwaha and Aghajanian, 1982; Mongeau et al., 1993).

The activation of α_2-adrenergic heteroceptors located on 5-HT terminals in cortex, hypothalamus, and the hippocampus, reduces 5-HT release in these areas (Gothert and Huth, 1980; Frankhuyzen and Mulder, 1980; Galzin et al., 1984; Starke et al., 1987). It has been demonstrated *in vitro* that exogenous, but not endogenously released NA, activates α_2-adrenergic heteroceptors in the human and rat neocortex (Feuerstein et al., 1993). *In vitro* studies suggest that an interaction between α_2-adrenergic heteroceptors and 5-HTT cannot be explained by the increased synaptic availability of NA. Rather, a functional link between these two systems depend on the level of activation of presynaptic 5-HT autoreceptors by endogenous 5-HT (Blier et al., 1990). However, from *in vivo* studies in the hippocampus this statement has to be reconsidered. Given that the firing activity of 5-HT neurons is modulated by fascilitatory α_1-adrenergic and inhibitory α_2-adrenoceptors, one may assume that both α_1- and α_2-adrenoceptor agents modulate 5-HT release *in vivo*, as in the case *in vitro* (Frankhuyzen and Mulder, 1980). In fact, microdyalisis studies in the rat ventral hippocampus have demonstrated that systemic administration of α_2-adrenergic agonists reduce the spontaneous release of 5-HT, an effect abolished by idazoxan (Tao and Hjorth, 1992). Local administration of α_2-adrenoceptor agonists clonidine or UK 14.304 into the hippocampus attenuated the firing activity of CA_3 pyramidal neurons and the K^+-evoked release of 5-HT in NA-lesioned rats, respectively (Curet and deMontigny 1989; Mongeau et al., 1994c). This suggest that these agonists activate α_2-adrenergic heteroceptors on 5-HT terminals These receptors appear to be coupled to G-proteins since the effect of UK 14.304 is abolished by pretreatment with pertussis toxin (Yoshioka et al., 1992; Numazawa et al., 1995). De Boer et al., (1996) using microdialysis in the rat ventral hippocampus demonstrated that local infusion of TTX attenuates 5-HT release and systemic administration of the α_2-adrenergic antagonist mirtazapine, but not mianserin or idazoxan, augments 5-HT levels. On the other hand, the α_1-adrenoceptor antagonist prazosin attenuated 5-HT release in this structure (Rouquier et al., 1994).

In order to distinguish the activations of α_2-adrenoceptor auto- and heteroceptors *in vivo*, it has been demonstrated that low doses of clonidine enhance the effectiveness of a high frequency (5 Hz) electrical stimulation of the ascending 5-HT pathway in the suppression of the firing activity of dorsal hippocampus CA_3 pyramidal neurons (Mongeau et al., 1994a). Also, comparison of the effectiveness of low (1 Hz) and high frequency stimulations of the afferent 5-HT bundle provides a means of assessing the sensitivity of terminal 5-HT_{1B} autoreceptors in the hippocampus. The inhibitory effects of these electrical stimulations is mediated by activation of postsynaptic 5-HT_{1A} receptors in this structure (Chaput et al., 1986b; Chaput and de Montigny et al.,1988). In contrast, high doses of clonidine attenuates the effectiveness of this high frequency stimulation (Mongeau et al., 1994a). Interestingly, only the enhancing effect of the low dose of clonidine was abolished in rats pretreated with the NA neurotoxin 6-OH-DA (Mongeau et al., 1993). This indicates that the increased effectiveness of the stimulation of the afferent 5-HT pathway to a low dose of clonidine results from the selective activation of α_2-adrenergic autoreceptors on NA terminals, thereby reducing the tonic activation of NA onto α_2-adrenergic heteroceptors on 5-HT terminals (Mongeau et al., 1994b). On the other hand, the attenuating effect of high doses of clonidine could be due to a direct activation of the α_2-adrenergic heteroceptors on 5-HT terminals (Mongeau et al., 1993), consistent with that of microdialysis studies in the rat hippocampus and frontal cortex (Cheng et al., 1993; De Boer et al., 1996). Thus, the NA system while inducing a tonic activation of 5-HT neuronal firing via α_1-adrenoceptors, can also increase and decrease 5-HT release in the hippocampus via α_2-adrenergic auto- and heteroceptors located on 5-HT neuron terminals, respectively (figure 9). Results from this experimental paradigm with different classes of antidepressant agents will be detailed in section 5. The inter-modulation of NA and 5-HT at the terminal level should be considered when pondering the mechanisms of action of antidepressant drugs selective for the NA system on the 5-HT (see Chapter IX).

Figure 9. Interactions between 5-HT and NA in the Hippocampus

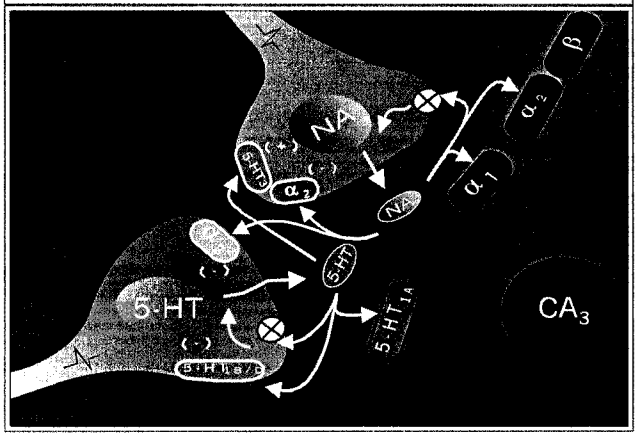


Figure 9. Iontophoretic application of NA mediates inhibitory effects on the firing activity of CA_3 pyramidal neurons via α_2-adrenoceptors. Electrical stimulation of the LC imparts inhibitory and excitatory effects on CA_3 neurons via α_1- and β-adrenoceptors, respectively. Iontophoretic application of 5-HT and electrical stimulation of the afferent 5-HT bundle mediates an inhibitory action on CA_3 neurons via 5-HT_{1A} receptors. 5-HT neuron terminals possess inhibitory 5-$HT_{1B/D}$ and α_2-heteroceptors modulating release. NA neuron terminals possess α_2-adrenoceptors inhibiting NA release. The cogwheels represent reuptake transporters. Note that 5-HT_3 receptors augment NA release in this structure and is purely placed on the NA terminals for simplicity. No data indicates or rejects thispossibility.

4.2. 5-HT innervation of the LC

There is a dense innervation of 5-HT containing fibers in the LC (Leger et al., 1980). Furthermore, a high density of 5-HTT, which is a valid index of 5-HT innervation (Soucy et al., 1994), is located in the LC of rodents and humans (Hrdina et al., 1990; De Souza and Kuyatt, 1987; Biegon and Mathis, 1993). Most studies have pointed to the DR and MR in the 5-HT innervation of the LC (Tork and Hornung, 1990). Beyond the raphe nuclei, 5-HT afferents to the LC also originate from the supralemniscal area (Maeda et al., 1991; Baumgarten and Grozdanovic, 1995) periventricular gray of the pons, and probably from 5-HT perikarya of the pericoerulear region (Aston-Jones et al., 1991a). A minute amount of 5-HT perikarya is located directly in the LC (Sladek and Walker, 1977; Leger et al., 1979; Iijima, 1993). Controversy exists with respect to if the LC core is innervated by the MR and DR or whether 5-HT neurons from these and other nuclei primarily innervate pericoerulear regions. Anterograde studies with proline indicate that both the MR and DR project only minimally to the core of the LC (Segal et al., 1973; Pickle et al., 1974). In keeping with this, lesions of the DR (Pieribone and Aston-Jones, 1988), but not the MR (Leger et al.,1980), do not induce any discernable decreases in the density of 5-HT immunoreactive fibers in the LC. The MR innervation to the LC proper would then appear to contribute to a greater extent than that of the DR, but is still rather minimal. These observations are at variance with results reported by

Luppi et al., (1995). In light of this, the group of Aston-Jones decided to use a combination of retrograde staining with 5-HT immunoreactivity showing that following an injection directly in the core of the LC nucleus, only a small number of cells were double stained in the DR whereas a greater proportion of labeling was observed in the MR cell group. It may be possible that the greater number of DR cells compared to MR cells observed following injection in the core of the LC in the study of Luppi et al., (1995) was in fact non-5-HT cells being that 1 out of every 3 cells located in the DR are 5-HT. Importantly, an injection into the pericoeruleus regions of the LC did yield a significant amount of staining in the DR and MR (Pieribone and Aston-Jones, 1988). When taken together, the majority of 5-HT to the LC innervates pericoerulear regions and corresponds to that of the raphe nuclei. Also, because LC NA neurons extend dendrites beyond the core of the LC into extranuclear zones, whether or not 5-HT innervation directly impacts upon the LC proper or dendrites in the pericoeruleus region seems of little importance in a functional perspective. 5-HT input from these regions can be expected to participate in the synaptic input to LC NA neurons.

The extracellular concentration of 5-HT has been monitored in the LC using the push-pull superfusion technique and is concluded to be of neuronal origin (Singewald et al., 1997). This is exemplified by the superfusion of TTX and veratridine in the LC was able to suppress and enhance the extracellular levels of 5-HT in this nucleus of freely moving rats, respectively (Singewald et al., 1997). It was later concluded with the same experimental techniques that under electrical, chemical, thermolitic, and 5,7-DHT injection in the DR, more than 50% of the 5-HT in the LC is derived from 5-HT neurons originating from the DR (Kaehler et al., 1999). Thus, in contrast to the excitatory effect LC NA neurons impart on DR 5-HT neurons monosynaptically via α_1-adrenocepetors, evidence suggests that the 5-HT system exerts an inhibitory influence on LC NA neurons. There is a void of knowledge with respect to intricacies and details of the circuitry(s) and receptors responsible for these effects. It is suspected that if it were a monosynaptic regulation, the mechanisms related to the 5-HT mediated inhibition on NA activity would most likely been elucidated and represents a complex effect. Interestingly, DR 5-HT neurons possess different characteristics than those in the MR

(table 2). In particular, the greater impact of 5,7-DHT on the DR versus MR 5-HT neurons will aid to pharmacologically elucidate the role of these nuclei and 5-HT receptor effects on the regulation of NA activity.

Table 2. Differences between the dorsal raphe nucleus and the median raphe nucleus

Dorsal Raphe Nucleus	Median Raphe Nucleus
Characteristics	
Less myelination	More myelination
Small axons	Larger axons
Small, irregular varicosities	Large, regular varicosities
More diffuse connections	More precise connections
May be damaged/destroyed by neurotoxins 5,7-DHT, MDA*, etc.	Not prone to damage by DR neurotoxins
More homotypic collaterals	Less homotypic collaterals
70% of neurons possess 5-HT	35% of neurons have 5-HT

*MDA = 3,4-methylenedioxyamphetamine
(reproduced from Tork and Hornung et al., 1990)

The presence of a tonic inhibitory action of 5-HT on the LC is demonstrated by the observation that both a PCPA pretreatment (Reader et al., 1986; Ferron, 1988) and a 5,7-DHT lesion (Haddjeri et al., 1997) lead to an increase in the spontaneous firing activity of LC NA neurons. This inhibitory input of 5-HT to the LC is further evidenced by the decrease in spontaneous and evoked firing of LC NA neurons by stimulation of the DR, an effect also abolished in PCPA or 5,7-DHT treated rats (Segal, 1979). The microiontophoretic application of 5-HT agonists in the LC was demonstrated in some studies (Segal 1979; Aston-Jones et al., 1991), but not others (Haddjeri et al., 1997; Gorea et al., 1991), to suppress the firing activity of NA neurons. This effect is antagonized by concurent microiontophoretic applications of the 5-HT receptor antagonist methysergide (Segal, 1979). Noteworthy is that studies which report a suppression of the spontaneous firing of LC NA neurons by microiontophoretic 5-HT used a higher 5-HT concentration and currents than in studies which report no change. This may be interpreted that the 5-HT receptors mediating the effects of 5-HT on LC activity are located away from the cell body, perhaps on terminals that project to the dendritic tree of NA neurons. This would be consistent with anatomical data showing

that the LC gives rise to an extensive network of dendrites located beyond the LC proper extending into pericoerulear zones (Shipley et al., 1996). Support for this is derived from *in vitro* studies in slices containing the LC that report a lack of effect of bath application of 5-HT or the non-selective agonist 5-CT on membrane potential, firing rate, and input resistance of NA neurons (Bobker and Williams, 1989). However, bath application of 5-HT elicits a decrease in the spontaneous firing activity of NA neurons accompanied by attenuated membrane potential (Chiu et al., 1995). Taken together, it appears that the bulk of the 5-HT input to the LC (derived from the DR), does not exert its effects directly on LC NA neurons.

4.3. 5-HT receptors and the LC

An abundant labeling of [^{3}H]5-HT is evidenced in the LC, however, that corresponding to 5-HT_1 receptors were much more scarce (Weissmann-Nanopoulos et al., 1985). Although often regarded as autoreceptors on 5-HT neurons, 5-HT_1 receptor subtypes are located postysynaptic to 5-HT neurons as well as being somatodendritic. The significant amount of 5-HT in the LC has been concluded to correspond to that of 5-HT_2 receptors (Pompeitto, 1999, 2001). The labeling of neither [^{3}H]5-HT, nor that of [^{3}H]8-OH-DPAT and ketanserine in the LC were significantly altered in 5,7-DHT treated animals (Weissmann-Nanopoulos et al., 1985). This indicates that these 5-HT receptors in the LC are located postsynaptic to 5-HT neurons and not on 5-HT nerve terminals. The fact that an mRNA hybridization signal for the presence of 5-HT_{1A} and 5-HT_2 receptors was not observed in the LC (Pompeiano et al., 1992; 1994), suggests that the 5-HT receptors are located on nerve terminals of other neuronal elements projecting to this nucleus, such as glutamatergic and GABAergic afferents (see Chapter IV). It is interesting that 5-HT_{1A} and 5-HT_{2A} receptors alter the activity of NA and 5-HT neurons and both these of monoamines are implicated in anxiety and affective disorders (Graeff et al., 1997). This may also represent an important link as to why more often than not a comorbidity of these disorders is observed. The next two sections will summarize the evidence regarding 5-HT_1 and 5-HT_2 receptors in the LC, as well as their functional impact.

4.3.1. 5-HT_1 receptor mediated effects

The acute systemic administration of various 5-HT_{1A} receptor agonists augment the firing activity of LC NA neurons (Sanghera *et al.* 1982; Sanghera *et al.* 1990; Piercy et al., 1994; Engberg 1992; Broderick and Piercey 1991). Although some of these compounds have 1-(2-pyrimidinyl)-piperazine (1-PP) as a common metabolite, which is a α_2-adrenergic antagonist, raised uncertainty of whether this was a true 5-HT receptor effect. The fact that systemic administration of 8-OH-DPAT augments the firing activity of NA neurons and is devoid of a 1-PP metabolite provides evidence that it is a 5-HT_{1A} receptor-mediated response (see section 5.1.; Engberg, 1992; Piercey et al., 1994). This enhancement of LC firing activity is also reflected by *in vivo* voltammetric experiments where systemic administration of 8-OH-DPAT elevated the DOPAC levels in the LC (Clement et al., 1992). These results, however, do not provide any indication on the location of the 5-HT_{1A} receptors mediating these responses on LC neurons. As mentioned earlier, a 5-HT destruction with 5,7-DHT does not alter the 5-HT_{1A} receptor binding profile in the LC (Weissman-Nanopoulous, 1985). Furthermore, these 5-HT_{1A} receptors are not directly situated on NA neurons as a hybridization signal for this subtype in the LC has not been observed (Pompeiano et al., 1992). Finally, microiontophoretic applications of 8-OH-DPAT, at low to moderate currents did not alter the firing activity of LC NA neurons (Gorea et al., 1991). Indeed, it appears plausible that the effects on NA activity observed through systemic administration of 5-HT_{1A} receptor agonists would inhibit the firing activity of 5-HT neurons in the DR and reduce the tonic 5-HT mediated inhibition in the LC. This would lead to an increase in NA neuron firing. In support of this contention, microinjection of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the DR led to a long-lasting reduction of the release rate of 5-HT in the LC (Kaehler et al., 1999) and in various terminal regions as measured by microdialysis (Hjorth and Sharp, 1991). On the other hand, locally applied 8-OH-DPAT in the LC attenuates the extracellular concentration of 5-HT in this nucleus as determined by push-pull cannulae experiments in freely moving rats (Singewald et al., 1997). The mechanism by which 5-HT_{1A} receptors are able to augment LC NA neuron activity remains to be elucidated.

An augmented endogenous 5-HT output by WAY 100,635 in the LC is supported by the fact that this suppression was demonstrated to be 5-HT_2-receptor mediated and presumably postsynaptic (Fornal et al., 1996). This supports that the 5-HT inhibitory input to the LC is mediated through 5-HT_2 receptors and will be further elaborated in the next section. It has also been demonstrated that the acute systemic administration of the selective and potent 5-HT_{1A} receptor antagonist WAY 100,635, in contrast to agonists, suppress the firing activity of LC NA neurons (Haddjeri et al., 1997). This effect of WAY 100,635 is dependent on 5-HT neurons as destruction of these neurons by 5,7-DHT abolishes its effects (Haddjeri et al., 1997). The inhibition of NA neuron firing from systemically administered WAY 100,635 may be due to an activity independent release mechanism of 5-HT in the LC as systemic administration of this compound fails to consistently alter DR activity. The 5-HT_{1A} receptor antagonists WAY 100,635 thereby augments 5-HT output in the LC and may activate 5-HT_2 receptors imparting an inhibitory influence in this nucleus to mediate attenuated NA activity. However, this needs to be experimentally assessed as well as the particular 5-HT_2 receptor subtypes responsible for this effect.

A 5-HT mediated attenuation on sensory evoked activity in the LC has been described (Chouvet et al., 1988). This observation is complemented by the demonstration that local activation of 5-HT_{1A} receptors attenuates glutamate-induced excitation of the firing activity of LC NA neurons (Aston-Jones et al., 1991b; Charléty et al., 1991). Presently, the physiological role of this regulation on NA activity is unclear. These authors have suggested that 5-HT in this nucleus would activate presynaptic 5-HT_{1A} receptors on LC NA neurons and reduce the excitatory effect of these neurons. More compelling, however, are observations of Bobker and Williams (1989) through using the *in vitro* LC slice preparation demonstrated the presence of functional 5-HT_{1A} and 5-HT_{1B} receptors modulating the release of glutamate in this nucleus, whereas only activation of the 5-HT_{1B} receptors modulate the release of GABA. That these 5-HT receptors are located on neurons in, or projecting terminals to the LC was further illustrated by the incapacity of these 5-HT receptors when activated to alter NA neuronal responses to exogenous GABA or glutamate (Bobker and Williams, 1989). The results of Bobker and Williams (1989) are thus incongruent with those of Aston-Jones et al.,

(1991) and Charléty et al., (1991) suggesting a presynaptic 5-HT_{1A} receptor mediated effect on NA neurons. The reason for this discrepancy is not currently known.

A unique feature of the expression pattern of 5-HT_{1B} receptors is the mismatch in the distribution of 5-HT_{1B} receptors with respect to 5-HT neurons (Boschert et al., 1994). Although present at much lower densities, theses receptors may function as autoreceptors in addition to heteroceptors controlling 5-HT firing and neurotransmitter release. As mentioned earlier, the 5-HT_{1D} receptor subtype corresponds more to the human form and the 5-HT_{1B} receptors is the orthologous receptor in the rat (see section 3.1.1.). However, these receptors have been detected in both species. 5-$HT_{1B/D}$ receptors attenuate the release of 5-HT from 5-HT neurons in many postsynaptic areas, being confirmed by different techniques (Moret and Briley, 1991; Piñeyro and Blier, 1996). These receptor subtypes do not appear to produce any tonic effects on 5-HT neuron activity, as antagonism of 5-$HT_{1B/D}$ receptors did not alter DR activity (Sprouse et al., 1997). In contrast, 5-$HT_{1B/D}$ receptor activation induce a 25% augmentation of 5-HT activity, of which the response to the preferential 5-HT_{1B} receptor agonist RU24969 is abolished in 5-HT_{1B} receptor knockout mice (Evard et al., 1999). The majority of evidence firmly supports that 5-HT_{1B} receptors act as an inhibitory modulator of 5-HT release but may also alter 5-HT neuronal firing activity minimally in some species. Furthermore, autoradiographic studies have proposed that 5-HT_1 receptor binding in the LC corresponds more to 5-$HT_{1B/1D}$ receptors (Weissmann-Nanopoulous et al., 1985). The lack of selective ligands for this receptor subtype, as well as selective agonists which permeate the blood brain barrier have hampered pharmacological studies of 5-$HT_{1B/D}$ receptor effects on LC NA activity. Given the likelihood of 5-HT_{1B} receptors in the LC, this receptor may be important in the overall 5-HT receptor mediated effects on NA activity.

4.3.2. 5-HT_2 receptor mediated effects

The systemic administration of LSD, mescaline, DOI, and other psychedelic hallucinogens induce a facilitation of the activation of LC neurons by sensory stimuli (Rasmussen and Aghajanian, 1986; Rasmussen et al., 1986). Paradoxically, these agents also attenuated the spontaneous firing activity of NA neurons in this nucleus

(Rasmussen and Aghajanian, 1986; Rasmussen et al., 1986). The suppressant effect of these hallucinogens on the firing activity of LC NA neurons is reversed by low doses of 5-HT_2 receptor antagonists (Rasmussen and Aghajanian, 1986; Rasmussen et al., 1986; Gorea and Adrien, 1988; Chiang and Aston-Jones, 1993). Blockade of these receptors, and maybe more so that of the 5-HT_{2A} receptor subtype, is postulated to be beneficial to the treatment of psychosis and schizophrenia (Jakab and Goldman-Rakic, 1998). Moreover, antipsychotic drugs reverse the actions of hallucinogens with doses that correlate with their affinity for 5-HT_2 receptors (Rasmussen et al., 1986b). Since the 5-HT system exerts a tonic inhibitory tone on the firing activity of LC NA neurons, and activation of 5-HT_2 receptors attenuates LC activity, it is possible that these receptors may be contributing to the tonic 5-HT inhibition on NA neurons (Haddjeri et al., 1997). Systemic administrations of 5-HT_2 receptor antagonists augment the firing activity of LC NA neurons (Gorea and Adrien, 1988; Rasmussen and Aghajanian, 1986; Aghajanian et al., 1990, VanderMaelen and Braselton, 1990). This is also observed in studies using *in vivo* voltammetry demonstrating that HVA levels (an index of NA cellular activity) are enhanced in the LC following systemic administration of the 5-$HT_{2A/2C}$ receptors antagonist ritanserin (Clement et al., 1992). *In vivo* microdialysis experiments have demonstrated that administration of 5-$HT_{2B/C}$ antagonists, but not that of the selective 5-HT_{2A} antagonist MDL 100,907, enhances the output of NA in the hippocampus and frontal cortex (Gobert and Millan, 1999), and may reflect increased firing activity of LC NA neurons with the former agents. These observations, when taken together, provides evidence for the involvement of the 5-HT_{2C} receptors in mediating a tonic inhibition on LC activity. This tonic inhibitory tone on NA neurons by 5-HT_{2C} receptors is probably not that pronounced as only a slight augmentation (16%) was observed upon their antagonism with ritanserin (VanderMaelen and Braselton, 1990).

Microiontophoretic application of 5-HT_2 receptor compounds on LC NA neurons do not alter firing activity (Chiang and Aston-Jones, 1993; Gorea et al., 1991), suggesting that these receptors are not located directly on the perikaria. There is convincing evidence that the decrease presented on NA neuron firing in the LC via systemic administration of 5-HT_2 receptor agonists reflect augmented GABA in this nucleus. This largely stems from *in vivo* data demonstrating a potent GABAergic

innervation to the LC arising from the prepositus hyppoglossi nucleus (PrH) in the rostral ventromedial medulla (Ennis and Aston-Jones, 1989). Electrical stimulation of this nucleus leads to a $GABA_A$ receptor mediated suppression on LC firing (Aston-Jones et al. 1991; Chiang and Aston-Jones, 1993). The presence of mRNAs coding for the $GABA_A$ as well as $GABA_B$ receptors in the LC has also been demonstrated (Luque et al., 1994). More specifically, evidence supports the notion of 5-HT_2 receptors being located in the PrH (Fay and Kubin, 1999), but are probably not responsible for the 5-HT_2-receptor mediated inhibition of NA firing activity in the LC (Gorea and Adrien, 1988; Gorea et al., 1991). This conclusion was drawn as direct application of DOI in the PrH and systemic administration of DOI in rats where the PrH fails to abolish the attenuation on LC NA neuron firing, respectively (Gorea et al., 1988). 5-HT_2 receptors exert an excitatory effect on various cell types (including GABAergic neurons) in numerous brain regions (Araneda and Andrade, 1991; McCormick and Wang, 1991; Shen and Andrade, 1998; Willins et al., 1997). Taken together, it appears that the 5-HT_2 receptor-mediated attenuation of LC NA neuron activity may involve GABAergic neuron terminals in this nucleus.

5. Impact of antidepressant treatments on 5-HT and NA systems

A considerable amount of work has been directed at the impact of antidepressants on the 5-HT and NA systems. This is partly because all antidepressant agents have consistently been shown to produce alterations on one or both of these systems during a long-term administration (for a recent review see Blier et al., 2001, located in the appendix). 5-HT and NA neurons innervate numerous structures in the forebrain (sections 2 and 3). Of particular interest, these monoamines project to the frontal cortex and hippocampus. These structures have been implicated in mediating the higher order brain functioning and some of the symptoms observed in affective and anxiety disorder patients (figure 8 and Szabo and Blier, 2001, located in the appendix). Specifically, in the brainstem, DR 5-HT and LC NA neurons reciprocally project to and modulate each other (see section 4). Thus, it must be evaluated whether the impact of antidepressants, even agents biochemically directed at one of these system, exert effects through connections at cell body and terminal levels. In section 2, steps pertaining to the biosynthesis and catabolism of 5-HT and NA were identified. The latter will now be emphasized as most antidepressant drugs involve efforts at maximizing the amount of neurotransmitter present, consistent with a monoamine deficiency hypothesis related to these disorders. For the sake of clarity and to be concise, antidepressant effects on NA and 5-HT neuron activity, as well as the impact of monoaminergic alterations in forebrain structures mainly that of the hippocampus will be highlighted. As antidepressants require a sustained administration of at least two weeks for beneficial effects to occur in patients, priority on the information presented in this section will be a reflection of this time-course.

5.1. 5-HT_{1A} agonists

5-HT_{1A} receptor agonists are used in the treatment of various anxiety and affective disorders. Nonetheless, these drugs have indications only for the former diagnosis. Currently, all 5-HT_{1A} receptor agonists made available for clinical use are of azapirone derivatives. This family is comprised of buspirone, gepirone, tandospirone, and ipsapirone. Systemic administration of these agents in rats rapidly suppress the firing activity of 5-HT neurons (Sprouse and Aghajanian, 1975; Scuvée-Moreau and Dresse, 1979; VanderMaelen et al., 1986; Blier and de Montigny, 1987; Godbout et al., 1991) and reduce 5-HT release (Sharp et al., 1989; Gobert et al., 1999). Short-term (2-day) treatments with 5-HT_{1A} receptor agonists also attenuate the firing activity of 5-HT neurons (Blier and de Montigny, 1990). As activation of 5-HT_{1A} receptors with these agents is prolonged, the initial suppression on 5-HT neuron firing gradually fades and activity of 5-HT neurons progressively recovers back to normal by 14-days of treatment (Blier et al., 1987). Desensitization of somatodendritic 5-HT_{1A} autoreceptors during long-term azapirone treatments has been observed *in vivo* via electrophysiological paradigms (Dong et al., 1997; Le Poul et al., 1997; Rueter and Blier et al., 1999). A long-term treatment with gepirone does not modify the effectiveness of the stimulation of the 5-HT pathway (Blier and de Montigny, 1987). Also, the 5-HT release-inhibitory capacity of buspirone is retained despite 10 weeks of repeated treatment with this compound (Soderpalm et al., 1993). This indicates that terminal 5-$HT_{1B/D}$ autoreceptors as opposed to 5-HT_{1A} receptors are normosensitive and not altered during a prolonged treatment with 5-HT_{1A} receptor antagonists.

The dose response curve for the suppressant effect on 5-HT neuron activity to intravenous administration of LSD, a 5-HT_{1A} receptor agonist, is shifted to the right in gepirone and ipsapirone treated rats (Blier and de Montigny, 1987; Dong et al., 1997). This, however, was not observed using the 5-HT_{1A} receptor agonist 8-OH-DPAT (Blier and de Montigny, 1987). These result prompted Blier and de Montigny (1987) to postulate that a systemic administration of 8-OH-DPAT would not solely mediate its inhibitory effects on 5-HT activity via somatodendritic 5-HT_{1A} receptors. This was later

experimentally supported by Hajos et al., (1998), indicating that activation of postsynaptic 5-HT_{1A} receptors in the medial prefrontal cortex is activated by 8-OH-DPAT and inhibits DR 5-HT neurons through a long neuronal loop. Recently, the neuronal mediators of this input from the cortex were pharmacologically dissected and involve ACh, $GABA_B$, and an NMDA receptor component (Haddjeri et al., 2000; Celeda et al., 2000).

The desensitization of G-protein coupled receptors, such as 5-HT_{1A} receptors, involves at least three distinct processes: uncoupling, sequestration, and downregulation (Hausdorff et al., 1990). Interestingly, treatment with ipsapirone does not alter the ability of 5-HT to inhibit the forskolin-stimulated accumulation of cAMP in the dorsal raphe (Varrault et al., 1991). Rather, the uncoupling of the receptors from its transduction system may account for this phenomenon (Fanelli et al., 1992; Schechter et al., 1990). It remains unclear whether this desensitization, which occurs following azapirone treatments are associated with a down-regulation of somatodendritic 5-HT_{1A} receptors. Results demonstrating non-5-HT mediated adaptations (eg., peptides and hormones) on 5-HT_{1A} receptors have been documented, but will not be mentioned further as it is out of the scope of this document.

Buspirone enhances the firing activity of LC NA neurons *in vivo* in the rat (Sanghera, 1982) and also *in vitro* in mouse brain slices (Trulson and Henderson, 1984). Since then, other 5-HT_{1A} receptor agonists part of the azapirone family have been demonstrated to augment LC firing activity (Sanghera et al., 1984) and NA release (Broderic et al., 1991; Gobert et al., 1999). The augmented NA activity produced by these agents was once thought to stem from the azapirone parent compound metabolite 1-(2-pyrimidinyl)-piperazine (1-PP; Hong et al., 1993; Sanghera et al., 1994). This metabolite is a α_2-adrenoceptor antagonist (Engberg, 1989; Sanghera, 1990; Blier et al., 1991). Since then, 5-HT_{1A} receptor agonists outside the azapirone family (i.e., 8-OH-DPAT) have been demonstrated to increase NA activity (Gorea and Adrien et al., 1988; Piercey et al., 1994) and release (Done and Sharp, 1994; Suzuki 1995; Hajos-Korcsok et al., 1999). It is concluded that 5-HT_{1A} receptor agonists independent of α_2-adrenoceptor affinity augment NA activity. This supports that an azapirone metabolite

with α_2-adrenergic antagonistic properties is probably not responsible for augmenting NA activity. Furthermore, a metabolic agent of this drug family is unlikely to produce these effects observed with *in vivo* electrophysiological experiments as the increase on LC NA neuron activity occurred seconds following i.v. administration. Experiments assessing the impact of prolonged treatments with various 5-HT_{1A} receptor agonists on LC NA activity are needed.

The response to iontophoretic application of 5-HT and 5-HT_{1A} receptor agonists on the firing activity of hippocampal CA_3 pyramidal neurons in rats treated with gepirone for 21-days did not differ as compared to controls (Blier and de Montigny, 1987). Long-term treatment with 5-HT_{1A} receptor agonists fail to alter the sensitivity of these receptors in the hippocampus when assessed by *in vivo* electrophysiological paradigms (Blier and de Montigny, 1987). On the other hand, *in vitro* experiments by Newman et al., (1992) demonstrated that short-term (8 days), but not acute treatment with buspirone, ipsapirone, or 8-OH-DPAT attenuates 5-HT inhibition of forskolin-stimulated adenylate cyclase in the rat hippocampus. As mentioned previously, distinct differences between pre- and postsynaptic 5-HT_{1A} receptors exist (see section 3.1.1.). Long-term treatments with azapirones alter neither the responsiveness of terminal 5-HT or NA autoreceptors on 5-HT terminals in the hippocampus nor the density or affinity of 5-HT_{1A} receptors located on pyramidal neurons (Blier and de Montigny, 1987; Welner et al., 1989; Schechter et al., 1990; Godbout et al., 1991; Fanelli et al., 1992; Dong et al., 1997). This is in accord with results from Wieland et al., (1993) indicating that a long-term tandospirone treatment did not alter the density of hippocampal 5-HT_{1A} receptors but attenuates 5-HT_{1A} receptor density in the frontal cortex. Of interest, long-term treatments with 5-HT_{1A} receptor agonists produce an enhanced tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus resulting from normalized 5-HT release in the presence of the exogenous agonist (Haddjeri et al., 1998; Rueter and Blier, 1999).

5.2. α_2-adrenoceptor antagonists

Mianserin and mirtazapine are two newer generation antidepressants that are both α_2-adrenoceptor antagonists, but significantly block 5-HT_2 receptors. Mianserin possesses affinity for α_1-adrenoceptors as well. A down-regulation of 5-HT_{2C} receptors with the paradoxical up-regulation of its mRNA levels (Sanders-Bush, 1990; Hamon et al., 1990), as well as a down-regulation of 5-HT_{2A} receptors without any change in mRNA levels (Roth et al., 1990), occurs in the rat brain after mianserin treatment. Given the pharmacological profile of mitazapine, an increase in LC NA neuronal firing activity was expected (Haddjeri et al., 1996). This effect of α_2-adrenoceptor antagonism on NA activity occurs presumably through a blockade of somatodendritic α_2-adrenoceptors located directly on LC NA neurons (see section 2.1.2. for review). Recurrent collaterals providing an auto-inhibitory influence onto NA neurons has been hypothesized (Cedarbaum and Aghajanian, 1978), however, anatomical verification is lacking. These antidepressants also enhance NA release in forebrain structures (Curet and deMontigny, 1989; Blier et al., 1991; Millan et al., 2000). Interestingly, α_2-adrenergic autoreceptors on NA terminals that normally induce a negative feedback regulation on release become slightly sensitized (Cerritto and Raiteri, 1981; Surgue et al., 1980). The increase in NA neuron firing activity and sensitization of α_2-adrenoceptors on NA terminals with α_2-adrenoceptor antagonists is sustained during a long-term administration (Blier et al., 1991; Haddjeri et al., 1997). This most likely represents an important homeostatic mechanism on the NA system. In contrast, (-) mirtazapine and (-) mianserin are preferential antagonists for α_2-adrenergic heteroceptors and enhance the effectiveness of electrically stimulated 5-HT in the hippocampus after a long-term treatment (Mongeau et al., 1993; Haddjeri et al., 1996). Interestingly, (-) mirtazapine as opposed to the racemic mixture attenuates NA activity when acutely administered (Haddjeri et al., 1996). This effect of the (-) enantiomer is dependent on alterations of 5-HT as lesioning of this system with 5,7-DHT abolished its effects (Haddjeri et al., 1996). The exact mechanism for the attenuation on NA activity with the (-) enantiomer of a α_2-adrenocepetor antagonist is not currently known.

Idazoxan is a α_2-adrenoceptor antagonist and purported antidepressant. This agent is used not generally prescribed to patients, but is primarily used for for pharmacological purposes in fundamental research. Recently, this agent has been reported to be effective in bipolar depressed patients (Grossman, 1999). Systemic administration of idazoxan augments the firing activity of 5-HT neurons (Garratt et al., 1991). Mirtazapine, but not mianserin, is also able to increase the firing activity of 5-HT neurons (Blier et al., 1984; Haddjeri et al., 1998). Augmented 5-HT activity with mirtazapine during a long-term administration is due to an alteration on the NA system as a 6-OH-DA lesion abolished this effect (Haddjeri et al., 1998). Given that α_1-adrenoceptors on DR 5-HT neurons receive a tonic activation from LC NA neurons, enhanced NA release to this nucleus with mianserin may not present an effect on 5-HT activity due to its α_1-adrenoceptor affinity. This highlights the importance of NA when assessing the effects of antidepressant drugs on the 5-HT system.

Acute administration of mirtazapine augments 5-HT efflux in the ventral hippocampus (de Boer et al., 1996). It has been demonstrated that a long-term treatment with mianserin does not alter basal 5-HT levels in hippocampal microdyalisates nor modifies the attenuating effect of 8-OH-DPAT on 5-HT release (Kreiss and Lucki, 1995). In addition, sustained treatments with mianserin and idazoxan fail to enhance the effectiveness of the stimulation of the 5-HT pathway monitored electrophysiologically (Mongeau et al., 1994a). This indicates that 5-$HT_{1B/D}$ receptors on 5-HT terminals are normosensitive. On the other hand, α_2-adrenergic heteroceptors located on 5-HT fibers in the dorsal hippocampus desensitize following a prolonged administration with mianserin (Mongeau et al., 1993). This effect stems from augmented NA concentrations that produce a desensitization of α_2-adrenoceptors on 5-HT terminals and enhance 5-HT release in postsynaptic structures (see figure 9). Mianserin treatments did not alter the responsiveness of terminal 5-HT autoreceptors, post-synaptic 5-HT_{1A} receptors, or α_2-adrenoceptors located on pyramidal cells of CA_3 neurons in the dorsal hippocampus (Mongeau et al., 1997). Desensitization of α_2-adrenergic heteroceptors is not nessesarily associated with down-regulation, but the lack of sensitivity is associated with increases in 5-HT release during a prolonged

administration. Thus, antidepressants targeting α_2-adrenoceptors can alter the 5-HT system during a long-term administration and may be important to their antidepressant effects.

5.3. Monoamine Oxidase Inhibitors

MAOIs were the first effective drugs in the treatment of depression and this class of antidepressants is still regarded as the "golden standard" for the treatment of GAD (Klein et al., 1980). However, dietary restrictions with a profound side effect profile as compared to that of newer antidepressant agents have hampered its usage. Recently, the non-selective MAOI deprenyl has been developed in patch form and circumvents many of the undesirable effects previously associated with this agent through by-passing first pass metabolism. MAO-A preferentially metabolizes NA and 5-HT, whereas DA is inactivated by both isoforms (Hall et al., 1969; Yang and Neff, 1974). The therapeutic efficacy of non-selective MAOIs is attributed to their effect on MAO-A (Blier and de Montigny 1987). This largely stems from clorgyline and moclobemide being a selective inhibitor of MAO-A and effective in the treatment of endogenous depression (Murphy et al., 1981; Cassachia et al., 1984), whereas the efficacy of MAO-B inhibitors is debatable (Murphy et al., 1991). The acute administration of MAO type A inhibitors suppress the firing activity of 5-HT neurons (Aghajanian et al., 1970; Blier and de Montigny, 1987). Over the course of a prolonged administration with MAOIs such as phenelzine, clorgyline, and amiflamine, the firing activity of DR 5-HT neurons slowly recovers over a prolonged treatment and is completely restored by 14 days (Blier and de Montigny, 1985; Blier et al., 1986). This phenomenon is attributed to desensitization of the somatodendritic 5-HT_{1A} receptors being indicated by a blunted response to the 5-HT_{1A} autoreceptor agonist LSD (Blier and de Montigny 1985; Blier et al., 1986). Somatodendritic 5-HT_{1D} receptors that modulate the release of 5-HT in raphe slices also become desensitized during a long-term administration with befloxatone (Piñeyro and Blier, 1996). Thus, autoreceptors controlling 5-HT activity and release are subsensitive during long-term MAOI treatments.

The sensitivity of postsynaptic 5-HT_{1A} receptors during long-term treatments with various MAOIs has been assessed using an *in vivo* electrophysiological approach.

Long-term administration of deprenyl and phenylzine do not alter the sensitivity of postsynaptic 5-HT_{1A} receptors (Blier et al., 1986). However, the efficacy of the stimulation of the ascending 5-HT pathway to the hippocampus is enhanced during a long-term treatment with clorgyline and phenelzine, but not with deprenyl (Blier et al., 1986). This indicates that there is most probably an enhanced synaptic release of 5-HT in this structure during electrical stimulation in phenelzyne treated animals. Deprenyl, by blocking not only MAO type A, does not enhance the releasable pool of 5-HT. These results are nonetheless at variance with those generated by the inhibition of adenylate cyclase activity by 5-HT in the hippocampal homogenate (Sleight et al., 1988; Varrault et al., 1991). These studies demonstrate that a long-term administration of tranylcypromine and clorgyline produces a reduction of the ability of 5-HT to inhibit the production of cAMP. This desensitization following a long-term administration of clorgyline was also observed using an *in vivo* electrophysiological paradigm also (Blier et al., 1986). A quantitative autoradiography study further substantiated these results and demonstrated a decreased number of [^{3}H]8-OH-DPAT binding sites following a clorgyline treatment (Mongeau et al., 1992). In contrast to the results obtained by Varrault et al., (1991) and Sleight et al., (1988), the desensitization of postsynaptic 5-HT_{1A} receptors measured *in vivo* is not observed with tranylcypromine (Haddjeri et al., 1998; located in the appendix).

It has been demonstrated, *in vivo*, that a long-term treatment with befloxatone, a reversible MAOI-A, augments the electrically-evoked release of [^{3}H]5-HT from the rat and guinea-pig hippocampus and hypothalamic slices (Blier and Bouchard, 1994). An altered sensitivity of terminal α_2-adrenergic heteroceptors is only observed in the hypothalamus (Blier and Bouchard, 1994). A study using microdyalisis revealed that a long-term treatment with the MAOI, MDL 72394, augments basal 5-HT levels in the rat frontal cortex without altering the sensitivity of terminal 5-$HT_{1B/D}$ autoreceptors (Sleight et al., 1989). In contrast, a long-term treatment with befloxatone augments the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons, but did not desensitize terminal α_2-adrenergic heteroceptors located on 5-HT fibers Mongeau et al., (1994b). There may be a region specific effect to the alterations of 5-HT and α_2-adrenergic heteroceptors controlling 5-HT release, not to mention agent specific variability within the MAOI class. *In vivo*, however, 5-$HT_{1B/D}$

receptors are not altered whereas α_2-adrenergic heteroceptors have the capacity to desensitize during prolonged MAOI treatments.

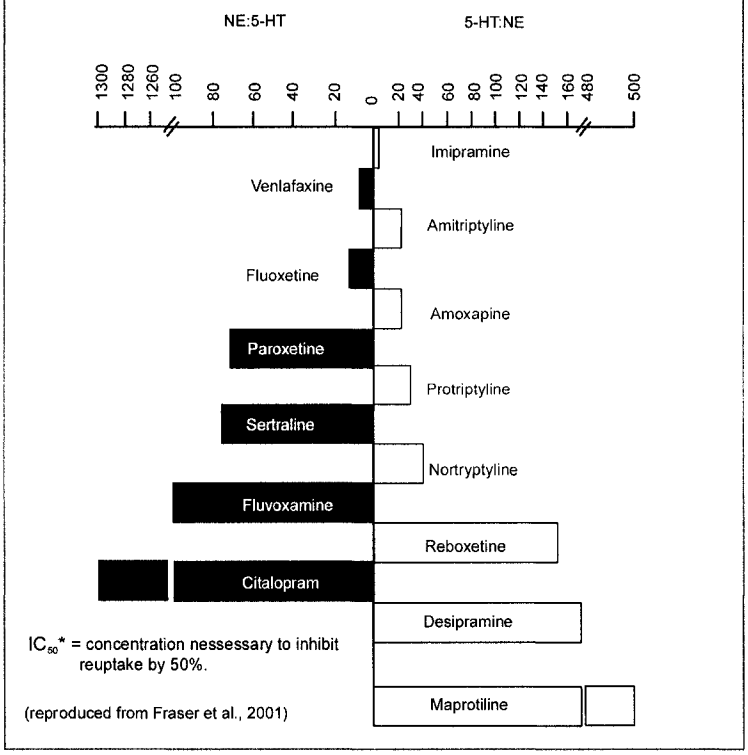
Systemic administration of MAOIs attenuate the firing activity of LC NA neurons (Blier and de Montigny, 1985). In opposition to that of 5-HT, these NA neurons did not recover their firing activity during a long-term administration (Blier et al., 1985). Repeated administrations of clorgyline or phenelzine yields an early and sustained decrease of more than 50% on the firing activity of rat LC NA neurons (Blier and de Montigny, 1985). This sustained attenuation on NA neuron activity is due to the overactivation of inhibitory α_2-autoreceptors via increased NA synaptic availability in the LC (Blier and de Montigny, 1985; 1987). Thus, MAOIs by inhibiting the catabolic metabolism of this neurotransmitter lead to subsequent overactivations of α_2-adrenoceptors to inhibit NA neuron firing. Although, amiflamine is a reversible MAOI-A, it has been reported induce effects selectivity on 5-HT (Ask et al., 1982; 1984). Thus, this would explain this agent failing to attenuate NA neuron firing upon acute administration. However, it is unclear as to why a 30% decrease on NA neuron firing is demonstrated during a 21-day treatment with amiflamine (Blier et al., 1986).

MAOI-induced NA alterations have also been reported in the hippocampus and other forebrain structures (Blier and de Montigny, 1985). A reduced cAMP response to NA occurs in the limbic forebrain following long-term MAOI treatments (Sleight et al., 1988). Most classes of antidepressant agents, including MAOIs, also downregulate β-adrenoceptors, but this effect may or may not be germane to efficacy as antagonists of these receptors are not antidepressants. Rather, β-adrenoceptor blockers are mildly effective by acting peripherally to attenuate the somatic manifestations of anxiety (Sullivan et al., 1999). The fact that LC neuron activity is decreased similarly during 2- and 21-day MAOI treatments sheds doubt that modifications of NA neurotransmission, *per se*, account for the delayed antidepressant effect of MAOIs. A long-term but not subacute administration of the MAOI tranylcypromine enhances the synaptic availability of NA in the rat frontal cortex (Greenshaw et al., 1989). The exact basis for this enhancement is not known, but is consistent with the delayed onset of antidepressant action of this non-selective MAOI.

5.4. Tricyclic Antidepressant Treatments

Chronic treatment with TCAs remarkably induces a sensitivity of postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian, 1978; Gallagher and Bunney, 1979) and α_1-adrenoceptors (Menkes et al., 1980; Menkes and Aghajanian, 1981). Blier and de Montigny (1980) investigated whether a similar mechanism would occur at the level of somatodendritic 5-HT_{1A} autoreceptors in the DR. It was reported that the sensitivity of these somatodendritic receptors to i.v. injection or iontophoretic application of LSD was unaltered during a long-term treatment with desipramine and imipramine (Blier and de Montigny, 1980). The inhibitory effect of TCAs on the firing activity of DR 5-HT neurons is correlated with their preferential ability to block 5-HTT rather than NAT. In fact, acute administration of imipramine and amitriptyline (IC_{50} for 5-HT uptake blockade: 80 and 40 nM, respectively) suppress the firing activity of 5-HT neurons, whereas nortiptyline and desipramine (IC_{50} for 5-HT uptake blockade: 160 and 180 nM, respectively) do not (Scuvée-Moreau and Dresse, 1979; Bolden-Watson and Richelson, 1993). Note that nortryptiline and desipramine are more potent NA reuptake inhibitors (figure 10). The firing activity of DR 5-HT neurons, and the sensitivity as well as density of somatodendritic 5-HT_{1A} autoreceptors in this nucleus remains unchanged following a long-term amitriptyline treatment (Blier and de Montigny, 1980; Welner et al., 1989). The sensitivity of terminal 5-HT_{1B} autoreceptors controlling 5-HT release does not become altered during a sustained treatment with TCAs (Blier et al., 1987; Chaput et al., 1991; Sleight et al., 1989).

Figure 10. Selectivity of Antidepressants in blocking NA or 5-HT reuptake: ratios of IC_{50}* values for uptake inhibition



Desipramine and other TCAs with a secondary amine in their side chain are more potent NA reuptake inhibitors then tertiary aminated analogues, which are more potent inhibitors of 5-HT reuptake (figure 10; Carlson et. al., 1969; Carlson, 1970, Shaskam and Snyder, 1970; Hamberger and Tuck, 1975; Ross and Reny, 1969, 1975). Most TCAs also block other receptor systems leading to significant side effects and hampers usage. Desipramine suppresses NA neuron firing activity during systemic injection or iontophoretic application in the LC (Lacroix, 1991). A prolonged treatment with desipramine does not produce an alteration on NA neuron activity, but induces a small shift to the right of the dose-response curve for the α_2-adrenoceptor clonidine (Lacroix et al., 1991). Other antidepressants that block the reuptake of NA are also able to produce a sustained attenuation on the activity of NA neurons activity during a long-term treatment (Mongeau et al., 1998; Béïque et al., 2000a). A plausible reason for the discrepancy on NA activity between desipramine and other antidepressants that block

NAT may be methodological. In the Lacroix et al., (1991) study, desipramine was administered to rats i.p. and the lack of effect on NA neurons activity during a prolonged treatment may reflect a steady state levels not being achieved. The effect of a sustained desipramine treatment in rats with minipumps may corroborate this assertion. Thus, the inhibitory effects on NA activity from TCAs, as with the MAOIs, are due to overactivation α_2-adrenocepetors.

Postsynaptic 5-HT_{1A} receptors in the lateral geniculate nucleus (Menkes and Aghajanian 1981; de Montigny and Aghajanian 1978) the amygdala (Wang and Aghajanian 1980), the facial motor nucleus (Menkes et al., 1980) and hippocampus (Chaput et al., 1991; Gravel and de Montigny, 1987; Gallager and Bunney, 1979; de Montigny and Aghajanian, 1978) were demonstrated to be sensitized following long-term, but not acute or subchronic administration of desipramine, imipramine, iprindole, amitriptiline, and chlorimipramine. Most TCAs studied *in vivo* and *ex vivo* appear to sensitize postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian, 1978; Gallager and Bunney, 1979; Gravel and de Montigny, 1987; Chaput et al., 1991; Dijcks et al., 1991; Bijak et al. 1997; Maj et al., 1996). This was not observed with other psychoactive drugs such as chlorpromazine and the sensitization effect was concluded to be specific to TCAs (de Montigny and Aghajanian, 1978). Most autoradiographic studies have failed to reveal alterations in 5-HT_{1A} receptor density in the hippocampus following TCA treatments (Watanabe et al., 1993; Hayakawa et al., 1994; Bijak et al., 1997), although increases have been reported (Welner et al, 1989; Burnet et al., 1994). Rather, an increased efficacy of post-receptor transduction mechanisms, such as G-protein coupling to the K+ channels may be postulated for the sensitization of the inhibitory effects of 5-HT_{1A} receptor agonists in the hippocampus following TCA treatments (Newman and Lerer, 1988; 1989; Varrault et al., 1991; Odagaki et al., 1991). Stimulation of the ascending 5-HT pathway is enhanced following a long-term administration of TCAs in the hippocampus (Blier and de Montigny, 1980). It is important to emphasize the sensitization effect on postsynaptic 5-HT_{1A} receptors induced by a long-term administration of iprindole. Indeed, this tricyclic compound presents antidepressant activity, but unlike the other TCAs is devoid of 5-HT or NA

reuptake inhibition affinity. It can thus be inferred from this contention that the sensitizing effect of prolonged administration of TCAs is independent of reuptake inhibition, and be related somehow to tricyclic moiety. The chemical structure of this antidepressant class may reflect its unique efficacy observed in the treatment of depression (see table 1., in the introduction). TCA treatments, including iprindole (Newman *et al.* 1992) do not alter the 5-HT_{1A}-mediated inhibition of adenylate cyclase activity in the hippocampal homogenate (Varrault *et al.* 1991). These results suggest that the response involved in the coupling of the 5-HT_{1A} receptor to the K^+ channel, and not that involved for the coupling of this receptor to adenylate cyclase would be sensitized. It is obviously tempting to assume that the functional sensitization of the 5-HT_{1A} receptor mediated response following treatment with various TCAs would be accompanied by an increase in 5-HT_{1A} receptor number. Thus, the mechanism underlying a phenomenon discovered more than 20 years ago is yet to be elucidated.

Systemic administration of desipramine fails to modify the firing activity of 5-HT neurons but attenuates the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons (Mongeau et al., 1993). A long-term administration of desipramine does not change the K^+ evoked release of [^{3}H] 5-HT from the rat cortex and hippocampal slices (Mongeau et al., 1993). Microdyalisis studies have reported that a long-term treatment with desipramine desensitizes α_2-adrenergic heteroceptors on 5-HT fibers modulating 5-HT levels in the rat hippocampus (Yoshioka et al., 1995). A recent study by Mateo et al., (2001) demonstrated that a prolonged treatment with desipramine is capable of desensitizing α_2-adrenoceptors in the LC. These receptors are different from those controlling NA firing as activity was still attenuated during a long-term treatment. This may reflect a different population of α_2-adrenergic autoreceptors on LC NA neurons and could possibly be putting into evidence α_2-autoreceptors on NA collaterals projecting back onto NA neurons (Aghajanian, 1978). The same phenomenon with desipramine has also been documented previously by Lacroix et al., (1991) and Mongeau et al.,(1998) using the NA/5-HT reuptake inhibitor milinacipran. Indeed, desensitization of α_2-adrenoceptors controlling NA release in forebrain regions during a prolonged treatment with

desipramine has been observed (Mateo et al., 2001). I*n vitro*, a long-term treatment with desipramine but not with the TCA trimipramine nor the SSRI fluoxetine desensitizes 5-HT_3 receptors modulating [^{3}H]NA release from rat hippocampal slices (Mongeau et al., 1994c). Of interest, chronic amitriptyline exposure in the hybrid cell line NG 108-15 reduces the affinity of 5-HT_3 receptors (Shimizu et al., 1996). The reason for such a desensitization is still unknown since amitriptyline possesses a moderate affinity for the latter receptor (Ki: 0.26 μM, Griebel, 1995). Augmented 5-HT response in the hippocampus during a long-term desipramine treatment does not occur via an altered sensitivity of terminal 5-HT_{1B} autoreceptors, as the sensitivity of these receptors remained unchanged (Blier et al., 1987; Chaput et al., 1991; Sleight et al., 1989). *In vitro* studies have also demonstrated that a long-term treatment with desipramine fails to alter the K^+-evoked release of tritiated 5-HT from both cortex and hippocampus slices (Schoffelmeer and Mulder, 1983). However, a microdialysis study indicates that a long-term treatment with desipramine in rats enhanced baseline 5-HT levels in the striatum but not in the hippocampus (Kreiss and Lucki, 1995). Some regional specific differences and discrepancies within the TCA class appear where no simple explanation can be proposed.

5.5. Selective 5-HT reuptake inhibitors

SSRIs are currently the most prescribed antidepressant drug class in the world. These agents rapidly penetrate the brain to inhibit 5-HTTs (Stark et al., 1985; Wikell et al., 1999). As a consequence, the firing activity of 5-HT neurons is attenuated upon systemic administration (Chaput et al., 1986b; Jolas et al., 1994; Arborelius et al., 1995; Hajos et al., 1995). This is due to an increase in somatodendritic 5-HT release that activates 5-HT_{1A} autoreceptors (Chaput, 1986). In fact, a single administration of SSRIs at low doses augment 5-HT levels and this effect is greater in the raphe nuclei than in projecting areas (Ble and Artigas, 1992; Invernizzi et al., 1992; Fuller et al., 1994; Malagie et al., 1995). Presumably, this reflects the high density of 5-HTT located on 5-HT cell bodies (Hrdina et al., 1990; Verge et al., 1985). Moreover, this increase of 5-HT

in raphe nuclei limits the antidepressant effects in 5-HT nerve terminal regions (Rutter and Auerbach, 1993). The release of 5-HT is blocked by TTX and reduced by 5-HT_{1A} receptor agonists (Sharp and Hjorth, 1990; Artigas, 1993; Fuller, 1994). It is fitting that the effects on 5-HT levels to low doses of SSRIs were increased by pre-treating animals with 5-$HT_{1A/B}$ receptor antagonists such as (-) penbutol, (-) pindolol, or methiothepin, as well as selective 5-HT_{1A} receptor antagonists such as UH-301 or WAY 100,635. These antagonists potentiate the ability of SSRIs to augment nerve terminal output of 5-HT by blocking the inhibitory effects of these agents on 5-HT neuron activity (Invernizzi et al., 1992; Hjorth; 1993, 1996; Hjorth et al., 1996; Hjorth and Auerbach, 1994, 1996; Artigas et al., 1996; Malagie et al., 1995; Gartside et al., 1995; Romero et al., 1996; Gardier et al., 1996).

A short-term treatment with SSRIs attenuates the firing activity of 5-HT neurons due to activation of 5-HT_{1A} autoreceptors (Chaput, et al., 1986). A complete recovery of 5-HT neuron firing activity in the DR occurs during 14-days of treatment (Blier et al., 1986; Chaput, 1986). The recovery of 5-HT neurons during a prolonged SSRI treatment results from a desensitization of 5-HT_{1A} receptors as *in vivo* and *in vitro* the reducing effect of 5-HT_{1A} receptor agonists are attenuated following prolonged treatments (Blier and de Montigny, 1987; 1990; Schechter et al., 1990; Godbout et al., 1991; Dong et al., 1997). Thus, SSRIs similar to 5-HT_{1A} receptor agonists and MAOIs return 5-HT firing back to normal after a prolonged administration. The combination of an SSRI with pindolol, a 5-HT_{1A} and β-adrenoceptor antagonist, is based on the concept that this agent preferentially inhibits 5-HT_{1A} autoreceptor blockade and would eliminate the need for these receptors to desensitize and therefore hasten the onset of action of SSRIs. Indeed, it has been indicated by numerous studies that pindolol addition to an SSRI can speed up the antidepressant response (Blier and Bergeron, 1998). Although pindolol is not selective for 5-HT_{1A} receptors, it is doubtful that a β-adrenocepetor component of this agent is responsible for the rapid onset as Zanardi et al., (1997) did not observe any change in response rate when metoprolol was given with paroxetine in depressed patients.

For the most part, 5-HT_{1B} receptors in the rat and 5-HT_{1D} receptors in the guinea pig are located on 5-HT terminals and modulate release (see section 3.1.1.). However, 5-HT_{1D} receptors are also located on cell bodies of 5-HT neurons in the DR and produce an inhibitory negative feedback influence on firing activity to attenuated neuron driven release (Piñeyro and Blier, 1995). The sensitivity of these autoreceptors was examined following a 21-day treatment with the SSRI paroxetine. After a 48 hour washout period, in order to allow complete drug elimination, mesencephalic slices (containing raphe nuclei) were prepared and loaded with [^{3}H]5-HT. The electrically evoked overflow was enhanced following a long-term SSRI treatment and the inhibitory effect of the 5-HT_{1D} receptor agonist sumatriptan was attenuated (Piñeyro and Blier, 1995). Thus, SSRIs have the capacity to desensitize 5-HT_1 autoreceptors at the cell-body and terminal level of 5-HT neurons. This is at variance from results obtained with the TCAs. Long-term administration of various 5-HT reuptake inhibitors does not alter the sensitivity of 5-HT_{1A} and adrenergic receptors located on CA_3 pyramidal neurons when assessed using an *in vivo* electrophysiological paradigm (Béïque et al., 2000a). At variance are the observations that prolonged treatments with zimelidine and fluoxetine attenuate the ability of 5-HT to inhibit forskolin-stimulated accumulation of cAMP (Newman et al., 1992). This outcome was not replicated by Varrault et al., (1991) using fluoxetine. Autoradiographic studies for whether an up- or downregulation of 5-HT_{1A} receptors occur in the hippocampus following a long-term administration of SSRIs have also generated conflicting results. By in large there is a stronger consensus that an SSRI administration does not alter 5-HT_{1A} receptor numbers in this structure (Pineyro and Blier, 1999; Mongeau et al., 1997).

There is paucity in the literature relating to the effects of SSRIs on NA neuron firing activity under acute conditions, and data during sustained administrations is non-existent. Systemic injection of fluoxetine and paroxetine did not alter the firing activity of LC NA neurons in the rat (Béïque et al., 1999). However, the effect of citalopram when administered directly in the LC increases extracellular levels of NA in this nucleus and attenuates LC activity (Mateo et al., 2000). This effect of citalopram was abolished by a pre-treatment with the 5-HT synthesis inhibitor PCPA, reflecting a 5-HT dependent

phenomenon. When fluoxetine was acutely administered, NA concentrations were enhanced in the cingulate cortex (Mateo et al., 1998) but not in the frontal cortex (Page and Abercrombie et al., 1999). This lack of an effect in the frontal cortex was also documented with citalopram (Hatanaka et al., 2000). Furthermore, Page and Abercrombie (1999) did not observe an increase in basal NA concentrations in the hippocampus as measured by microdyalisis, but a potentiated NA concentration in this structure after a foot shock in rats receiving a long-term but not acute administration was observed. When rats were treated with the SSRI fluoxetine and MAOI-A befloxatone for 21-days, the evoked release of [^{3}H] NA in the hippocampus was augmented (Mongeau et al., 1994c). This effect was blunted by administration of the 5-HT_3 receptor antagonist odansetron (Mongeau et al., 1994c). It appears that an increase in NA concentration during a systemic SSRI administration occurs in the hippocampus and some cortical regions. The increase of NA would be mediated in the presence of unaltered LC activity. On the other hand, local application of SSRIs in the LC attenuates NA activity while augmenting NA concentrations in this nucleus. Thus, regional specific differences in forebrain structures appear to exist on the effects of SSRIs to augment NA levels. This may reflect the density of 5-HTT as well as 5-HT_3 receptors in these areas. The divergent results when comparing local to systemic administration of SSRIs on NA neuron firing and release may reflect the latter administration also recruiting the influence of DR 5-HT neurons projecting to the LC. The assessment of the effects of SSRIs on NA levels in the LC may be clarified with experiments where these agents are also administered systemically.

5.6. 5-HT/NA reuptake inhibitors

Recently, data has accumulated on the *in vivo* characterization of dual 5-HT/NA reuptake inhibitors. Similar to SSRIs, the acute and subacute administration of venlafaxine and duloxetine, which are both preferential 5-HTT inhibitors attenuate the firing activity of DR 5-HT neurons (Kasamo et al., 1996; Béïque et al., 2000a). Furthermore, a 2-day treatment with the partial 5-$HT_{1A/B}$ adrenoceptor antagonist (-)

pindolol reverses the suppressant effect of a systemic injection of venlafaxine on the firing activity of 5-HT neurons (Béïque et al., 2000b). In rats undergoing a long-term treatment with duloxetine for 21-days, the firing activity of 5-HT neurons regain activity due to desensitization of 5-HT_{1A} autoreceptors (Rueter et al., 1998). This supports that the inhibitory effect presented on 5-HT neuron activity is mediated through overactivation of 5-HT_{1A} autoreceptors in the presence of transporter reuptake blockade. On the other hand, the acute injection of milnacipran suppressed the firing activity of 5-HT neurons, but the effect was not abolished in NA-denervated rats (Mongeau et al., 1998). The firing activity of 5-HT neurons in rats treated with milnacipran for 14-days did however recover back to basal firing rates. The inhibitory effect of clonidine on 5-HT neuron firing activity was markedly reduced by long-term milnacipran treatments whereas the inhibition of electrically evoked release of [^{3}H]NA and of [^{3}H]5-HT produced by the α_2-adrenoceptor agonist UK 14.304 from preloaded mesencephalic slices containing the DR was unaltered (Mongeau et al., 1998). The latter results indicate that α_2-adrenergic autoreceptors and heteroreceptors were unaffected in the raphe area by milnacipran. In conclusion, milnacipran had profound effects on the function of 5-HT neurons emulating that of duloxetine and venlafaxine. In contrast to the other dual reuptake inhibitors, the mechanisms by which 5-HT neurons regained firing activity during a milnacipran treatment reflects that of an NA alteration. Indeed, because of the reciprocal connections between the 5-HT and NA systems, milnacipran may be imparting its primary action on the NA system.

The effects obtained with dual reuptake inhibitors on NA neuron firing is dose related. As in the case of venlafaxine and duloxetine, the NA reuptake property of these agents is recruited only by a higher dose (Rueter et al., 1998; Béïque et al., 1998; Béïque et al., 2000a). Thus, NA neurons become attenuated as a result of increased availability of NA and overactivation of α_2-adrenoceptors. The same remains true for milnacipran (Mongeau et al., 1998). A similar phenomenon on NA neuron activity is observed with that of the TCA desipramine and MAOIs (but not with amiflamine). Acute administration of duloxetine augments the effectiveness of the stimulation of the 5-HT pathway to suppress the firing activity of CA_3 pyramidal neurons *in vivo* (Blier et al.,

1986). Duloxetine also enhances the release of 5-HT and NA in the frontal cortex and hippocampus following a sustained administration (Rueter et al., 1998). The venlafaxine induced desensitization of terminal 5-$HT_{1B/D}$ autoreceptors occurs only at a high dose in this region (Béïque et al., 2000). Moreover, an enhancement of 5-HT neurotransmission by venlafaxine was only achieved under conditions whereby the desensitization of the terminal 5-HT_{1B} autoreceptor is in occurrence of that of the somatodendritic 5-HT_{1A} receptors. Augmented 5-HT as a result of prolonged duloxetine treatments is due to 5-HT reuptake blockade and α_2-adrenergic heteroceptor desensitization. Duloxetine appears to produce an increase in NA in the hippocampus via desensitization of terminal α_2-adrenoceptors whereas the enhancement in the frontal cortex is due to desensitization of NA transporters (Rueter et al., 1998). Augmented NA as opposed to that of 5-HT with dual reuptake inhibitors is more complicated and represents as with the other antidepressant classes regional specific differences.

The antidepressant effects on the NA system during a prolonged administration has been considerably less evaluated (probably due to the success of SSRIs) and a clear picture of their effects have yet to surface. Future research endeavours should be directed at clarifying the inter-relationship between the 5-HT and NA systems being linked to antidepressant effects as it may be critical to their efficacy or lack thereof. As for now, table 3., summarizes the effects that major classes of antidepressant impart on the 5-HT system during a long-term administration, of which augmented 5-HT transmission is common to all. In the appendix lie a review article by Szabo and Blier, 2001, of which the effects of antidepressants on NA neuron activity is highlighted in table 1.

Table 3. Effects of lopng-term administration of antidepressant treatments on 5-HT neurotransmission

	Responsiveness of somatodendritic 5-HT_{1A} autoreceptors	Function of terminal $5\text{-HT}_{1B/D}$ autoreceptors	Function of terminal α_2-adrenergic heteroreceptors	Responsiveness of postsynaptic 5-HT_{1A} receptors	Net effect on 5-HT neurotransmission
SSRI	↓	↓	↔	↔	↑
MAOI	↓	↔	↓	↔ or ↓	↑
5-HT_{1A} agonists	↓	↔	n.d.	↔	↑
TCA	↔	↔	n.d.	↑	↑
ECT	↔	↔	↔	↑	↑

↓: decrease ↔: no change n.d.: not determined ↑: increase

(reproduced from Blier and de Montigny, 1994; with permission)

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CHAPTER II: First article

PREFATORY REMARKS

Extensive data from our laboratory, and others, indicate that LC NA neurons modulate DR 5-HT neurons. Furthermore, antidepressant agents that selectively block NA reuptake transporters alter many 5-HT parameters during a long-term administration postulated to be beneficial to the antidepressant response. There are many presynaptic modifications that occur onto 5-HT neurons during a prolonged SSRI administration leading to a net enhancement of 5-HT availability in postsynaptic structures. Given that 5-HT neurons present a tonic inhibitory tone on LC NA firing, as well as hyperactivity of these neurons correspond to anxiety levels, we focused in this paper on whether sustained SSRI treatments can modify LC NA neuron firing. Interestingly, these alterations correlated with a time-course for the antidepressant induce effects observed in anxiety and affective disorder patients.

The article entitled "Modulation of noradrenergic neuronal firing by selective serotonin reuptake blockers" by myself, Claude de Montigny, and Pierre Blier was published in the British Journal of Phamacology (1999, vol. 126, pp. 568-71) as a *Special Report*. A reprint of this article is located in the appendix.

MODULATION OF NORADRENERGIC NEURONAL FIRING BY SELECTIVE SEROTONIN REUPTAKE BLOCKERS

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Summary

Using in vivo extracellular unitary recording, the effect of short term (2-day) and long-term (21-day) administration of the selective 5-HT reuptake inhibitor (SSRI) paroxetine (10 mg kg^{-1} day^{-1}, s.c. using osmotic minipumps) was examined on the spontaneous firing activity of LC noradrenergic neurons. Long-term but not short-term treatment significantly decreased firing activity. Thus, it appears that enhancing 5-HT neurotransmission by sustained SSRI administration leads to a reduction of the firing rate of noradrenergic neurons. The SSRI paroxetine therefore alters the activity of noradrenergic neurons with a delay that is consistent with its therapeutic action in depression and panic disorder.

KEYWORDS: antidepressant; noradrenaline; major depression; panic disorder; serotonin (5-HT); selective serotonin reuptake inhibitor (SSRI).

Introduction

The pathophysiology underlying major depression and panic disorder is poorly understood, however, more is known about the mechanisms of action of the antidepressant drugs used to treat these disorders (reviewed by Blier and de Montigny, 1997). For instance, selective 5-HT reuptake inhibitors (SSRIs) have been shown to enhance 5-HT neurotransmission in projecting brain areas by increasing 5-HT release as a result of a progressive desensitization of somatodendritic and terminal 5-HT autoreceptors which normally exert a negative feedback influence on the function of 5-HT neurons. Since SSRIs and other antidepressant drugs require an administration of about two weeks before exerting a detectable therapeutic effect, the blockade of 5-HT uptake *per se* cannot account for their therapeutic efficacy in major depression and panic disorder. In the treatment of panic disorder, when a SSRI is administered at a starting dose equivalent to that utilized in the treatment of major depression, an exacerbation of the symptoms often occurs (van Vilet *et al.,* 1996). Consequently, the starting dose is routinely decreased by at least half to avoid this deterioration and then it is progressively titrated to the upper range of the therapeutic window. These clinical observations suggest that panic disorder patients, contrary to depressed patients, might have an increased hypersensitivity of certain 5-HT receptor subtypes. The beneficial effects of the drugs in panic disorder occur gradually at about the same rate as for the treatment of major depression.

It is well established that noradrenergic neurons modulate the 5-HT system. Dorsal raphe 5-HT neurons receive noradrenergic projections from the LC (Baraban and Aghajanian, 1980; Anderson *et al.,* 1977; Loizou, 1969), a nucleus which gives rise

to more than 90% of noradrenergic innervation of the brain. The noradrenergic neurons located in the LC modulate the activity of 5-HT neurons in the dorsal raphe nucleus via excitatory α_1-adrenoceptors (Baraban and Aghajanian, 1980). In turn, noradrenergic neurons of the LC receive dense 5-HT projections which have revealed an inhibitory role of 5-HT using different experimental approaches (Vertes and Kocsis, 1994; Léger and Descarries, 1978; Cedarbaum and Aghajanian, 1978). This modulation is indicated by several lines of evidence. For instance, lesioning of 5-HT neurons with a selective 5-HT neurotoxin produces an elevation of firing rate of noradrenergic neurons (Haddjeri *et al.,* 1997). The noradrenergic system is in itself a neuronal system which has been implicated in the antidepressant response. Consequently, the therapeutic effect of drugs selective for the 5-HT system, like SSRIs, could in fact be mediated in part by a modification of the efficacy of 5-HT transmission in the LC. Changes in noradrenergic function in various brain areas by antidepressant drugs may play a crucial role in controlling 5-HT output, and noradrenergic /5-HT interactions may ultimately be relevant to onset antidepressant efficacy and/or to their side effects. In the present study, electrophysiological experiments were performed in male rats undergoing short-term (2-day) and long-term (21-day) treatment with the SSRI paroxetine where the spontaneous neuronal firing rate of LC noradrenergic neurons was determined since this parameter controls in large part the release of noradrenaline in the brain.

Methods

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g, kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water *ad libitum*). Two groups of rats were treated with paroxetine (10 mg kg^{-1} day^{-1}) for either three weeks or two days and one group of rats were treated with citalopram (20 mg kg^{-1} day^{-1}) for three weeks delivered by osmotic minipumps (ALZA, Palo Alto, CA) inserted subcutaneously. Two groups of rats were treated with a vehicle (a 50% v/v ethanol/water solution) for three weeks or two days via osmotic minipumps implanted subcutaneously to act as respective controls for the treated groups. The rats were tested with the minipumps in place. Electrophysiological experiments were performed on rats anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instuments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiment utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.).

Extracellular unitary recording of noradrenergic neurons of the LC were conducted with single-barrelled glass micropipettes preloaded with fiberglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1-3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for LC neurons recordings. LC noradrenergic neurons were recorded with micropipettes lowered at -0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active

noradrenergic neurons of the LC were identified using the following criteria: regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. Noradrenergic neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of noradrenergic neurons, four to five electrode descents were carried out through this nucleus in control and treated rats.

All results were expressed as mean (± S.E.M.) of single neuron values. Statistical comparisons of values obtained in control and paroxetine treated rats were carried out using one-way analysis of variance followed by posthoc Tukey Test.

Results

Systematic electrode descents into the LC were carried out in rats treated with paroxetine for 2- and 21-days as well as with their respective controls. The spontaneous firing activity of LC noradrenergic neurons in treatment and control groups were recorded (figure 1). The 2-day paroxetine treated rats (n = 5) did not significantly differ in spontaneous firing rate activity when compared to control rats (n = 7). However, the 21-day paroxetine treated rats (n = 8) resulted in a significant 52% decrease in the mean spontaneous firing rate when compared to that of the control rats (figure 2). Similar results were obtained with the other SSRI citalopram after a 21-day treatment (n= 5 rats;1.69 ± 0.08 Hz, n = 54 neurons) resulting in a 36% significant decrease when compared to that of control rats. However, this attenuation of firing activity was significantly different from that obtained in the paroxetine group.

Discussion and conclusion

Previous results from our laboratory have demonstrated that acute injection of SSRIs like paroxetine has no effect on the spontaneous firing activity of LC noradrenergic neurons (Béïque *et al.,* 1998). The results of the present study indicate that the long-term 21-day treatment but not the short-term 2-day paroxetine treatment greatly reduced the spontaneous firing rate of the LC noradrenergic neurons. In contrast, the acute and short-term administration of a SSRI reduces the firing rate of 5-HT neurons of the dorsal raphe nucleus in the rodent brain (de Montigny *et al.,* 1981; Quinaux *et al.,* 1982). However, these neurons regain their normal firing rate after long-term treatment (Blier and de Montigny, 1983). This has been shown to be due to desensitization of the somatodendritic 5-HT_{1A} autoreceptor which controls their firing activity (Blier and de Montigny, 1983). The terminal 5-HT autoreceptor controlling 5-HT release also desensitizes following long-term SSRI administration (Blier *et al.,* 1988). These two modifications, in the presence of sustained 5-HT reuptake blockade, result in an increased amount of 5-HT release per action potential in the forebrain.

The difference observed between the paroxetine and citalopram groups after 21 days of treatment cannot be attributed to different degrees of 5-HT reuptake blockade as these regimens were shown to produce a similar effect on the 5-HT reuptake process (Piñeyro *et al*, 1994; Mongeau *et al*, 1998). The difference may rather stem from the weak but significant anti-cholinergic potency of paroxetine. Indeed, since acetylcholine exerts an excitatory effect on noradrenergic neurons firing (Guyenet *et al*, 1979), then the antagonism of an endogenous acetylcholine activation by paroxetine, but not citalopram, might have lead to the greater decrease of noradrenergic firing by

the former drug.

The present findings are interesting when taken into the context of the time course needed for SSRIs to exert their therapeutic efficacy of major depression and panic disorder. The increase in 5-HT release resulting from long-term SSRI treatment would theoretically lead to an increased activation of 5-HT_{2A} receptors on noradrenergic LC neurons (Haddjeri *et al.,* 1997). This would yield an increased inhibitory response and ultimately a decrease in firing activity of LC noradrenergic neurons which is what we have observed. SSRIs thus decrease the LC firing rate and may ultimately also attenuate noradrenaline release in projection areas. This in turn may have a profound impact on the α_2-adrenergic heteroreceptors on the 5-HT terminals, thus diminishing the inhibitory influence on these noradrenergic receptors and contributing to the increase of 5-HT neurotransmission by the SSRI.

The present findings might also be related to the initial exacerbation of panic disorder generally observed with usual starting doses of SSRI for major depression. The acute and short-term administration of SSRIs produces in general a small increase in extracellular 5-HT concentration in several postsynaptic structures (Romero *et al.,* 1996), but has no effect on noradrenergic neuronal firing rate (Béïque *et al.,* 1998). It is thus possible that increased symptoms upon SSRI treatment initiation symptoms may in fact be attributable to an increase in 5-HT synaptic availability not counteracted by an attenuation of noradrenergic firing activity. However, as the treatment is prolonged, 5-HT neurotransmission is further increased but noradrenergic neurotransmission is attenuated. The latter effect may contribute to the anxiolytic and anti-panic effect of SSRI since an enhancement of noradrenergic firing and release achieved with the α_2-

adrenoceptor antagonist yohimbine can produce anxiety in healthy volunteers and trigger panic attacks in patients the with panic disorder (Charney *et al.,* 1984). The decrease in firing activity of LC noradrenergic neurons combined with the increase in 5-HT neurotransmission may thus be the adaptive mechanisms whereby SSRIs eventually exert their therapeutic effect in some anxiety disorders. In contrast, this attenuated noradrenergic tone could explain in part the fatigue and asthenia sometimes reported following long-term SSRI treatment in major depression. Indeed, these symptoms occasionally remain in the presence of markedly improved mood (Feighner *et al*, 1991).

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Legends to figure 1.

Figure 1. Integrated firing rate histograms of LC noradrenergic neurons, recorded in one electrode descent in the LC showing their spontaneous firing activity in control (A), 2-day paroxetine treatment (10 mg kg^{-1} day^{-1}) (B), and 21-day paroxetine treatment (10 mg kg^{-1} day^{-1}) (C). The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

Legends to figure 2.

Figure 2. Effects of 2- and 21-day paroxetine treatments (10 mg kg^{-1} day^{-1}) on the firing activity of LC neurons. The *shaded area* represents the range (SEM x 2) of the mean firing activity of neurons recorded in control rats. *$P < 0.05$ (Tukey Test) when compared to the control value. The number of neurons recorded is displayed in each box.

Figure I.

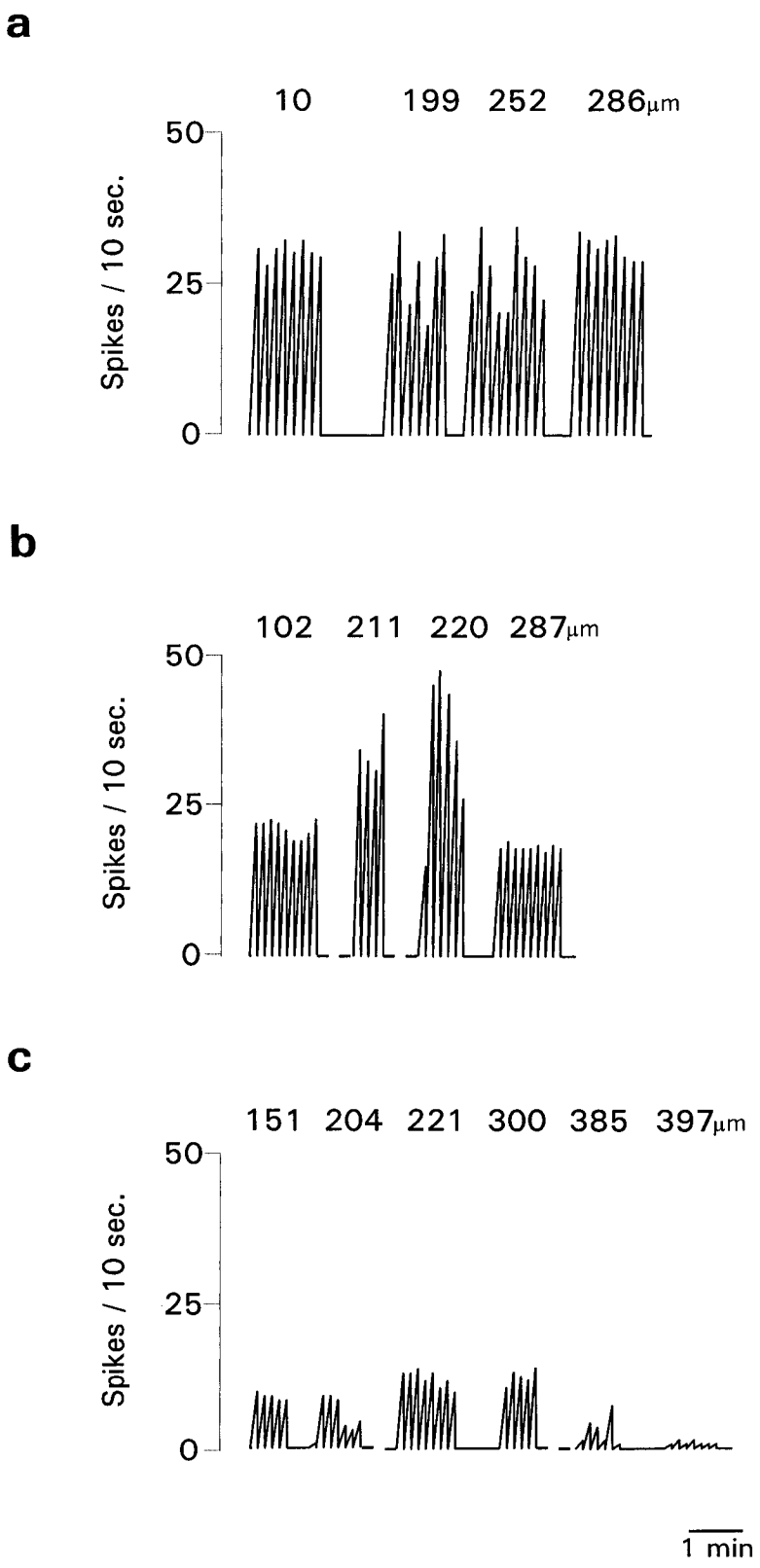
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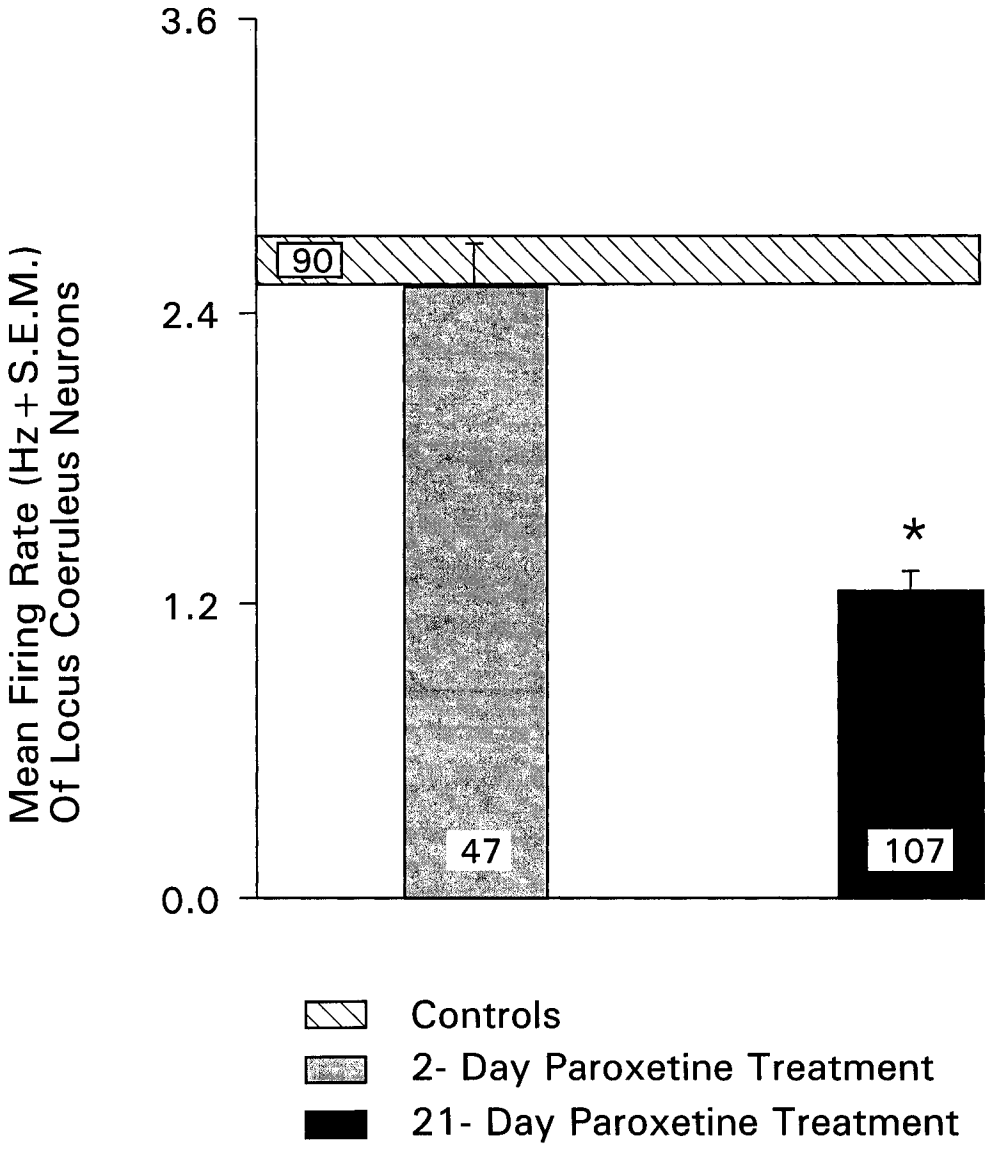
Figure II.


3.6
2.4
1.2
0.0
Mean Firing Rate (Hz + S.E.M.)
Of Locus Coeruleus Neurons
90
47
107
*
Controls
2- Day Paroxetine Treatment
21- Day Paroxetine Treatment

CHAPTER III: Second article

PREFATORY REMARKS

Given the findings of our previous study (Chapter II) indicating that LC NA neuron firing activity is attenuated after a prolonged but not subacute paroxetine administration, it was mandatory to use another SSRI and assesses whether this was a class-specific property or one that is solely indicative of paroxetine. SSRIs undergo many neuronal adaptations that lead to increased 5-HT transmission during a sustained administration. We assessed whether 5-HT receptors that control LC firing become altered after sustained SSRI administration and may be responsible for the attenuation on presented onto NA neuron activity.

The article entitled "Progressive attenuation of the firing activity of LC noradrenergic neurons by sustained administration of selective serotonin reuptake inhibitors" by myself, Claude de Montigny, and Pierre Blier was published in the International Journal of Neuropsychopharmacolology (2000, vol. 3, pp. 1 -11). A reprint of this article is located in a section after the appendix.

PROGRESSIVE ATTENUATION OF THE FIRING ACTIVITY OF LC NORADRENERGIC NEURONS BY SUSTAINED ADMINISTRATION OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS

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Statistical Summary: 248 words **(Abstract)**; 3776 words **(Manuscript)**
References: 58
Figures: 8
Tables: 1

Short Running Title: SSRI And Noradrenergic Neuronal Firing

Note: Some preliminary results were presented before (Szabo et al., 1999).

Summary

Sustained administration of the selective serotonin (5-HT) reuptake inhibitors (SSRIs) citalopram for 2, 14, and 21 days and paroxetine for 2 and 21 days (20 and 10 mg kg^{-1} day^{-1}, respectively, s.c. using osmotic minipumps) produced a gradual decrease in spontaneous firing activity of LC (LC) noradrenergic neurons. In contrast, sustained desipramine administration for 2 and 21 days (10 mg kg^{-1} day^{-1}) robustly reduced LC firing activity, but to the same extent following these two treatment periods. The enhancement of the firing rate of LC neurons produced by the 5-HT_{1A} agonist 8-OH-DPAT (10 - 50 μg/kg, i.v.) in desipramine and citalopram treated rats was abolished, indicating a desensitization of 5-HT_{1A} receptors. However, the attenuation of the firing rate of LC neurons induced by the 5-HT_2 agonist DOI (5 - 50 μg/kg, i.v.) was decreased approximately two-fold in citalopram-treated rats but not significantly altered in desipramine-treated rats. Since 5-HT neurons exert a tonic inhibitory effect on LC neurons, it appears that enhancing 5-HT neurotransmission by sustained SSRI administration leads to a reduction of the firing rate of noradrenergic neurons. In conclusion, SSRIs attenuate the activity of noradrenergic neurons with a delay that is consistent with their beneficial effect in depression and some anxiety disorders, such as panic, generalized and social anxiety disorders. However, given the hyperadrenergic state often observed in anxiogenic conditions the latter phenomenon is believed to contribute more to the anxiolytic effect of SSRI than to their antidepressant action.

KEYWORDS: antidepressant; noradrenaline; serotonin: SSRI; major depression; panic disorder.

Introduction

The noradrenergic and the 5-HT systems modulate the activity of various structures of the CNS. The biological functions in which 5-HT and noradrenaline participate are numerous, and disturbances associated with perturbations of these two monoaminergic systems are diverse. The noradrenergic and 5-HT systems have both been implicated in anxiety and affective disorders, with panic disorder being more closely linked to the former and major depression to the latter system. The exact pathophysiology of these two disorders remains however elusive. In contrast, more is known about the mechanisms of action of the antidepressant drugs used to treat these disorders (Blier and de Montigny, 1997). Selective 5-HT reuptake inhibitors (SSRIs) promptly prevent the reuptake of 5-HT, but they require an administration of at least two weeks before exerting a significant therapeutic effect, just like other antidepressant agents. Therefore, the rapid blockade of 5-HT reuptake by antidepressant drugs *per se* cannot account for their therapeutic effect in major depression and panic disorder. SSRIs, however, have been shown to enhance 5-HT neurotransmission in projecting brain areas by increasing 5-HT release as a result of a progressive desensitization of somatodendritic and terminal 5-HT autoreceptors, which normally exert a direct negative feedback influence on the firing rate of 5-HT neurons and on 5-HT release, respectively. In the treatment of panic disorder, when a SSRI or a tricyclic antidepressant (TCA), affecting the 5-HT and/or the noradrenergic reuptake process is administered at a starting dose equivalent to that utilized in the treatment of major depression, an exacerbation of the symptoms often occurs (Taylor, 1995; Westenberg, 1996). Consequently, the starting dose is routinely decreased by at least half to avoid

this deterioration and then it is progressively titrated to the upper range of the therapeutic window. The beneficial effects of the drugs in panic disorder occur gradually at about the same rate as for the treatment of major depression. These clinical observations suggest that panic disorder patients, contrary to depressed patients, might have an increased hypersensitivity of certain 5-HT receptor subtypes and/or a heightened noradrenergic neuronal activity.

It is well established that noradrenergic neurons modulate the 5-HT system. Dorsal raphe 5-HT neurons receive noradrenergic projections from the LC (Clement et al., 1992; Baraban and Aghajanian, 1980; Anderson et al., 1977; Loizou, 1969). For instance, noradrenergic neurons located in the LC modulate the activity of 5-HT neurons in the dorsal raphe nucleus via excitatory α_1-adrenoceptors (Baraban and Aghajanian, 1980). At the level of 5-HT terminals, noradrenergic agonists modulate 5-HT release via α_2-adrenergic heteroceptors (Göthert et al., 1981). In turn, noradrenergic neurons of the LC receive dense 5-HT projections most probably coming from pericoerulear 5-HT neurons (Aston-Jones et al., 1991), which exert an inhibitory role. In particular, lesioning 5-HT neurons with a selective 5-HT neurotoxin (5,7-DHT) produces an elevation of firing rate of noradrenergic neurons (Haddjeri et al., 1997).

In vivo studies in the rat have demonstrated that although iontophoretically applied 5-HT does not consistently affect LC spontaneous discharge, 5-HT markedly attenuates LC responses to iontophoretic glutamate mediated via a 5-HT_{1A} receptor (Aston-Jones et al., 1990; Charlety et al., 1991). This is consistent with 5-HT agonists inhibiting excitatory amino acid-mediated synaptic potentials in LC, but not with data showing that systemic administration of the selective 5-HT_{1A} agonist 8-hydroxy-2(di-n-

proplylamino)-tertralin (8-OH-DPAT) augments the spontaneous firing activity of LC neurons (Piercey et al., 1993). Furthermore, systemic injection, but not iontophoretic application, of the selective 5-HT_{1A} antagonist WAY 100635 decreases firing activity of LC neurons (Haddjeri et al., 1997). Similarly, systemic administration of the preferential 5-HT_{2A} agonist (±) 2,5-dimethoxy-4-iodoamphetamine hydrochloride (DOI) produces a dose-dependent decrease in the firing activity of LC neurons (Rasmussen et al., 1988; Chiang et al., 1993). It is thus conceivable that the therapeutic effect of drugs selective for the 5-HT system, like SSRIs, could be mediated in part by a modification of the efficacy of 5-HT transmission in the LC.

Preliminary results indicated that a 21-day treatment but neither acute nor a 2-day administration of the SSRI paroxetine decreased the spontaneous firing activity of LC noradrenergic neurons in the rat (Béïque et al.,1998a; Szabo et al., 1999). The present studies were designed to further characterize the effect of sustained 5-HT and NE reuptake blockade on the spontaneous firing activity of LC noradrenergic neurons.

Methods

Animals

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water ad libitum). Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instuments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, rats were inserted with a catheter in a lateral tail vein for systemic i.v. injection of drugs.

Short- and Long-term Treatments

Rats were anaesthetized with halothane for subcutaneous implantation of osmotic minipumps (ALZA, Palo Alto, CA). Three groups of rats were treated with citalopram (20 mg kg^{-1} day^{-1}), for three weeks, two weeks, or two days, and four other groups of rats were treated with paroxetine (10 mg kg^{-1} day^{-1}) or desipramine (10 mg kg^{-1} day^{-1}) for either three weeks or two days. Control rats were treated with the vehicle for citalopram and paroxetine (a 50% ethanol/water solution) for each treatment group and another set of controls were treated with a water vehicle for either three weeks or two

days via osmotic minipumps to act as controls for the desipramine treated group. The rats were tested with the minipumps in place.

Electrophysiological experiments

Extracellular unitary recording of noradrenergic neurons of the LC were conducted with single-barrelled glass micropipettes preloaded with fiberglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1-3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ which provides stable recordings and a good signal-to-noise ratio. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for LC neurons recordings. LC noradrenergic neurons were recorded with micropipettes lowered at -0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active noradrenergic neurons of the LC were identified using the following criteria: regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. Noradrenergic neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of noradrenergic neurons in treated animals, four to five electrode descents were carried out through this nucleus in control and treated rats. Cell position within the LC did not appear to correlate with firing rate.

Dose-response curves for the alteration of LC neuron firing activity were obtained for systemic (i.v.) administration of the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT and the 5-HT_2 receptor agonist DOI which *in vivo* but not *in vitro* acts as a preferential 5-

HT_{2A} agonist (Mazzola-Pomietto et al., 1995; Aulakh et al., 1995; Yamada et al.,1995). Changes in the firing activity are expressed as percentages of the baseline firing rate and were measured after administration of the agonists. After systemic injection of 8-OH-DPAT and DOI, the selective 5-HT_{1A} antagonist WAY 100635 and the 5-HT_2 receptor antagonist ritanserin were systemically administered in attempts to reverse the effects of each of the agonists, respectively. Dose-response curves of 8-OH-DPAT and DOI were constructed, where only one dose was injected to each rat to generate an effective dose 50 (ED50).

Drugs

The following drugs were used: citalopram hydrobromide (H. Lundbeck A/S, Copenhagen, Denmark), desipramine HCL, DOI, 8-OH-DPAT, ritanserin, idazoxan, clonidine (RBI, Natick, MA, U.S.A.), WAY 1000635 (Wyeth Research, Berkshire, U.K.). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water while ritanserin was solubilized in acetic acid and then diluted with distilled water to the appropriate concentration.

Statistical comparisons

All results were expressed as mean (± S.E.M.) of single neuron values. Statistical comparisons of values obtained in treated and control rats were carried out using Kruskal-Wallis One-Way Analysis of Variance followed by post-hoc Dunn's Method where multiple comparison versus control group was performed.

Results

Saline and 50% ethanol vehicle control groups resulted in no significant difference in LC spontaneous firing activity when compared to each other and these data were therefore merged to make up one control group. Systematic electrode descents into the LC yielded a similar mean number of neurons per trajectory discharging spontaneously in paroxetine (2- and 21-days), citalopram (2-, 14- and 21-days) and desipramine (2-days) treated rats when compared to their respective controls (figures 1 and figure 3). The 21-day paroxetine and citalopram treatments resulted in a significant 52% (range of firing: 0.1 to 4.6 Hz) and 36% (range of firing: 0.7 to 3.1 Hz) decrease in mean spontaneous firing activity, respectively, whereas their corresponding two-day treatments did not differ significantly from the control group (range of firing: 0.8 to 5.3 Hz). The 14-day citalopram treatment resulted in a significant 24% decrease in mean spontaneous firing activity of LC neurons (range of firing: 0.5 to 3.8 Hz), when compared to that of the control rats (figure 2). The 2- and 21-day desipramine treatments resulted in a robust but identical decrease in the mean spontaneous firing activity (range of firing: 0.1 to 2.6 Hz; figure 4). The slight but statistically significant greater number of spontaneously active noradrenergic neurons in the 21-day desipramine treated rats was likely reflecting an increased proficiency in recording given the identical firing activity in the 2- and 21 day desipramine groups (table 1).

A dose-response curve of systemic 8-OH-DPAT administration in control rats yielded an ED_{50} of 15 μg kg^{-1} (figures 5 and 6). After systemic injection of 8-OH-DPAT, the selective 5-HT_{1A} antagonist WAY 100635 (100 μg kg^{-1}, n = 5) reversed the effects of

the agonist, thus confirming the 5-HT_{1A} nature of the enhancing effect of 8-OH-DPAT on LC neuronal firing. However, systemic injection of 8-OH-DPAT (5-100 μg kg^{-1}) in 21-day citalopram and desipramine treated rats (n = 6 and n = 5, respectively) failed to induce any augmentation of LC neuron firing activity. Whenever possible, the α_2-adrenergic agonist clonidine was injected at the end of the experiment to confirm the noradrenergic nature of the neuron recorded (figure 5).

Systemic administration of the preferential 5-HT_{2A} agonist DOI dose-dependently reduced LC neuron firing activity to the same extent in controls (ED_{50} = 20 μg kg^{-1}; figure 7) and desipramine (ED_{50} = 35 μg kg^{-1}; figure 8) treated rats. In citalopram-treated rats, there was a significant shift to the right of the DOI dose response curve (ED_{50} = 70 μg kg^{-1}). After systemic injection of DOI in controls, citalopram, and desipramine treated rats, the 5-HT_2 receptor antagonist ritanserin (500 μg kg^{-1}, n = 3, n = 2, n = 4, respectively) reversed the agonistic effect of DOI, thus establishing the 5-HT_2 nature of the latter phenomenon.

Discussion and conclusion

The results of the present study indicate that sustained administration of the SSRI citalopram significantly reduced the spontaneous firing activity of LC noradrenergic neurons in a time-dependent manner. Similarly, a 21-day but not a 2-day paroxetine treatment greatly reduced the spontaneous firing activity of LC noradrenergic neurons, indicating that this phenomenon is a class specific effect and not merely a drug specific effect (Szabo et al., 1999). A 33% decrease of LC firing had also been previously reported using long-term sertraline administration. This effect did not, however, reach statistical significance most likely due to a small sample size as only ten neurons were recorded (Valentino et al., 1990). In contrast, the acute and short-term administration of SSRI greatly reduces the firing activity of 5-HT neurons of the dorsal raphe nucleus in the rodent brain (de Montigny et al., 1981; Quinaux et al., 1982). Contrary to noradrenergic neurons, 5-HT neurons regain their normal firing rate after long-term treatment (Blier and de Montigny, 1983). This has been shown to be due to the desensitization of the somatodendritic 5-HT_{1A} autoreceptor which control their firing activity (Blier and de Montigny, 1994).

Previous experiments have shown that systemic injection of the 5-HT_{1A} agonist 8-OH-DPAT produces a dose-dependent increase in LC firing activity with an ED_{50} similar to that reported herein (Piercey et al., 1993). In an attempt to better understand the possible basis for the progressive decrease of LC firing activity following sustained citalopram treatment, the sensitivity of this 5-HT_{1A} receptor involved in enhancing noradrenergic firing activity was assessed in long-term 21-day citalopram treated rats. The response to systemic 8-OH-DPAT after long-term 21-day citalopram treatment was

abolished indicating a marked desensitization of such 5-HT_{1A} receptors normally mediating an excitatory effect on LC neuronal firing. The desensitization of this 5-HT_{1A} receptor could thus account for the alteration of firing activity of LC neurons: an increase in 5-HT release per action potential, as was documented for the ascending 5-HT pathway projecting to the forebrain (Blier and de Montigny, 1983) most likely attributable to the fact that this 5-HT_{1A} receptor can no longer be activated following long-term SSRI treatment. This might would thus explain the decreased firing rate of noradrenergic LC neurons following SSRI long-term administration. This pharmacological condition would then mimic that of the administration of the 5-HT_{1A} antagonist WAY 100,635 which produces a suppression of firing of LC neurons (Haddjeri et al., 1997). Indeed, blocking a receptor or desensitizing it should have the same physiological consequence.

In addition to the abovementioned 5-HT_{1A} receptor mediating an excitatory effect on noradrenergic neuron firing activity, a postsynaptic 5-HT_{2A} receptor is likely present in the LC (Haddjeri et al., 1997). Systemic injection of DOI produces a dose-dependent decrease in LC firing activity consistent with previous results (Rasmussen et al., 1988; Chiang et al., 1993). Since direct microiontophoretic application of DOI onto noradrenergic neurons does not affect their firing activity, 5-HT_{2A} receptors would thus not be located in the immediate vicinity of their cell body where the recordings and applications of DOI were carried out. Nevertheless, these results would still be compatible with the localization of these 5-HT_{2A} receptors on the dendrites of noradrenergic neurons which may extend outside the core of the LC. The response to systemic DOI after long-term 21-day citalopram treatment was reduced by approximately two-fold indicating a desensitization of 5-HT_{2A} receptors, but to a much

lesser magnitude than that observed for the 5-HT_{1A} receptors (figures 6 and 8). Wherever the localization of these 5-HT_{2A} receptors, their attenuated inhibitory role on LC firing would not be sufficient to overcome the enhanced 5-HT release resulting from the apparent complete desensitization of the 5-HT_{1A} receptors following long-term SSRI administration.

In rats treated in a sustained fashion with desipramine, regardless of whether treatment duration was short (2-day) or long (21-day), LC firing activity was reduced to the same extent. The increase of auto-inhibitory effect of noradrenaline, mediated by α_2-adrenergic autoreceptors on LC neurons as a result of noradrenergic reuptake blockade, likely mediates in large part the dramatic effect of desipramine on LC firing. The response to systemic 8-OH-DPAT after the long-term 21-day desipramine treatment was abolished, as for was the case following long-term SSRI administration, thus indicating a desensitization of 5-HT_{1A} receptors and a possible contribution of the latter receptors to maintain LC firing attenuated. Indeed, despite a decreased responsiveness of LC neurons to the α_2-adrenoceptor agonist clonidine during sustained noradrenergic reuptake blockade, spontaneous firing remains markedly decreased (Svensson and Usdin, 1978; Mongeau et al., 1998). Consistent with the present results, sustained desipramine administered orally has been shown to induce a functional down-regulation of postsynaptic 5-HT_{1A} receptors in radioligand binding experiments (Newmann et.al., 1988). However, it is important to emphasize that the somatodendritic 5-HT_{1A} receptors in the dorsal raphe remain normosensitive after long-term desipramine administration (Blier and de Montigny, 1980). The location of the 5-HT_{1A} receptor modulating LC firing activity therefore remains to be identified. However,

the response to systemic DOI after long-term 21-day desipramine treatment was similar to that of the controls, indicating fully functional 5-HT_{2A} receptors. These results stand in contrast with the down-regulation of 5-HT_{2A} receptors in the cerebral cortex following sustained administration of desipramine (Todd et al., 1995), thus implying different adaptive properties of 5-HT_{2A} receptors depending on the brain region where they are located.

The present findings showing a gradual decrease of LC neuronal firing during SSRI administration may be relevant to the notion that panic disorder patients may have increased noradrenergic function (Charney and Heninger, 1986), while depressed patients may have decreased 5-HT neurotransmission (Stockmeier et al., 1997). A putative increased noradrenergic activity is not specific to panic disorder as it has been reported in mania (Post et al., 1989; Swann et al., 1983; Swann et al., 1991). Since 5-HT imparts an inhibitory modulatory effect on noradrenaline neurotransmission, abnormally low 5-HT levels possibly may allow a permissive noradrenergic dyscontrol during stressful events (Depue and Spoont, 1986). This may in turn contribute to the genesis of panic attacks. The increase in 5-HT release resulting from long-term SSRI treatment would theoretically lead to an increased activation of 5-HT_{2A} receptors inhibiting the firing of noradrenergic LC neurons (Haddjeri et al., 1997; figure 1). However, in long-term SSRI treated rats, 5-HT_{2A} receptor sensitivity was decreased approximately two-fold. It appears that long-term administration of either SSRI or the tricyclic agent desipramine markedly desensitizes the 5-HT_{1A} response of LC neurons but to a lesser extent their 5-HT_{2A} response. SSRIs thus progressively decrease the LC firing rate and possibly noradrenaline release in projection areas. This may also

have a significant impact on the α_2-adrenergic heteroreceptors on the 5-HT terminals, diminishing the inhibitory influence on these noradrenergic receptors and possibly contributing to the increase of 5-HT neurotransmission observed with SSRI treatment. This possibility highlights the notion that when considering changes in overall efficacy in neurotransmission, factors at both the cell body and the terminal levels must be taken into consideration. For instance, the present data obtained with desipramine, i.e. a rapid and sustained decrease in firing of noradrenergic neurons, does not imply that it should exert a rapid anti-panic effect. Indeed, desipramine despite attenuating noradrenergic impulse flow also blocks noradrenergic reuptake throughout the brain, unlike SSRI's.

The initial exacerbation of panic disorder symptoms generally observed with usual starting doses of SSRI for major depression might also be related to the present findings. The acute and short-term administration of SSRIs produces in general a small increase in extracellular 5-HT concentration in most postsynaptic structures (Fuller et al., 1994), but has little effect on noradrenergic neuronal firing rate. It is thus possible that increased symptoms upon SSRI treatment initiation may in fact be attributable to an increased activation of some subtypes of 5-HT receptors not counteracted by an attenuation of noradrenergic firing activity. In analogy, one may think of the 5-HT_3-mediated nausea sometimes produces upon initiation of a SSRI treatment in the absence of any antidepressant effect (Bergeron and Blier, 1993; Bailey et al., 1995). However, as the treatment is prolonged, 5-HT neurotransmission is further increased but noradrenergic neurotransmission would be progressively attenuated. The latter effect may contribute to the anxiolytic and anti-panic effect of SSRI since an

enhancement of noradrenergic firing and release achieved with the α_2-adrenoceptor antagonist yohimbine can produce anxiety in healthy volunteers and trigger panic attacks in patients with panic disorder (Charney et al., 1984). On the other hand, a recent study by Page and Abercrombie (1997) reported that while acute and chronic blockade of 5-HT reuptake did not alter basal extracellular levels of noradrenaline, chronic fluoxetine administration resulted in an increased of stress-induced noradrenaline efflux in the rat hippocampus. This may be explained by a lack of absolute selectivity of fluoxetine for the 5-HT transporter at higher doses, with noradrenaline reuptake being blocked to a significant extent in the presence of marked 5-HT reuptake blockade (Ki = 143 nM and 14 nM, respectively; Bolden-Watson et al., 1993). Indeed, the affinity ratio of fluoxetine being one of the lowest among the drugs considered as SSRI's (Hyttel, 1982; Owen et al., 1997; Stanford, 1996). This interpretation of these data would, however, not be compatible with an enhancement of extrasynaptic noradrenaline in the rat frontal cortex following long-term sertraline administration because the latter drug is highly selective for the 5-HT transporter (Thomas et al., 1998).

The decrease in firing activity of LC noradrenergic neurons combined with the increase in 5-HT neurotransmission may thus be an adaptive mechanism whereby SSRIs eventually exert their therapeutic effect in some anxiety disorders, such as panic and generalized as well as social anxiety disorders (Connor and Davidson, 1998; Davidson, 1998; Jefferson, 1998). In contrast, this putative attenuated noradrenergic tone could explain in part the fatigue and asthenia sometimes reported following long-term SSRI treatment in major depression (Montgomery et al., 1993). Indeed, these

symptoms occasionally remain in the presence of markedly improved mood (Feighner et al., 1991). In addition, this delayed reduction of LC neuronal firing may account for the lesser efficacy of SSRI than dual 5-HT/noradrenergic reuptake blockers in some depressed patients, although the latter agents often produce more side effects (Einarson et al., 1999; Danish University Antidepressant group, 1990; Danish University Antidepressant group, 1986; Schaeffer et al., 1998; Silverstone and Ravindran, 1999). In fact, despite decreasing noradrenergic neuronal firing like SSRI's, drugs like venlafaxine in addition block noradrenergic reuptake in projection areas (Béïque et al.,1998b), which would contribute to enhance noradrenergic transmission.

Acknowledgements

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Legends to figure 1.

Figure 1. Integrated firing rate histograms of LC noradrenergic neurons, recorded in single electrode descents in the LC showing their spontaneous firing activity in control (n = 6)(A), 2-day citalopram treatment (20 mg kg^{-1} day^{-1}; n = 5) (B), 14-day citalopram treatment (20 mg kg^{-1} day^{-1}; n = 3) (C), and 21-day citalopram treatment (20 mg kg^{-1} day^{-1}; n = 4). The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

Legends to figure 2.

Figure 2. Effects of 2-, 14- and 21-day citalopram treatments (20 mg kg^{-1} day^{-1}) on the spontaneous firing activity of LC neurons. The *shaded area* represents the range (SEM x 2) of the mean firing activity of neurons recorded in control rats. *P < 0.05 (Dunn's Method) when compared to the control value. The number of neurons recorded is displayed in each box.

Legends to figure 3.

Figure 3. Integrated firing rate histograms of noradrenergic neurons, recorded in single electrode descents in the LC showing their spontaneous firing activity in control (n = 6)(A), 2-day desipramine treatment (10 mg kg^{-1} day^{-1}; n = 3) (B), and 21-day desipramine treatment (10 mg kg^{-1} day^{-1}; n = 3) (C). The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

Legends to figure 4.

Figure 4. Effects of 2- and 21-day desipramine treatments (10 mg kg^{-1} day^{-1}) on the firing activity of LC neurons. The *shaded area* represents the range (SEM x 2) of the mean firing activity of neurons recorded in control rats. *P < 0.05 (Dunn's Method) when compared to the control value. The number of neurons recorded is displayed in each box.

Legends to figure 5.

Figure 5. Integrated firing rate histogram of a LC noradrenergic neuron illustrating the effects of intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT producing an increase in the firing activity, a subsequent injection the 5-HT_{1A} antagonist of WAY100635 reversed the effects in a control rat (A). Integrated firing rate histogram of a LC noradrenergic neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with citalopram (20 mg kg^{-1} day^{-1}; *n* = 6) for 21 days (B). Note that a subsequent injection of the 5-HT_2 agonist DOI produced a lesser inhibition of firing when compared to controls (see figures 7 and 8). Integrated firing rate histogram of a LC noradrenergic neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with desipramine (10 mg kg^{-1} day^{-1}) for 21 days (C). The identity of the noradrenergic neuron recorded in the desipramine treated rat was assessed by showing the suppressant effect of a subsequent intravenous administration of the α_2-adrenoceptor agonist clonidine.

Legends to figure 6.

Figure 6. Relationship between the degree of augmentation of LC noradrenergic firing activity and doses of 8-OH-DPAT administered intravenously in controls and treated rats. Only the initial response of a single noradrenergic neuron to the first dose of 8-OH-DPAT in each rat was used to construct the curves. Outer lines represent the standard error of the regression line.

Legends to figure 7.

Figure 7. Integrated firing rate histograms of LC noradrenergic neurons illustrating the effects of intravenous administration of the preferential 5-HT_{2A} agonist DOI producing suppressions of the firing activity, whereby a subsequent injection of the 5-HT_2 antagonist ritanserin reversed the effects in a control rat (A), in a rat treated with citalopram (20 mg kg^{-1} day^{-1}; n = 6) for 21 days (B), and in a 21- day desipramine (10 mg kg^{-1} day^{-1}; n = 6) treated rat (C). The identity of the noradrenergic neuron recorded in the citalopram treated rat was also assessed by showing the suppressant effect of a subsequent intravenous administration of the α_2-adrenoceptor agonist clonidine (n = 3).

Legends to figure 8.

Figure 8. Relationship between the degree of suppression of LC noradrenergic firing activity and doses of DOI administered intravenously in controls and rats treated with citalopram (20 mg kg^{-1} day^{-1}) and desipramine (10 mg kg^{-1} day^{-1}). Only, the initial response of a single noradrenergic neuron to the first dose of DOI in each rat was used to construct the curve. Outer lines represent the standard error of the regression line

and the hatched area represents the mean (SEM x 2) of the responses obtained in controls. The shift to the right of the dose-response curve was significant.

Table 1. Firing activity of Locus Coeruleus noradrenergic neurons in controls and treated rats

		Average number of noradrenergic neurons per descent	No. of descents
Control		2.7 ± 0.2	33
Paroxetine	(10 mg/kg/day)		
	2 days	2.8 ± 0.6	17
	21days	2.7 ± 0.2	39
Citalopram	(20 mg/kg/day)		
	2 days	3.1 ± 0.3	35
	14 days	3.4 ± 0.4	18
	21days	3.4 ± 0.4	16
Desipramine	(10 mg/kg/day)		
	2 days	2.8 ± 0.4	18
	21days	4.8 ± 0.5*	21

*P<0.05, when compared to the control group, using ANOVA followed by posthoc Dunn's method

Figure I.

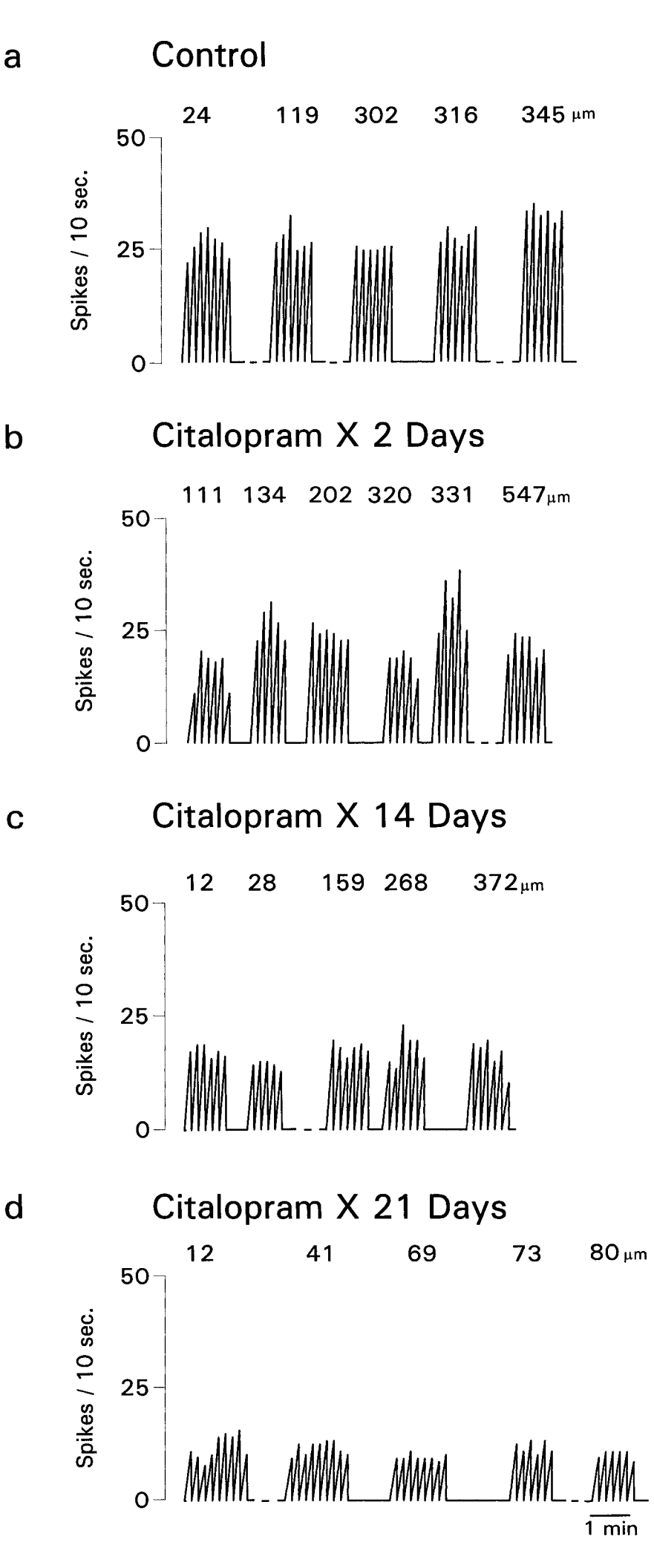

a
Control
24
119
302
316
345 µm
50
25
0
Spikes / 10 sec.
b
Citalopram X 2 Days
111
134
202
320
331
547µm
50
25
0
Spikes / 10 sec.
c
Citalopram X 14 Days
12
28
159
268
372µm
50
25
0
Spikes / 10 sec.
d
Citalopram X 21 Days
12
41
69
73
80 µm
50
25
0
Spikes / 10 sec.
1 min

Figure II.

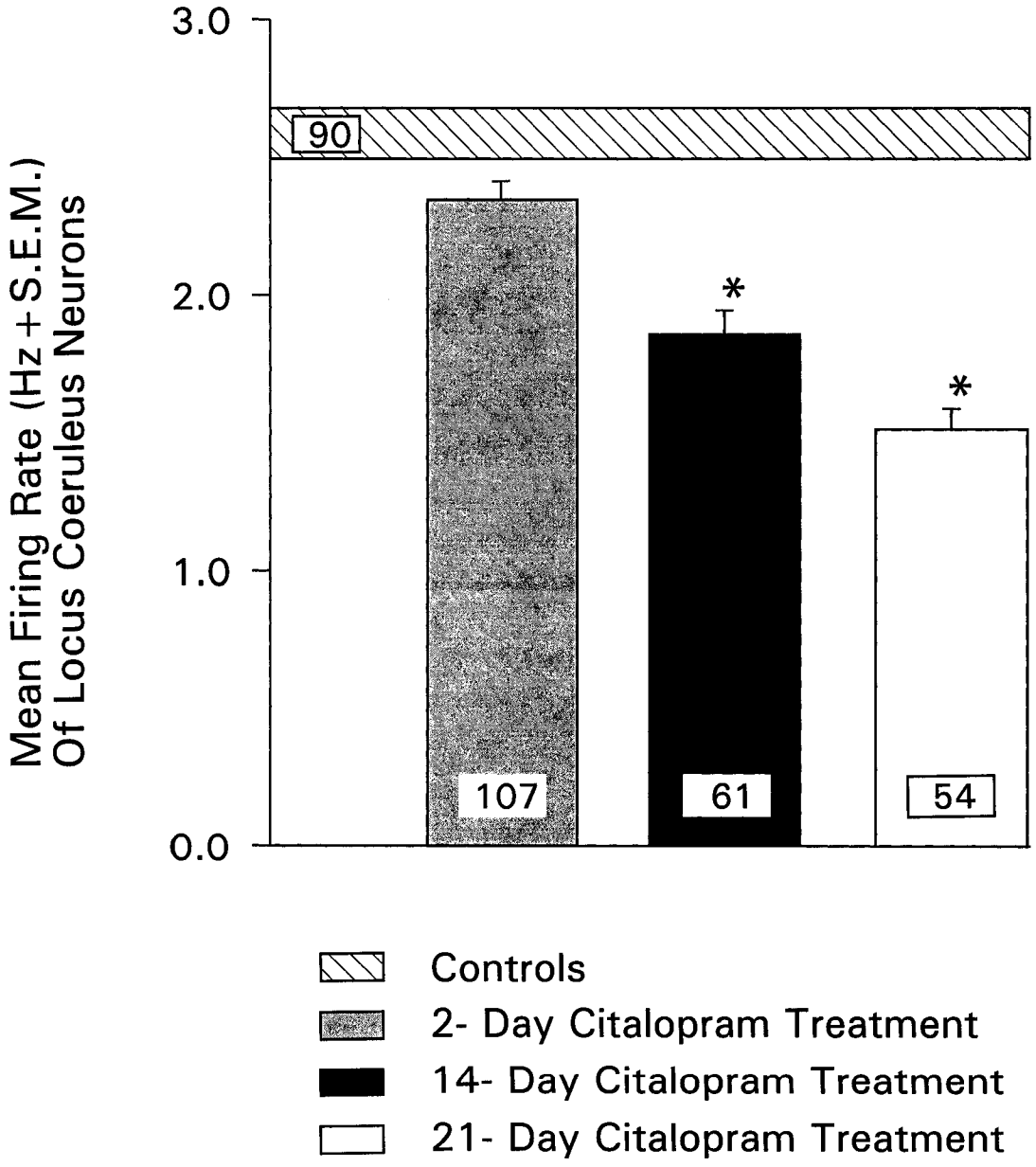

3.0
2.0
1.0
0.0
Mean Firing Rate (Hz + S.E.M.)
Of Locus Coeruleus Neurons
90
107
61
54
*
*
Controls
2- Day Citalopram Treatment
14- Day Citalopram Treatment
21- Day Citalopram Treatment

Figure III.

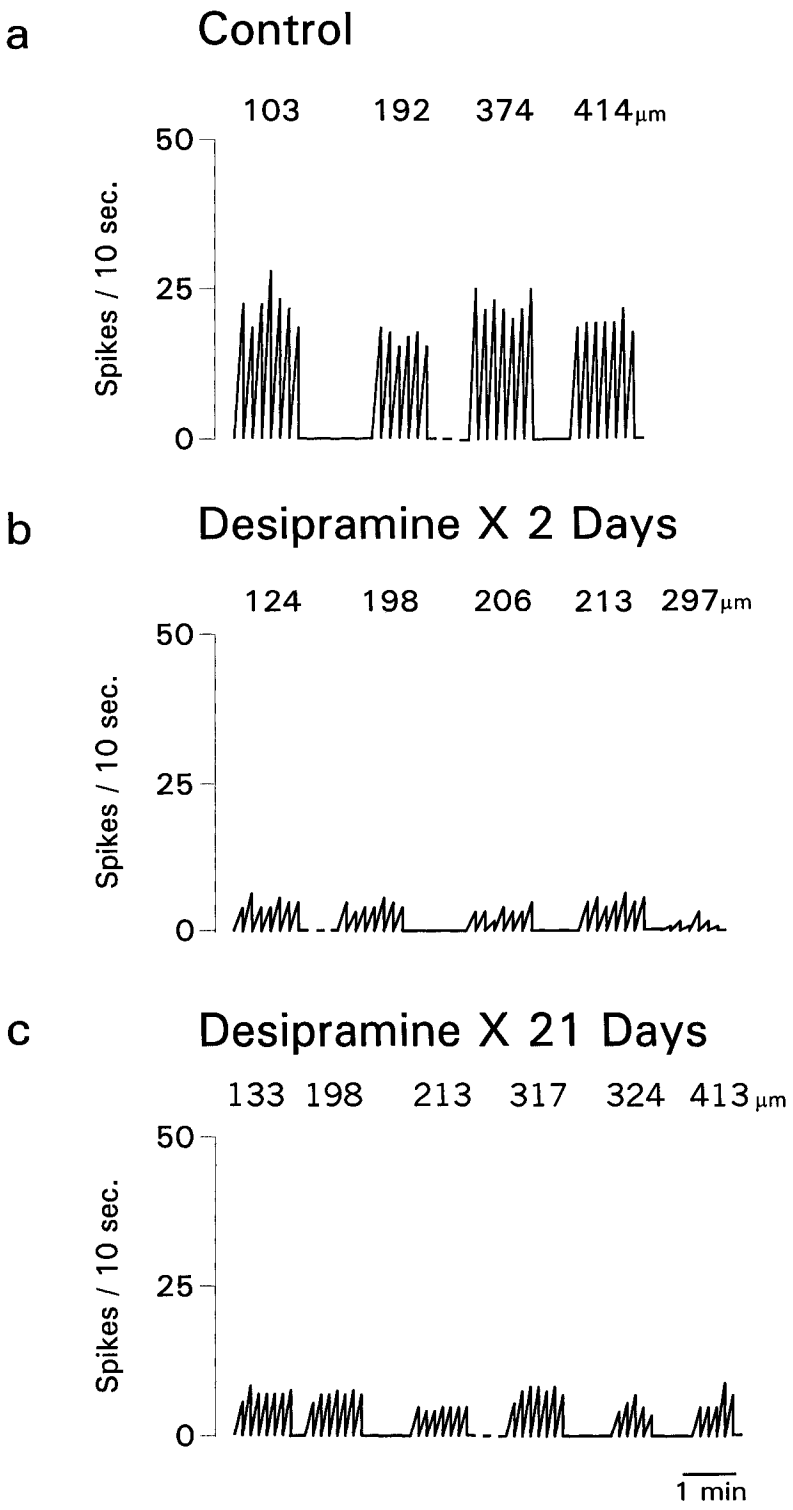

a
Control
103
192
374
414μm
50
25
0
Spikes / 10 sec.
b
Desipramine X 2 Days
124
198
206
213
297μm
50
25
0
Spikes / 10 sec.
c
Desipramine X 21 Days
133
198
213
317
324
413μm
50
25
0
Spikes / 10 sec.
1 min

Figure IV.

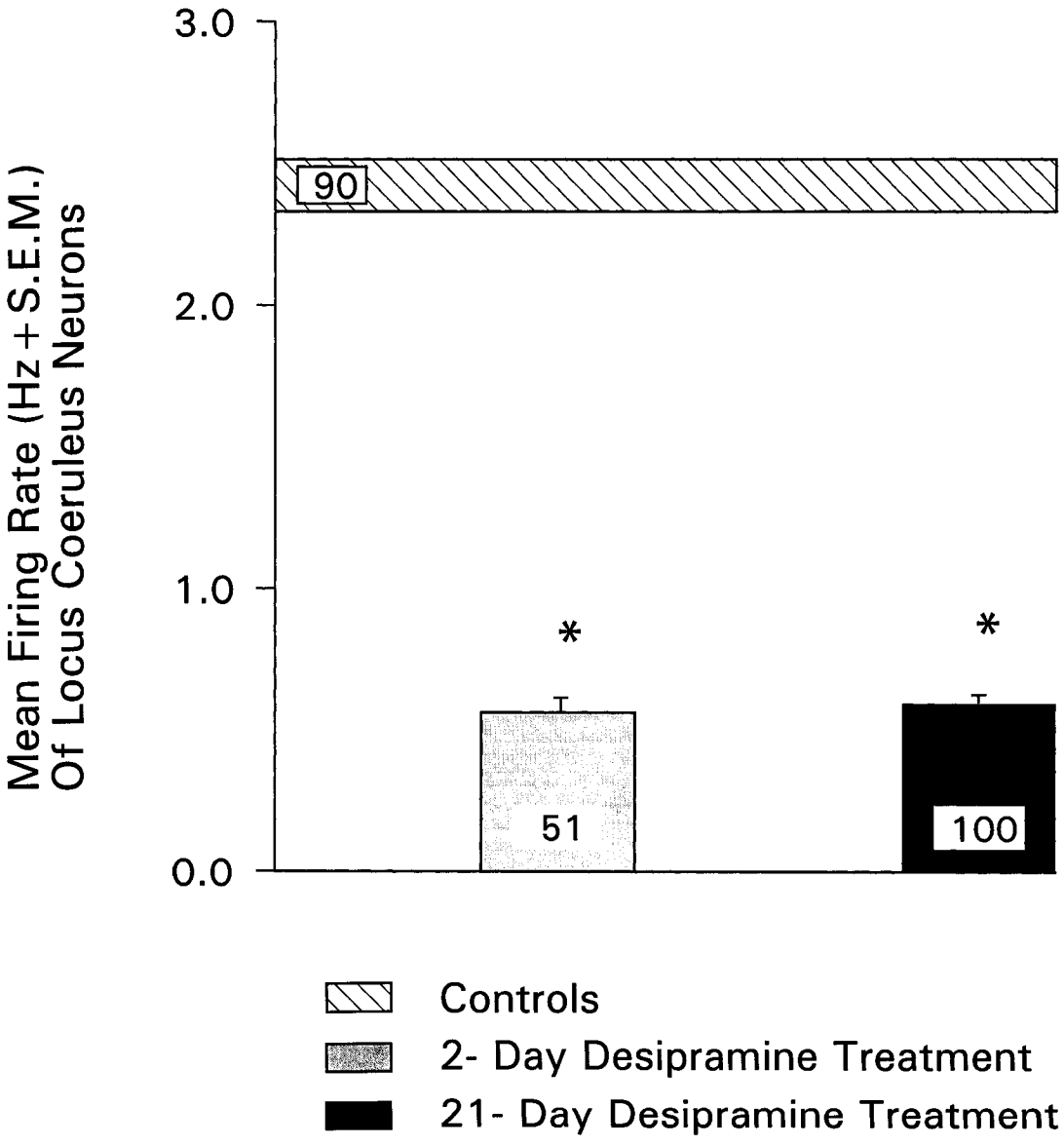

Mean Firing Rate (Hz + S.E.M.)
Of Locus Coeruleus Neurons
3.0
2.0
1.0
0.0
90
51
100
*
*
Controls
2- Day Desipramine Treatment
21- Day Desipramine Treatment

Figure V.

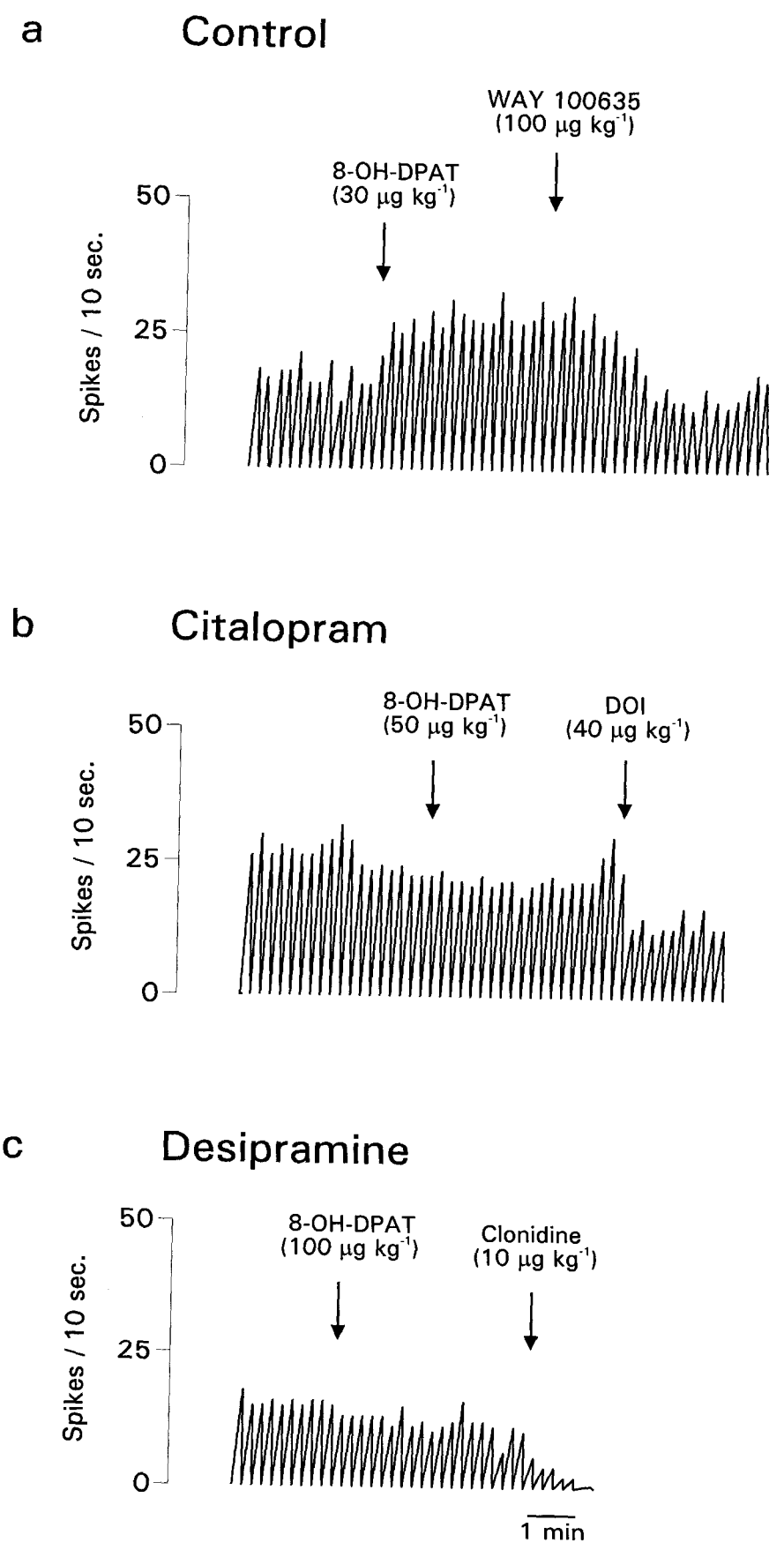

a
Control
WAY 100635
(100 µg kg⁻¹)
8-OH-DPAT
(30 µg kg⁻¹)
50
25
0
Spikes / 10 sec.
b
Citalopram
8-OH-DPAT
(50 µg kg⁻¹)
DOI
(40 µg kg⁻¹)
50
25
0
Spikes / 10 sec.
c
Desipramine
8-OH-DPAT
(100 µg kg⁻¹)
Clonidine
(10 µg kg⁻¹)
50
25
0
Spikes / 10 sec.
1 min

Figure VI.

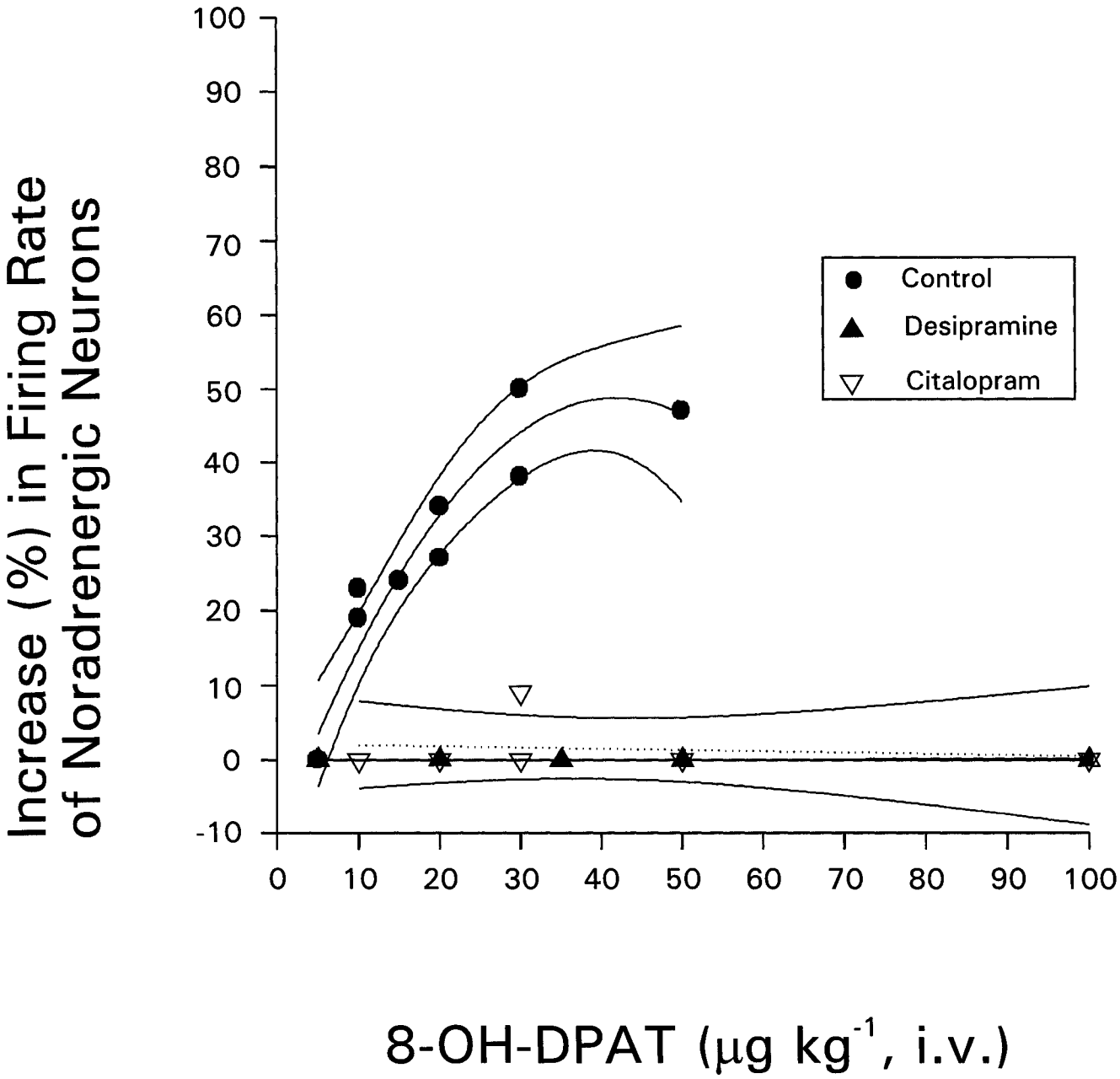

Increase (%) in Firing Rate of Noradrenergic Neurons
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100
Control
Desipramine
Citalopram
8-OH-DPAT (μg kg-1, i.v.)

Figure VII.

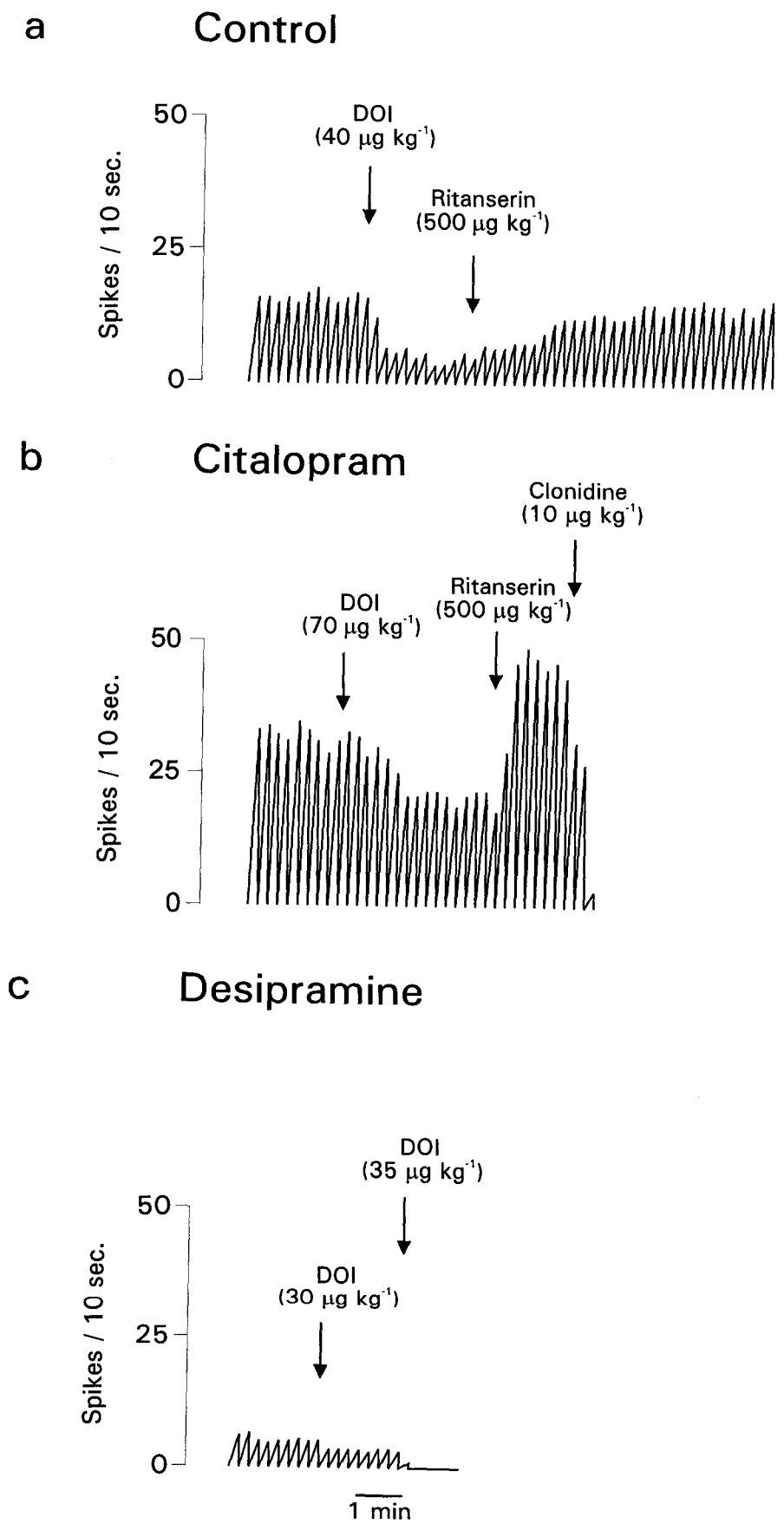
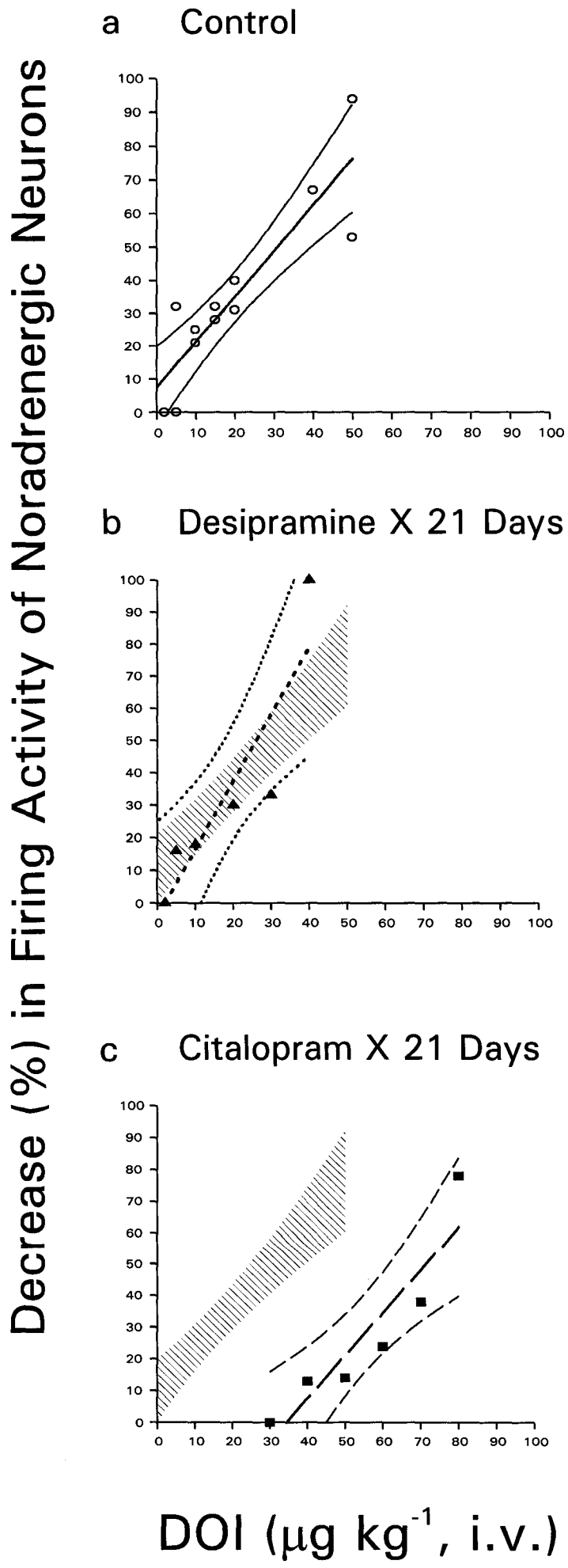

a
Control
50
25
0
Spikes / 10 sec.
DOI
(40 µg kg-1)
Ritanserin
(500 µg kg-1)
b
Citalopram
Clonidine
(10 µg kg-1)
DOI
(70 µg kg-1)
Ritanserin
(500 µg kg-1)
50
25
0
Spikes / 10 sec.
c
Desipramine
DOI
(35 µg kg-1)
DOI
(30 µg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure VIII.


a Control
b Desipramine X 21 Days
c Citalopram X 21 Days
Decrease (%) in Firing Activity of Noradrenergic Neurons
DOI (μg kg-1, i.v.)

Chapter IV: Third Article

Prefatory Remarks

The attenuation on LC firing activity reported with SSRIs is a class--specific effect given that citalopram, the most selective of the SSRIs, and paroxetine, the most prescribed antidepressant in Canada, both induced this effect. This attenuation may be relevant to beneficial effects observed in anxiety disorders as a decrease in LC activity corresponds to the delay in onset of these agents in affective and anxiety disorders. 5-HT_{1A} receptors regulating NA neuron firing are desensitized after a prolonged SSRI administration. This phenomenon by itself may be responsible for the inhibitory effects on LC activity. Indeed, these receptors when activated by 8-OH-DPAT augment NA neuron firing, whereas blockade of these receptors via WAY 100,635 attenuated their activity. As a result of prolonged SSRI administration, the increase in 5-HT availability desensitized 5-HT_2 receptors that mediate an inhibitory effect on LC firing activity. It is not clear whether these 5-HT_2 receptors controlling LC NA firing are of the 5-HT_{2A} or 5-HT_{2C} subtype and whether these receptors are mediating a tonic effect on LC activity as with the 5-HT_{1A} receptors. Also, whether these SSRI-induced effects are mediated through adaptations on the 5-HT system, and due to their projections to the LC, would subsequently alter NA neuron firing had to be assessed. These aspects were experimentally evaluated in this next chapter.

This article entitled "Functional and pharmacological characterization of the modulatory role of serotonin on the firing activity of locus coeruleus norepinephrine neurons" by myself and Pierre Blier was submitted for publication in Brain Research. The results in this manuscript allowed me to obtain the American Psychiatry Association Junior Investigator Award, 2001; to present this data at the annual meeting that was held in New Orleans.

FUNCTIONAL AND PHARMACOLOGICAL CHARACTERIZATION OF THE MODULATORY ROLE OF SEROTONIN ON THE FIRING ACTIVITY OF LOCUS COERULEUS NOREPINEPHRINE NEURONS

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Abstract

Previous studies, using *in vivo* extracellular unitary recordings in anaesthetized rats, has shown that the selective 5-HT_{1A} receptor antagonist WAY 100,635 suppressed the firing rate of locus coeruleus (LC) norepinephrine (NE) neurons and that this effect was abolished in rats by lesioning 5-HT neurons. In the present experiments, the selective 5-HT_{2A} receptor antagonist MDL 100,907, while having no effect on the spontaneous firing activity of LC neurons in controls, was able to restore NE neuronal discharges following the injection of WAY 100,635. The 5-HT_{1A} receptor agonist 8-OH-DPAT enhanced the firing activity of NE neurons, and this action was entirely dependent on intact 5-HT neurons, unlike the inhibitory effect of the 5-HT_2 receptor agonist DOI. Taken together, these data indicate that 5-HT_{2A} but not 5-HT_{1A} receptors controlling LC firing activity are postsynaptic to 5-HT neurons. Prolonged, but not subacute, administration of selective 5-HT reuptake inhibitors (SSRIs) produces a decrease in the spontaneous firing activity of LC NE neurons. MDL 100,907 partially reversed this suppressed firing activity of LC neurons in SSRI-treated rats. Although the α_2-adrenoceptor antagonist idazoxan also enhanced the firing activity of NE neurons in paroxetine-treated rats, this increase was similar to that obtained in controls. In conclusion, prolonged SSRI treatment enhances a tonic inhibitory influence by 5-HT on LC neurons through postsynaptic 5-HT_{2A} receptors that are not located on NE neurons. A speculative neuronal circuitry accounting for these phenomena on LC NE activity is proposed.

Theme: Neurotransmitters, Modulators, Transporters, and Receptors

Topic: Interactions between neurotransmitters

Keywords: 5-HT_{1A} receptors; 5-HT_{2A} receptors; α_2-adrenoceptors; antidepressants; anxiety disorders; firing activity

Introduction

It is well established that norepinephrine (NE) neurons modulate the serotonin (5-HT) system. In the brainstem, dorsal raphe (DR) 5-HT neurons receive ascending NE neuron afferents originating from the locus coeruleus (LC), a nucleus almost exclusively responsible for the NE innervation of the forebrain [4, 1, 37, 47]. Interactions between LC-DR impart a significant NE influence on the 5-HT system [47, 32] and evidence has recently accumulated for the inverse relationship between these two nuclei as well, for review see [31] and [48]. For instance, Kaehler et al., [35] reported that descending projections from the DR to the LC account for at least 50% of the 5-HT innervation of this nucleus. These reciprocal monoaminergic interactions have been linked to the efficacy of antidepressant drugs in anxiety and affective disorders, in which treatment, but not necessarily etiology, of these disorders relies on altered NE and 5-HT transmission. Interestingly, not all antidepressant drugs induce these neurochemical changes via the same mechanisms and agents that selectively target only one of these systems almost invariably produce alterations in both monoaminergic systems after chronic administration [9].

The selective serotonin reuptake inhibitors (SSRIs) are now considered as a first line therapeutic approach in the treatment of affective and many anxiety disorders. However, as with all antidepressants, they require an administration of at least two weeks before a clear beneficial effect can manifest itself. Long-term, but not acute or short-term SSRI administration increases the net output of 5-HT neurons, via the desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors in the presence of 5-HT reuptake blockade, and induces an attenuation in the spontaneous firing activity of LC NE

neurons [6, 54, 55] with a time-course that correlates with their anxiolytic and antidepressant responses (see [53] for review).

Various 5-HT receptor subtypes, especially the 5-HT_{1A} and 5-HT_{2A} receptors, are believed to be implicated in the antidepressant and anti-panic effects of long-term SSRI treatment. Activation of these two receptor subtypes, which are present in the LC [39, 57], alters NE neuron firing, however, in opposite directions. On the one hand, the enhancement of the firing rate of LC neurons produced by the intravenous administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT is abolished in rats treated with antidepressants which selectively inhibit either 5-HT or NE transporter reuptake [50, 51, 55]. On the other hand, the attenuation of the firing rate of LC neurons induced by the 5-HT_2 receptor agonist DOI is decreased in long-term SSRI-treated rats, but unaltered by a NE reuptake blocker [55]. Thus, the therapeutic effect of drugs selective for the 5-HT system, like SSRIs, could be mediated in part by a modification of the efficacy of 5-HT transmission to the LC via the desensitization of 5-HT_{1A} and 5-HT_2 receptors, but this possibility needed to be better documented.

In a first series of experiments, selective lesions of 5-HT neurons were performed to further delineate whether 5-HT neurons are necessary to mediate the 5-HT_{1A} and 5-HT_{2A} receptor effects that modulate NE neuron firing. In a second series of experiments, the effects of the selective 5-HT_{2A} and α_2-adrenoceptor antagonists MDL 100,907 and idazoxan, respectively, were assessed on the firing activity of LC NE neurons in controls and 21-day paroxetine treated rats. The latter study was performed to ascertain whether enhanced 5-HT availability and 5-HT receptor activation/alterations

in the LC from prolonged SSRI administration [33, 54] may be mediating the attenuation of NE neuron activity by overactivating inhibitory 5-HT_{2A} receptors [52]. Given the reciprocal interactions between these two monoaminergic systems, it also had to be ensured that the attenuation on NE activity from sustained SSRI administration was truly a 5-HT mediated event and not to another receptor alteration. Thus, the response to idazoxan on LC firing activity was also evaluated in control and 21-day paroxetine treated rats.

Materials and Methods

Animals

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water ad libitum). Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instuments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, rats were inserted with a catheter in a lateral tail vein for systemic injection of drugs.

Sustained Treatment

For the sustained treatment regimens, rats were anaesthetized with halothane containing a 2 to 1 O_2/N_2O mixture for subcutaneous implantation of osmotic minipumps (ALZA, Palo Alto, CA). Rats were treated with paroxetine (10 mg/kg/day) or saline for 21 days delivered by osmotic minipumps. These rats were tested with the minipumps in place in order to mimic the clinical condition whereby patients present an antidepressant response while taking their medication. Lesions of 5-HT neurons were performed on separate group of rats that received, under chloral hydrate anesthesia (400 mg/kg, i.p.), intracerebroventricular (i.c.v.) injections of 5,7-dihydroxytryptamine (5,7-DHT; 200 μg of free base in 20 μl of 0.9% NaCl and 0.1% ascorbic acid) 1 hour after the injection of

desipramine (25 mg/kg, i.v.) to protect NE neurons from the neurotoxic action of 5,7-DHT. This protocol has previously been demonstrated to effectively decrease brain 5-HT levels [19]. A group of rats acting as controls for this experimental group underwent the same procedures but the solution injected i.c.v. only contained 0.9% saline. These rats were tested 10 days following the i.c.v. injection.

Electrophysiological experiments

Extracellular unitary recordings of NE neurons were conducted with single-barrelled glass micropipettes preloaded with fiberglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1-3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for NE neurons recordings. Bleeding from disruption of the sagittal sinus was immediately stopped using bone wax. NE neurons were recorded with micropipettes lowered at -0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active NE neurons of the LC were identified using the following criteria: regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. Furthermore, by first locating the mesencephalic fifth motor nucleus neurons that respond to lower jaw depression, moving medially to record LC NE neurons provided additional indication for the validity of the recordings. NE neurons were recorded for at least 1 min to establish basal firing rate.

The selective 5-HT_{2A} and α_2-adrenoceptor antagonists MDL 100,907 (200 μg/kg, i.v.) and idazoxan (1 mg/kg, i.v.), respectively, were injected in control and 21-day paroxetine treated rats while recording the spontaneous firing activity of the first NE neuron encountered before and after injection. Dose-response curves for the alteration of LC neuron firing activity were obtained for systemic (i.v.) administration of MDL 100,907 (200 μg/kg) after a pre-injection of the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg) in untreated rats. In 5,7-DHT and control treated rats, dose-response effects to the preferential 5-HT_{2A} receptor agonist DOI [3, 42, 58, 50] and the 5-HT_{1A} receptor agonist 8-OH-DPAT on NE neuron firing was assessed. Changes in the firing activity are expressed as percentages of baseline firing rate and were measured after systemic drug administration. Dose-response curves were constructed for only one dose of 8-OH-DPAT and DOI in each rat. However, in experiments where WAY 100,635 and MDL 100,907 were systemically administered, only one dose of MDL 100,907 preceded by the WAY 100,635 injection in each rat was used to generate an effective dose 50 (ED_{50}). Similarly, only one dose of DOI following an MDL 100,907 pre-injection was used to generate an ED_{50}.

Drugs

The following drugs were used: MDL 100,907 from Marion Merrell Dow Inc. (Cincinnati, OH, U.S.A.); 8-OH-DPAT, WAY 100,635, DOI, clonidine, ritanserin, desipramine and idazoxan from RBI (Natick, MA, U.S.A.). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water while ritanserin was dissolved dropwise by acetic acid then titrated with distilled water to the appropriate concentration.

Statistical comparisons

All results were expressed as mean (± S.E.M.) of single neuron values. Statistical comparisons between the difference in NE neuron firing activity before and after intravenous drug administration were carried out using a paired *t*-test. Statistical comparisons of values obtained in treated and control rats were carried out using either one-way or two-way analysis of variance. All post-hoc pairwise multiple comparisons were done by the Student-Newman-Keuls method to assess the differences between controls and treated groups. Correlational coefficients (*r* values) for the dose-response relationship observed in the LC were calculated using simple linear regression analysis. The SEM for the ED_{50} values were calculated using regression analysis, with the Y value of 50 used as the regressor. Difference between the two regressions were assessed by comparing their ED_{50} values using the confidence intervals method. The Student's *t* distribution was used to determine the 95% confidence limit, as well as for

assessing the differences reported in mean NE neuron firing activity between the control and treated groups (table 1).

Results

Effect of MDL 100,907 on WAY 100,635-mediated attenuation of NE neuron firing activity

Systemic injection of the selective 5-HT_{1A} receptor antagonist WAY 100,635 decreases the firing rate of NE neurons which can be prevented by a previous administration of drugs with 5-HT_{2A} antagonistic properties, but that were not selective for the latter receptor [31]. Given that MDL 100,907 is currently the only selective 5-HT_{2A} receptor antagonist available, it was deemed mandatory to assess whether this compound could reverse, rather than prevent, the effect of WAY 100,635 on LC firing activity. In the present study, acute injection of WAY 100,635 decreased the firing activity of NE neurons (*ca.*, 91%), as previously reported (figure 1A; 1B). This inhibitory effect of WAY 100,635 was reversed to the initial basal discharge values by a subsequent injection of the selective 5-HT_{2A} receptor antagonist MDL 100,907 (figure 1B), thus providing evidence of the latter receptor mediating these effects.

Effect of 8-OH-DPAT and DOI on NE neuron firing activity in 5,7-DHT treated rats

The 5-HT_{1A} receptor agonist 8-OH-DPAT enhances the firing activity of LC NE neurons in a dose-dependent manner (figure 2A)[45, 55]. The injection of the selective 5-HT_{1A} receptor antagonist WAY 100,635 shuts down firing activity following the injection of 8-OH-DPAT, an example of which is provided in figure 2A. This figure also highlights that a final injection of MDL 100,907 reversed the inhibitory effects of WAY 100,635 and returned NE firing activity back to basal values. The preferential 5-HT_{2A}

receptor agonist DOI suppresses the firing activity of NE neurons [17, 55] and a following injection of the 5-$HT_{2A/2C}$ receptor antagonist ritanserin counteracted these effects (figure 3A). 5,7-DHT is a neurotoxin that destroys 5-HT neurons [19] as well as producing an increase in LC NE neuronal firing rate [31]. To assess whether the effects of the aforementioned 5-HT agonists on NE neuron firing are mediated via 5-HT neurons projecting to the LC, lesions with 5,7-DHT were performed. Consistent with previous findings [31], the mean spontaneous firing activity of NE neurons in 5,7-DHT treated rats was increased as compared to controls (table 1). Dose-response curves for systemic 8-OH-DPAT and DOI administration were obtained in control and 5,7-DHT treated rats (figure 4). The responsiveness of NE neurons to 8-OH-DPAT in 5,7-DHT treated rats was abolished over wide a range of doses, even at doses two times higher than the maximal excitatory dose obtained in the control group (figures 2A, 4A). To ascertain the integrity of the α_2-adrenoceptors on NE neurons following the lack of 8-OH-DPAT response observed in 5,7-DHT treated rats, subsequent injections of the α_2-adrenoceptor agonist clonidine suppressed the firing activity of NE neurons, being reversed by the α_2-adrenoceptor antagonist idazoxan (n = 4; figure 2B). In fact, the injection of idazoxan (1 mg/kg; i.v.) following 8-OH-DPAT and clonidine in treated rats increased NE neuron firing to the same extent as that observed in control rats when idazoxan was the first drug injected (p = 0.844; figure 7A and 8).

The response to injections of DOI in 5,7-DHT treated rats was blunted (ED_{50} = 68 μg/kg) as compared to controls (ED_{50} = 30 μg/kg; figure 4B). The dose-response curve for DOI in 5,7-DHT treated rats was thus shifted approximately 2-fold to the right (figure

4B). As in controls, an injection of ritanserin (n = 2) effectively reversed the inhibitory effects of DOI on NE neuron firing activity in 5,7-DHT treated rats (figure 3B).

Effect of MDL 100,907 on the firing activity of NE neurons in control and long-term paroxetine-treated rats

Acute injections of the selective 5-HT_{2A} receptor antagonist MDL 100,907 did not alter the firing activity of NE neurons in control rats, but blocked the inhibitory effects of a subsequent injection of the preferential 5-HT_{2A} receptor agonist DOI (figures 4B, 6). In fact, MDL 100,907 was able to antagonize the suppressant effects of DOI on NE neuron firing activity at doses as high as 120 μg/kg of the agonist. After observing the lack of effect of DOI on NE neuron firing activity, clonidine was still able to decrease NE neuron firing activity while idazoxan reversed this effect (n = 2), an example of which is provided in figure 5A. The suppressant effects of long-term paroxetine treatment on the basal firing rate of the NE neurons was significant as compared to that of controls (table 1; p = 0.01). The enhancing effect of MDL 100,907 (*ca.*, 25%) on the firing activity of attenuated NE neurons in long-term paroxetine treated rats was statistically significant (p = 0.003), but this antagonist did not modify NE neuron basal firing rate at all in controls (figure 6). Interestingly, following the injection of MDL 100,907 in rats treated with paroxetine for 21-days, the injection of idazoxan further increased the firing activity of LC neurons (n = 2 rats; figure 5B).

Effect of idazoxan on the firing activity of NE neurons in control and long-term paroxetine treated rats

The blockade of α_2-adrenoceptors, which exerts a negative feedback influence on NE neuron firing, has been shown to disinhibit these neurons resulting in an increase in firing rate in naive animals [26]. Indeed, the selective α_2-adrenoceptor antagonist idazoxan increased the firing activity of NE neurons, an example of which is provided in figure 7A. The enhancing effect of idazoxan on NE neuron firing activity in control (*ca.* 54%) and 21-day paroxetine treated rats (*ca.* 62%) were significant ($p = 0.004$ and $p = 0.007$, respectively), but not statistically different from each other ($p = 0.673$; figure 8). Following the acute administration of idazoxan, which increased the attenuated NE neuron firing activity in 21-day paroxetine treated rats, a subsequent injection of MDL 100,907 ($n = 3$) was still able to enhance further the firing rate (figure 7B). When the basal firing rates of NE neurons in control and paroxetine treated rats that later received either MDL 100,907 or idazoxan, were merged within their respective groups, a statistically significant decrease was observed (table 1).

Discussion

The results of the present study first indicate the inhibitory effects of the selective 5-HT_{1A} receptor antagonist WAY 100,635 on LC NE neuron firing is mediated through an augmented 5-HT transmission at 5-HT_{2A} receptors. This is based on the premise that the suppressant effect of WAY 100,635 on NE neuron firing is dependent on intact 5-HT neurons, as their destruction prevents its inhibitory action [33]. Then, acute administration of the selective 5-HT_{2A} receptor antagonist MDL 100,907 by itself did not alter the firing activity of NE neurons (figure 6), but reversed the inhibitory effect of a prior injection of WAY 100,635 (figure 1). This finding is consistent with prior results whereby non-selective 5-HT_2 receptor antagonists, as well as the 5-HT_{2A} receptor antagonist spiperone, prevented the suppressant effect of WAY 100,635 on NE neuron firing [31]. Thus, administration of WAY 100,635 most likely increases 5-HT transmission [20, 25] and decreases NE firing activity by an overactivation of excitatory 5-HT_{2A} receptors on inhibitory neurons projecting to the LC (figure 9) because neurons in this nucleus do not possess mRNA for 5-HT_{2A} receptors [44], and these receptors are not located in the raphe nuclei [24, 18].

The spontaneous firing activity of LC NE neurons in 5,7-DHT treated rats was significantly increased as compared to that of their respective controls recorded in the present study (table 1). This degree of enhancement is fully consistent with that previously reported [31], and therefore with the putative inhibitory role of 5-HT on the firing activity of these neurons. As expected, and contrary to results obtained with the 5-HT_{1A} antagonist WAY 100,635, activation of 5-HT_{1A} receptors using the 5-HT_{1A} agonist 8-OH-DPAT produced an excitatory effect on NE neuron firing activity which

was also dependent on intact 5-HT neurons (figure 2; 4A). This lack of effect of 8-OH-DPAT on LC firing activity in 5,7-DHT lesioned rats indicates that these receptors are not on LC neurons. Interestingly, these 5-HT_{1A} receptors desensitize after long-term administration with antidepressant drugs that selectively block either 5-HT or NE transporters [50, 51, 55]. Given that 5-HT neurons exert an inhibitory role on LC activity, and that 5-HT_{1A} autoreceptors located on the cell body of 5-HT neurons exert a negative feedback control on the firing activity of 5-HT neurons and ultimately the impulse-dependent release of 5-HT (figure 9; for review see [12]), these somatodendritic 5-HT_{1A} autoreceptors appear as candidates for mediating the effect of 8-OH-DPAT on NE neuron firing. In addition, it is widely accepted that this receptor population becomes subsensitive after long-term SSRI treatment and is important to produce a net increase in 5-HT transmission in projection structures during this time period [11, 34, 36, 49]. However, there is considerable evidence indicating that the excitatory effects of 8-OH-DPAT on NE neuron firing is not mediated by somatodentritic 5-HT_{1A} receptors, but rather by the activation of a different population of 5-HT_{1A} receptors. First, a dose of 5 μg/kg of 8-OH-DPAT completely shuts off DR 5-HT neuronal activity but leaves unaffected that of NE neurons (figure 4A). Second, acute administration of WAY 100,635 does not consistently alter 5-HT neuron firing activity by itself but shuts down that of NE neurons in the LC (figure 1A)[31]. Third, the selective NE reuptake blockers desipramine and reboxetine, which do not alter the sensitivity of somatodendritic 5-HT_{1A} autoreceptors [10, 51], desensitize 5-HT_{1A} receptors controlling LC firing rate [55, 51]. Nevertheless, due to the disappearance of the effect of 8-OH-

DPAT and WAY 100,635 on LC firing in 5,7-DHT treated rats (figure 2B and [32]), it can be concluded that the latter action of these two 5-HT_{1A} receptor ligands depends on the integrity of 5-HT neurons.

The location of the 5-HT_{1A} receptors, which control NE neuron firing, is probably not on the cell body of NE neurons, because neurons in the LC lack mRNA for 5-HT_{1A} receptors [45]. Considerable evidence has accumulated for a glutamate regulation of 5-HT release [41, 5]. In fact, Van Bockstaele [56] recently used immunogold-silver labeling of an antibody that recognizes kainate receptors and peroxidase labeling for 5-HT neurons. These results showed that 5-HT terminals were labeled with kainate receptor immunoreactivity in the LC. Surprisingly however, 5-HT attenuates the activation of LC neurons by exogenous glutamate applications [16, 2, 14]. This dampened response of LC neurons was initially deemed to act through 5-HT_{1A} receptors on NE neurons as the iontophoretic application of 8-OH-DPAT produced the same effect [13]. Another study did not, however, confirm the latter observation [30], possibly due to methodological differences. Indeed, the use of high ejection currents in the former study certainly lead to a large outflow of chloride ions through the barrel used to counterbalance them. Such a large ionic flow may alter on its own neuronal responsiveness. The possibility that 8-OH-DPAT could alter the excitatory action of glutamate on NE neuronal firing appears somewhat puzzling given the absence of mRNA for 5-HT_{1A} receptors in the LC proper [44]. On the other hand, Weissman-Nanopoulos et al., [57] reported that following a 5,7-DHT lesion in rats, 5-HT_{1A} binding density in the LC was unchanged. Moreover, glutamate release from terminals in the LC is attenuated by perfusion of 8-OH-DPAT of LC slices [7]. Taken together, these

data suggests the presence of inhibitory 5-HT_{1A} receptors on a glutamate neuron projecting to the LC. This interpretation is consistent with that proposed by Singewald and Phillippu [48] whereby the impact of 5-HT_{1A} ligands on LC activity is dependent on intact 5-HT neurons (figure 4A and [31]).

One of the major afferent glutamatergic inputs to the LC is from neurons in the nucleus paragigantocellularis. In light of their importance in mediating sensory information to the LC as well as controlling NE neuron firing [21], 5-HT_{1A} receptors which impart an influence on LC firing may be located on neurons from the paragigantocellularis nucleus projecting to the LC. The activation of these 5-HT_{1A} receptors by systemic administration of 8-OH-DPAT would then decrease the amount of glutamate available to depolarize 5-HT terminals, thereby inducing an attenuated activation of excitatory 5-HT_{2A} receptors on GABA, finally resulting in an increase in NE neuron firing (figures 2A; 9). Consistent with this proposed mechanism, it was recently reported that microiontophoretic application of the glutamate antagonist kynurenate and bicuculline antagonize the modulatory effects of 8-OH-DPAT and WAY 100,635, respectively, on LC NE neurons firing [52].

The suppressant effect of the preferential 5-HT_{2A} receptor agonist DOI on NE neuron firing activity was only decreased in 5,7-DHT treated rats as evidenced by a small but still significant shift to the right of its dose-response curve (figure 4B), when compared to the excitatory effect of 8-OH-DPAT which was abolished (figure 4A). Thus, 5-HT_{2A} receptors mediating the inhibitory effects of DOI on NE neuron firing activity can be considered as being predominantly postsynaptic to 5-HT neurons, contrary to the 5-HT_{1A} receptors which control LC firing that are entirely dependent on

intact 5-HT neurons to exert their action. Also, it was previously shown that 5-HT depletion with the synthesis inhibitor p-chlorophenylalanine did not alter the inhibitory response of 5-HT_2 ligands on LC activity [29], but increased LC activity [46]. A high density of 5-HT_{2A} receptor labeling has been visualized in the rat and primate LC area using [^{3}H]MDL 100,907 [38, 39]. These 5-HT_{2A} receptors are, however, probably not located directly on NE neurons because cell bodies labeled for the presence of the 5-HT_{2A} receptor protein were not detected within this nucleus, although the pericoerulear area was labeled and the nucleus prepositius hypoglossi as well [23]. The latter structure is another major afferent to the LC and exerts an inhibitory role on LC firing through $GABA_A$ receptors [24]. In addition to the anatomical evidence demonstrating a lack of 5-HT_{2A} receptors on LC neurons, microiontophoretic application of DOI on LC neurons does not alter spontaneous or glutamate-induced NE neuron firing activity [30, 13]. It has previously been reported that the inhibitory effects of intravenously injected DOI on LC firing can be blocked with the microiontophoretic application of the $GABA_A$ receptor antagonist bicuculline on LC neurons [17], and systemic injection of MDL 100,907 [50]. Furthermore, the inhibitory effect of DOI on LC neuronal firing is abolished upon hypoglossal nucleus destruction [30], therefore emphasizing the importance of this structure. However, DOI injected directly into the hypoglossal nucleus does not alter NE neuron activity [30]. Interestingly, 5-HT neurons synapsing on axon terminals making contact with tyrosine hydroxylase labeled neurons in the LC accounted for the greatest proportion of 5-HT elements in the LC [56]. It is thus proposed that, on the basis of these prior results and those of the present study, an

excitatory 5-HT_{2A} receptor located on a hypoglossal GABA terminal projecting to the LC is primarily responsible for mediating the inhibitory effect of DOI on LC firing (figure 9).

MDL 100,907 was able to reverse the inhibitory effects of WAY 100,635 on NE neurons firing activity presumably by counteracting the enhanced activation of such 5-HT_{2A} receptors (figure 9). This served as the impetus to assess whether an increase in 5-HT_{2A} receptor activation resulting from enhanced 5-HT transmission in long-term SSRI-treated rats was contributing to the attenuation in LC firing activity observed after a 21-day treatment [54, 55]. Consistent with the latter effect of SSRIs, Freuo et al., [27] observed that blood flow was attenuated to the greatest extent attenuated by the same treatment in this nucleus. Administration of MDL 100,907 in paroxetine-treated rats did produce an increase in NE neuron firing activity, as compared to controls where MDL 100,907 was totally inactive (figure 6). Even though the response to DOI in long-term SSRI treated rats is decreased [55], antagonism of these 5-HT_{2A} receptors could still enhance the firing activity of NE neurons, and perhaps not exactly to the control level precisely because of their partial desensitization (figure 6). In analogy, the increased availability of 5-HT in mice lacking 5-HT transporters results in a 3-fold decrease in 5-HT neuron firing activity, despite a marked desensitization of 5-HT_{1A} autoreceptors. Nevertheless, administration of WAY 100,635 reverses the attenuated firing activity in these mice [28]. It is important to mention that the decrease in LC firing activity observed in long-term paroxetine [54] and citalopram treated rats [55] is not likely due to increased α_2-adrenoceptor activation because administration of the selective α_2-adrenoceptor antagonist idazoxan produced the same increase in LC firing activity as

compared to controls (figure 8). Furthermore, MDL 100,907 still increased the firing of LC neurons after the injection of idazoxan in paroxetine-treated rats (see figure 7B), thus supporting the possibility of enhanced 5-HT_{2A} receptor activation by long-term SSRI administration.

It appears that the desensitization of the 5-HT_{1A} receptors which control LC firing activity is common to all antidepressant drug classes tested thus far and may represent an important finding with respect to the treatment of certain anxiety disorders. Prolonged administration of SSRIs, which are effective in panic, generalized, and social anxiety disorders, desensitizes not only somatodendritic 5-HT_{1A} autoreceptors, but also 5-HT_{1A} receptors which normally augment LC firing, presumably through a decrease in GABA transmission. This is a possible mechanism that may explain, in part, the discrepancy between the rapid anxiolytic action of benzodiazepines, as a result of their facilitatory action on the $GABA_A$ receptor, and the delayed response obtained with SSRIs. Consistent with this possibility are reports of decreased GABA levels and/or $GABA_A$/benzodiazepine receptor binding in several brain areas of depressive and panic disorder patients [41,8].

In conclusion, the present study unveiled complex interactions between 5-HT and NE neurons. These also appear to involve other neurotransmitters, such as GABA and glutamate through which the modulatory effect of SSRIs on spontaneous LC neuronal firing would be exerted. Further experiments should help establish the precise localization of the various receptors involved in this complex circuitry.

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Legends to figures

Figure 1. (A) Integrated firing rate histogram of a LC NE neuron illustrating the effect of intravenous administration of the selective 5-HT_{1A} antagonist WAY 100,635 in a control rat, producing a decrease in the firing activity, and that of a subsequent injection of the selective 5-HT_{2A} antagonist MDL 100,907 reversing it. (B) Mean effects of systemic administration of WAY 100,635 and then MDL 100,907 on the firing activity of LC NE neurons. *$P < 0.001$ (Paired t-test) when compared to NE neuron basal firing activity of the before WAY 100,635 drug injections. † $P<0.001$ (Paired t-test) when compared to NE neuron basal firing activity before MDL 100,907 drug injections.

Figure 2. (A) Integrated firing rate histogram of a LC NE neuron illustrating the effects of intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT producing an increase in the firing activity, a subsequent injection of the selective 5-HT_{1A} antagonist WAY 100,635 attenuating the firing activity, and a final injection of the selective 5-HT_{2A} antagonist MDL 100,907 reversing the inhibitory action of WAY 100,635 in a control rat. (B) Integrated firing rate histogram of a LC NE neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with the selective 5-HT neurotoxin 5,7-DHT. Note that the subsequent intravenous injection of the α_2-adrenoceptor agonist clonidine was still able to attenuate NE neuron firing activity which was reversed by the α_2-adrenoceptor antagonist idazoxan.

Figure 3. (A) Integrated firing rate histogram of a LC NE neuron, recorded in a control rat illustrating the effects of intravenous administration of the preferential 5-HT_{2A} agonist DOI producing a decrease in the firing activity, and a subsequent injection of the 5-HT_2 antagonist ritanserin reversing the latter effect. (B) Integrated firing rate histogram of a LC NE neuron, recorded in a rat treated with the selective 5-HT neurotoxin 5,7-DHT illustrating the effect of intravenous administration of the preferential 5-HT_{2A} agonist DOI, and the subsequent injection of the 5-HT_2 antagonist ritanserin reversing the latter effect.

Figure 4. (A) Relationship between the degree of augmentation of LC NE firing activity and doses of 8-OH-DPAT administered intravenously in controls and rats treated with the 5-HT neurotoxin 5,7-DHT. (B) Relationship between the degree of suppression of LC NE firing activity and doses of DOI administered intravenously in controls, 5,7-DHT treated rats, and rats receiving a pre-injection of MDL 100,907. Only the initial response of a single NE neuron to the first dose of 8-OH-DPAT or DOI in each rat was used to construct the curves, with exception to rats that received a prior injection of MDL 100,907, DOI was the next drug injected. Outer lines represent the standard error of the regression lines. The shift to the right in the DOI dose- response curve in 5,7-DHT treated rats was significant as compared to the control. The 8-OH-DPAT and the DOI dose-response curves obtained in control rats were previously reported in [47], and used herein because these experimental series were performed concurrently.

Figure 5. (A) Integrated firing rate histogram of a LC NE neuron illustrating the lack of effect of the intravenous administration of the selective 5-HT_{2A} receptor antagonist MDL 100,907 on firing activity and a subsequent injection the preferential 5-HT_{2A} agonist DOI (see figure 3., for an example of the inhibitory action of DOI). Intravenous injection of the α_2-adrenoceptor agonist clonidine was still able to attenuate NE neuron firing activity and the α_2-adrenoceptor antagonist idazoxan reversed this effect. (B) Integrated firing rate histogram of a LC NE neuron showing a increase in firing activity to intravenous MDL 100,907 in a rat treated with paroxetine (10 mg kg^{-1} day^{-1}).

Figure 6. Effect of systemic administration of the selective 5-HT_{2A} receptor antagonist MDL 100,907 on the firing activity of LC NE neurons in control and paroxetine treated (10 mg kg^{-1} day) rats. † $P < 0.05$ (Student-Newman-Keuls method) when comparing LC basal firing activity in controls to paroxetine treated rats. * $P = 0.003$ (Paired t-test) when compared to NE neuron firing activity before MDL 100,907 drug injections. The number of neurons recorded is displayed in each box.

Figure 7. (A) Integrated firing rate histograms of LC NE neurons illustrating the excitatory effect of the intravenous administration of the selective α_2-adrenoceptor antagonist idazoxan recorded in a control rat. (B) Note that the subsequent injection of the selective 5-HT_{2A} antagonist MDL 100,907 in the rat treated with paroxetine produced a further enhancement of firing.

Figure 8. Effect of systemic administration of the α_2-adrenoceptor antagonist idazoxan (1 mg kg^{-1}) on the firing activity of LC NE neurons in control and paroxetine treated (10 mg kg^{-1} day) rats. *P = 0.004 and P = 0.007 (Paired t-test) when compared to the basal firing activity of the before idazoxan injection value in control and paroxetine treated rats, respectively. The number of neurons recorded is displayed in each box.

Figure 9. Speculative neuroanatomical and neurochemical bases for the interactions between 5-HT neurons and LC NE neurons. This diagram was prepared on the basis of the results obtained by several groups of investigators as well as data generated in our laboratory (see discussion).

Table 1. Spontaneous firing activity (Hz) of LC NE neurons in controls and pre-treated rats

	Basal firing activity of NE neurons	Range of Firing Activity	Number of Neurons †
Control	2.3 ± 0.2	0.9 - 3.6	22
5,7-DHT	3.2 ± 0.3*	2.1 - 5.6	10
Control	2.6 ± 0.2	1.6 - 3.9	12
Paroxetine	1.9 ± 0.2*	1.1 - 2.8	10

Basal values refer to firing rates prior to any acute drug challenge.

*$P < 0.05$ when comparing treatment groups to controls using Student's t-test.

† Each value corresponds to the firing activity of single LC NE neurons recorded in each rat before drug injection. These values were obtained from all the data points in the dose-response curves.

Figure I.

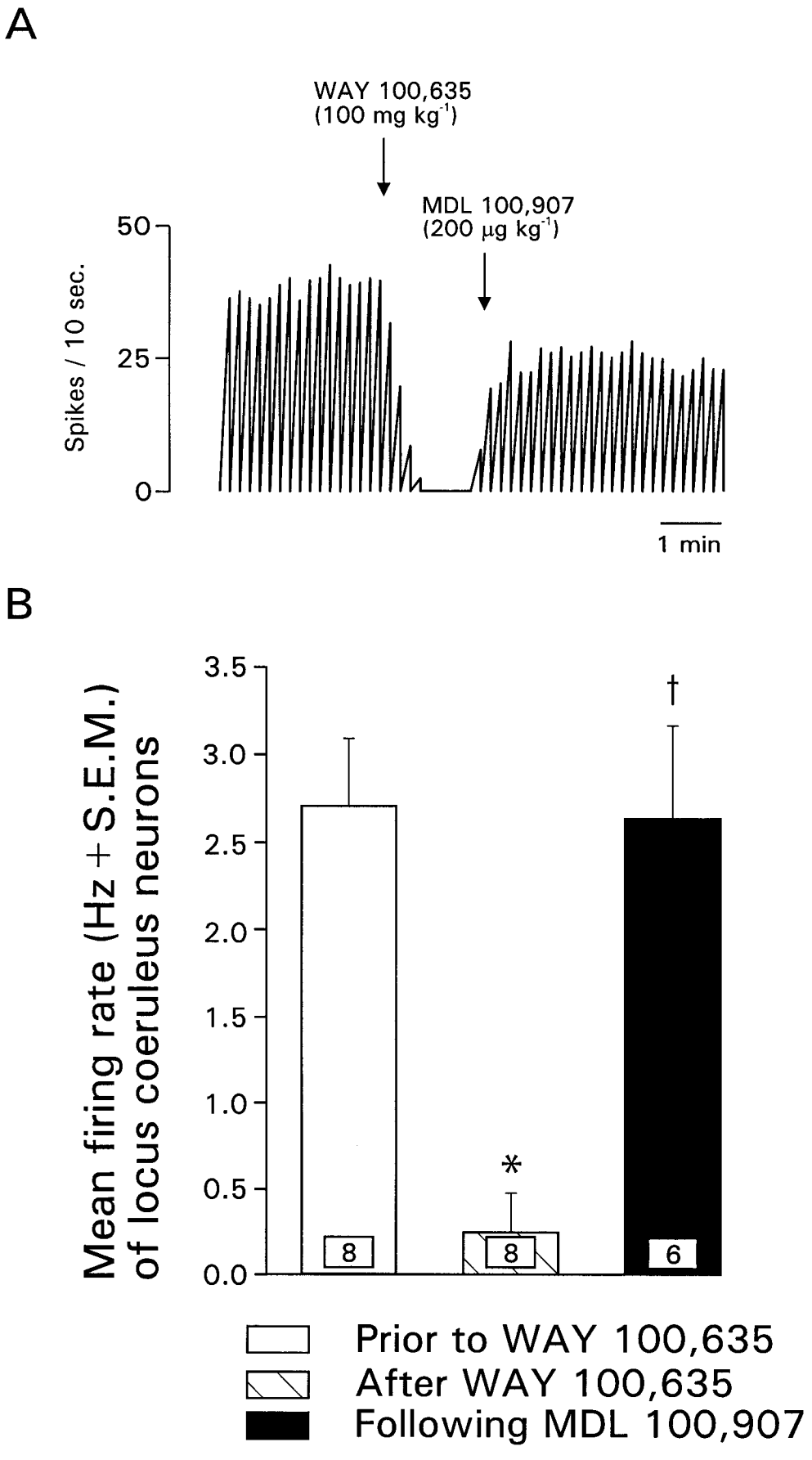

A
WAY 100,635
(100 mg kg-1)
MDL 100,907
(200 µg kg-1)
50
25
0
Spikes / 10 sec.
1 min
B
Mean firing rate (Hz + S.E.M.)
of locus coeruleus neurons
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
†
*
8
8
6
Prior to WAY 100,635
After WAY 100,635
Following MDL 100,907

Figure II.

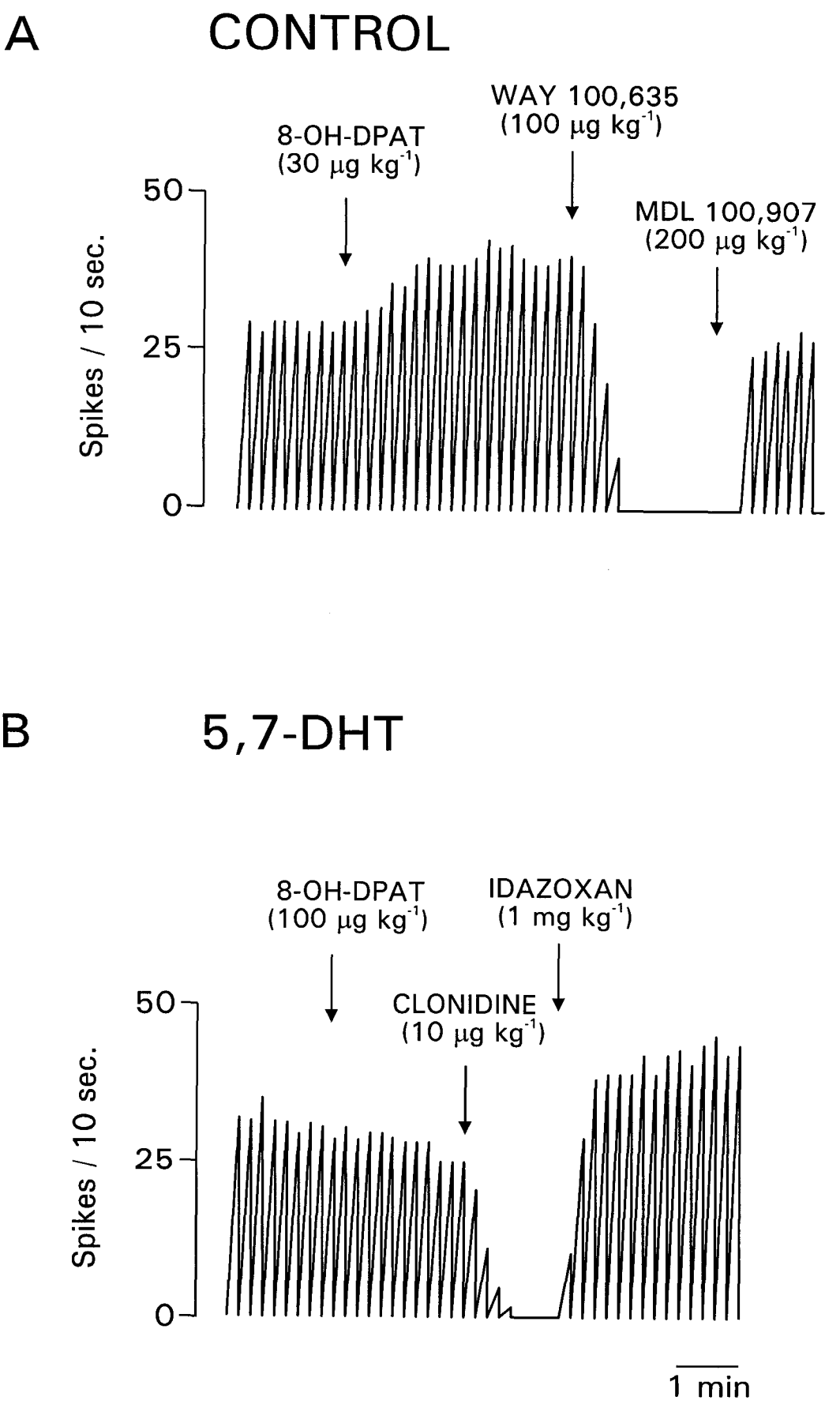

A
CONTROL
8-OH-DPAT
(30 μg kg-1)
WAY 100,635
(100 μg kg-1)
MDL 100,907
(200 μg kg-1)
Spikes / 10 sec.
50
25
0
B
5,7-DHT
8-OH-DPAT
(100 μg kg-1)
IDAZOXAN
(1 mg kg-1)
CLONIDINE
(10 μg kg-1)
Spikes / 10 sec.
50
25
0
1 min

Figure III.

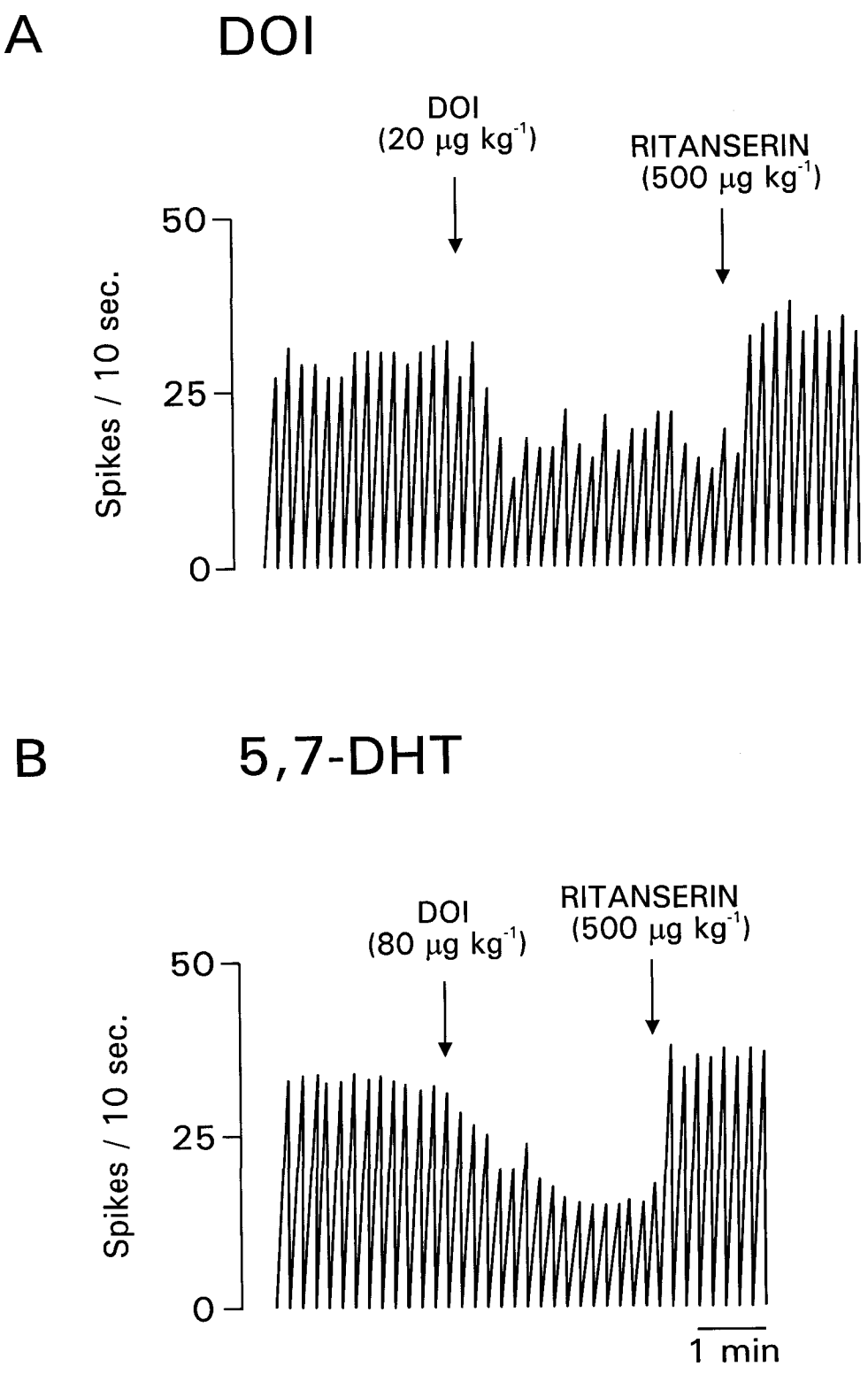
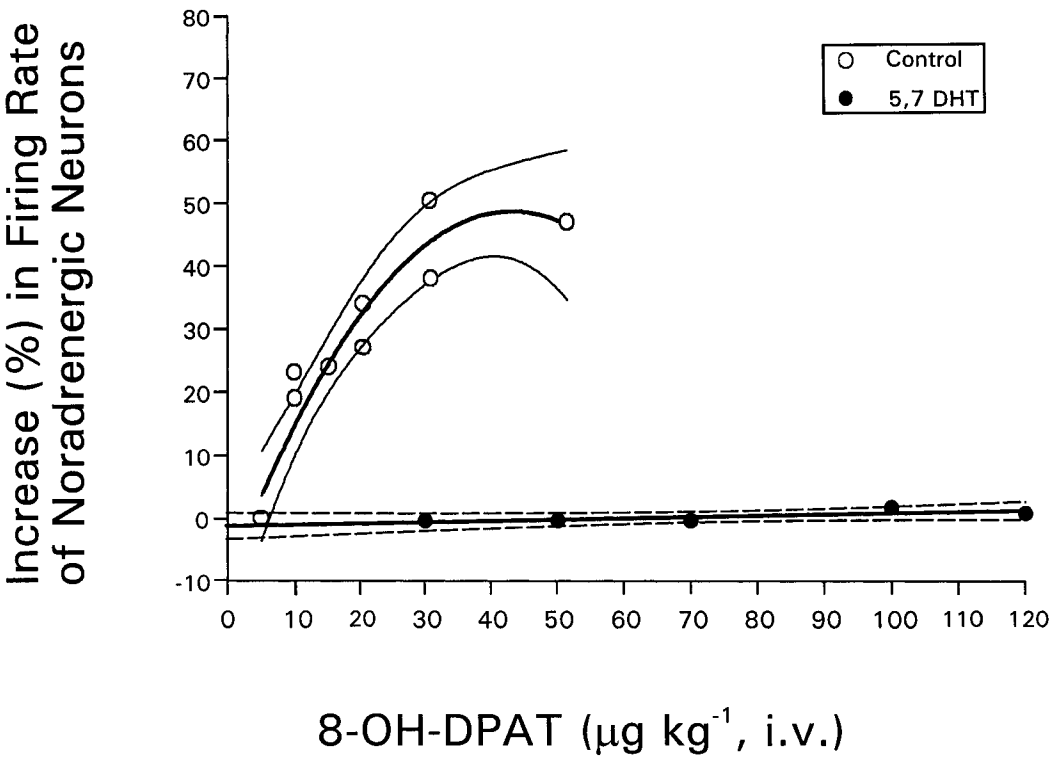

A
DOI
DOI
(20 μg kg-1)
RITANSERIN
(500 μg kg-1)
50
25
0
Spikes / 10 sec.
B
5,7-DHT
DOI
(80 μg kg-1)
RITANSERIN
(500 μg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure IV.

A


Increase (%) in Firing Rate of Noradrenergic Neurons
Control
5,7 DHT
80
70
60
50
40
30
20
10
0
-10
0 10 20 30 40 50 60 70 80 90 100 110 120
8-OH-DPAT (µg kg-1, i.v.)

B

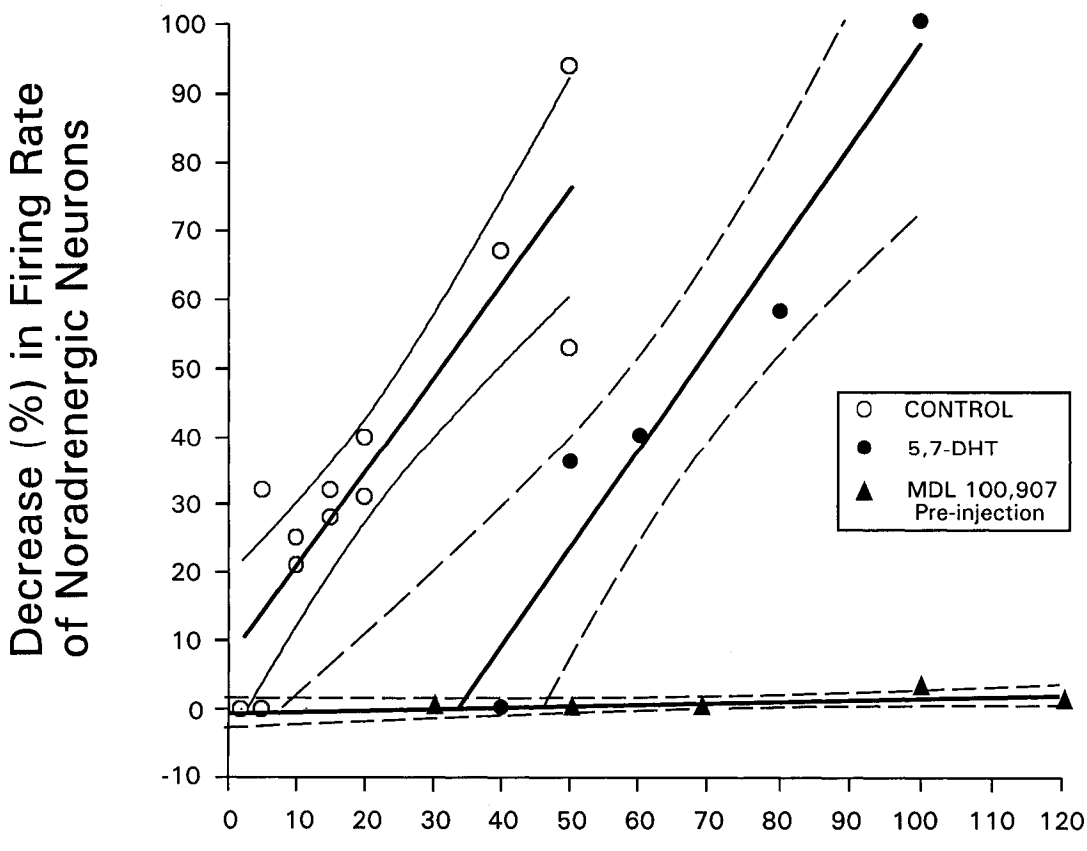

Decrease (%) in Firing Rate of Noradrenergic Neurons
CONTROL
5,7-DHT
MDL 100,907 Pre-injection
100
90
80
70
60
50
40
30
20
10
0
-10
0 10 20 30 40 50 60 70 80 90 100 110 120

Figure V.

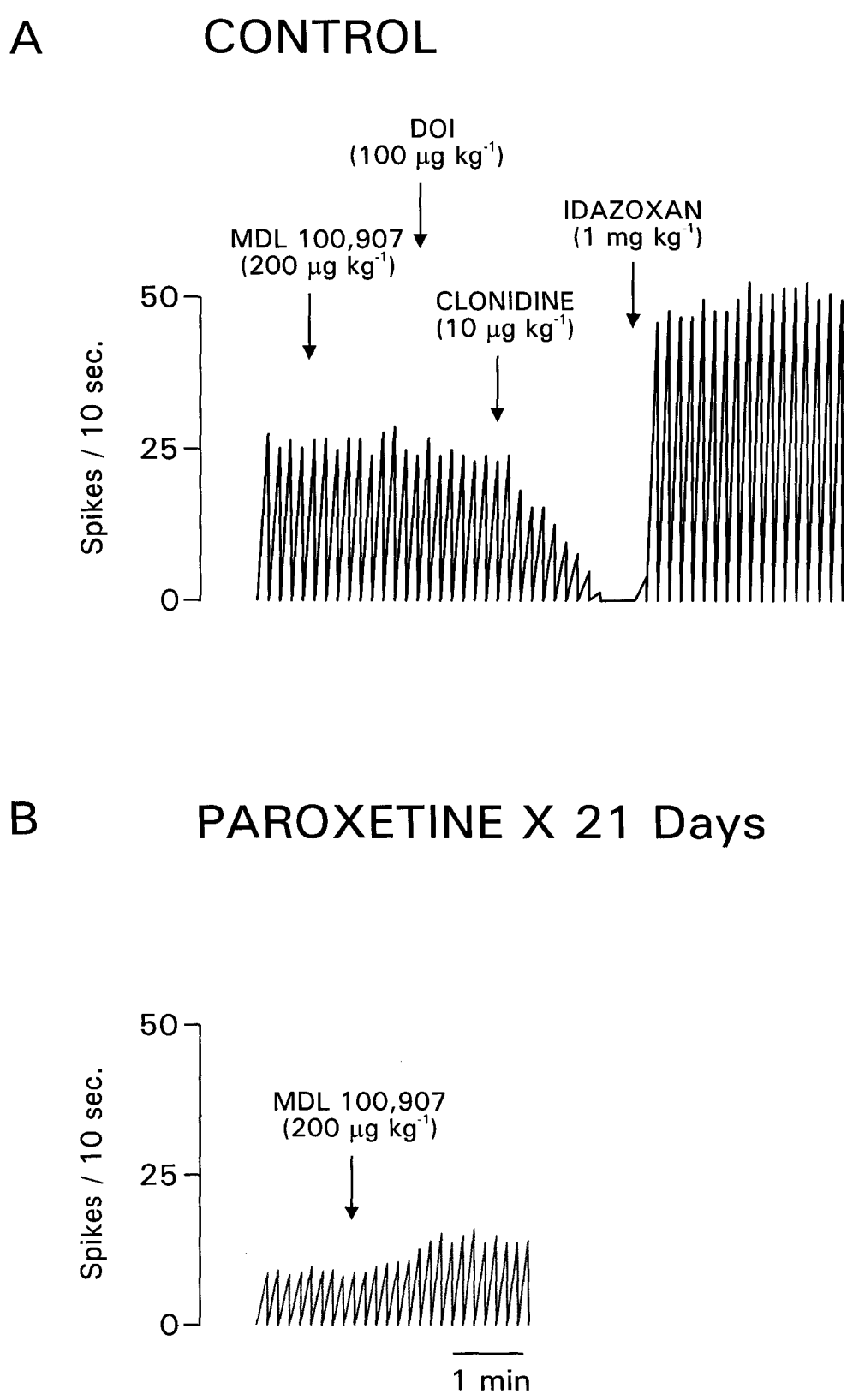

A
CONTROL
DOI
(100 μg kg-1)
MDL 100,907
(200 μg kg-1)
IDAZOXAN
(1 mg kg-1)
CLONIDINE
(10 μg kg-1)
50
25
0
Spikes / 10 sec.
B
PAROXETINE X 21 Days
50
25
0
Spikes / 10 sec.
MDL 100,907
(200 μg kg-1)
1 min

Figure VI.

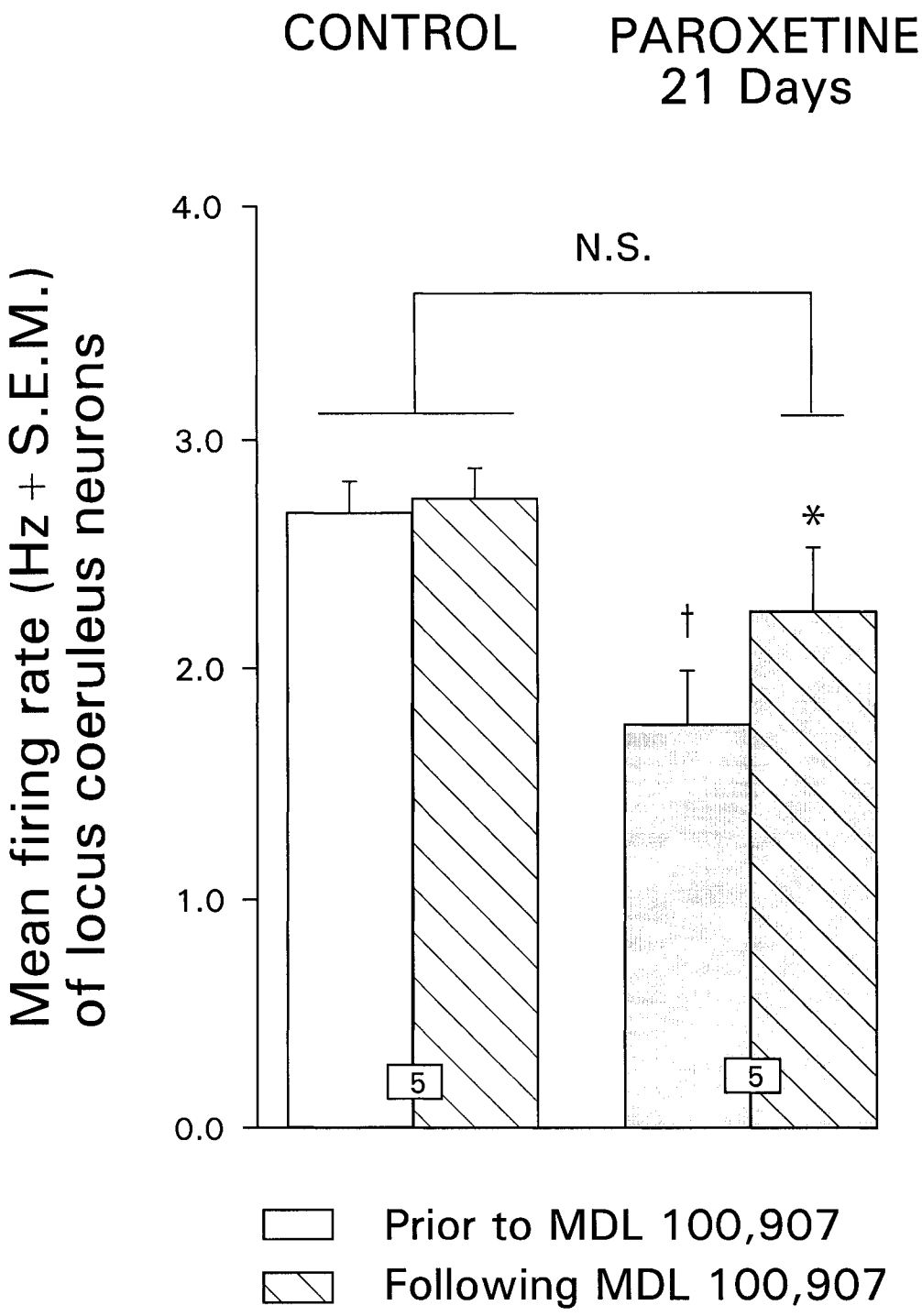

CONTROL
PAROXETINE
21 Days
N.S.
4.0
3.0
2.0
1.0
0.0
Mean firing rate (Hz + S.E.M.)
of locus coeruleus neurons
†
*
5
5
Prior to MDL 100,907
Following MDL 100,907

Figure VII.

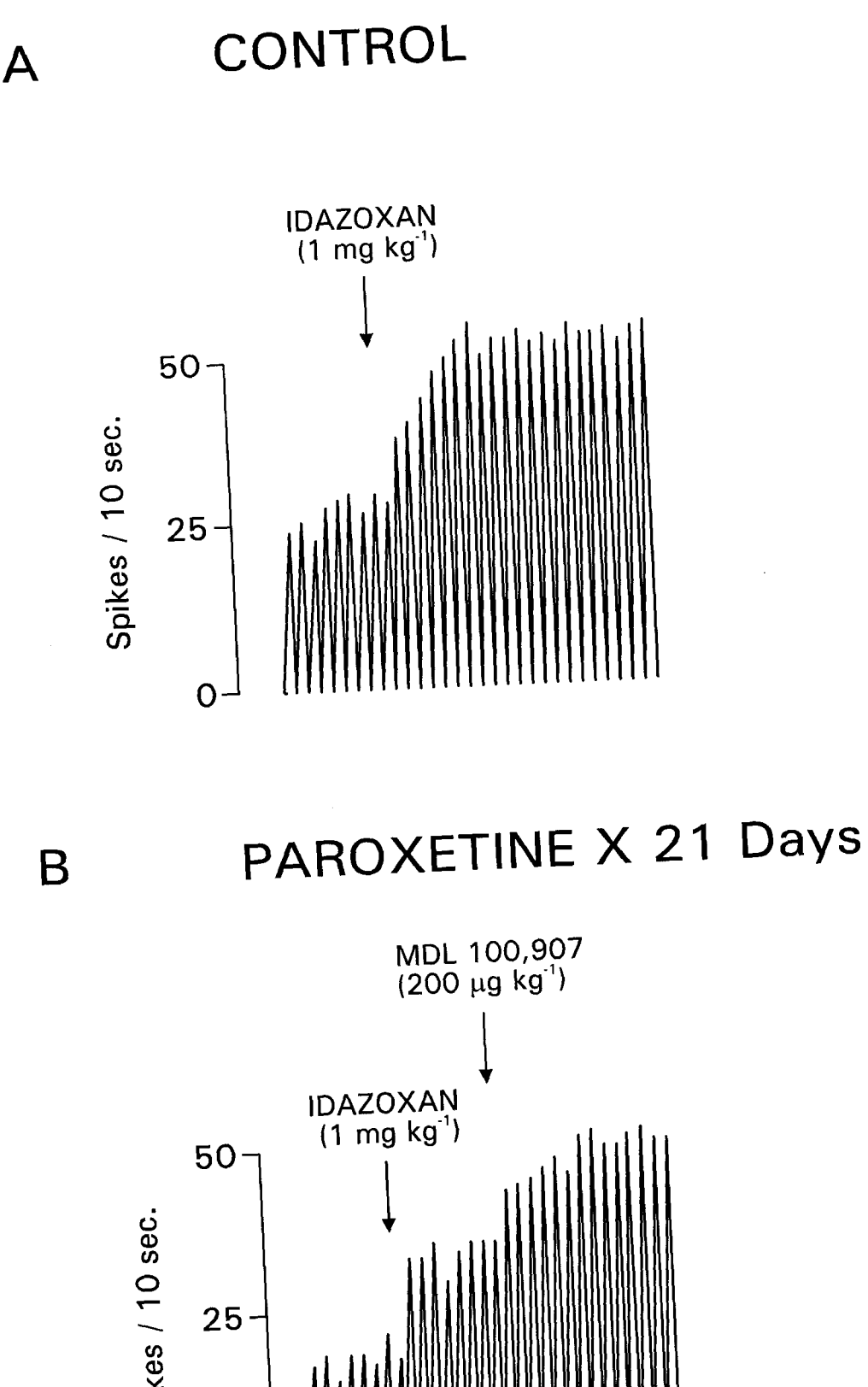

A
CONTROL
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
B
PAROXETINE X 21 Days
MDL 100,907
(200 µg kg-1)
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure VIII.

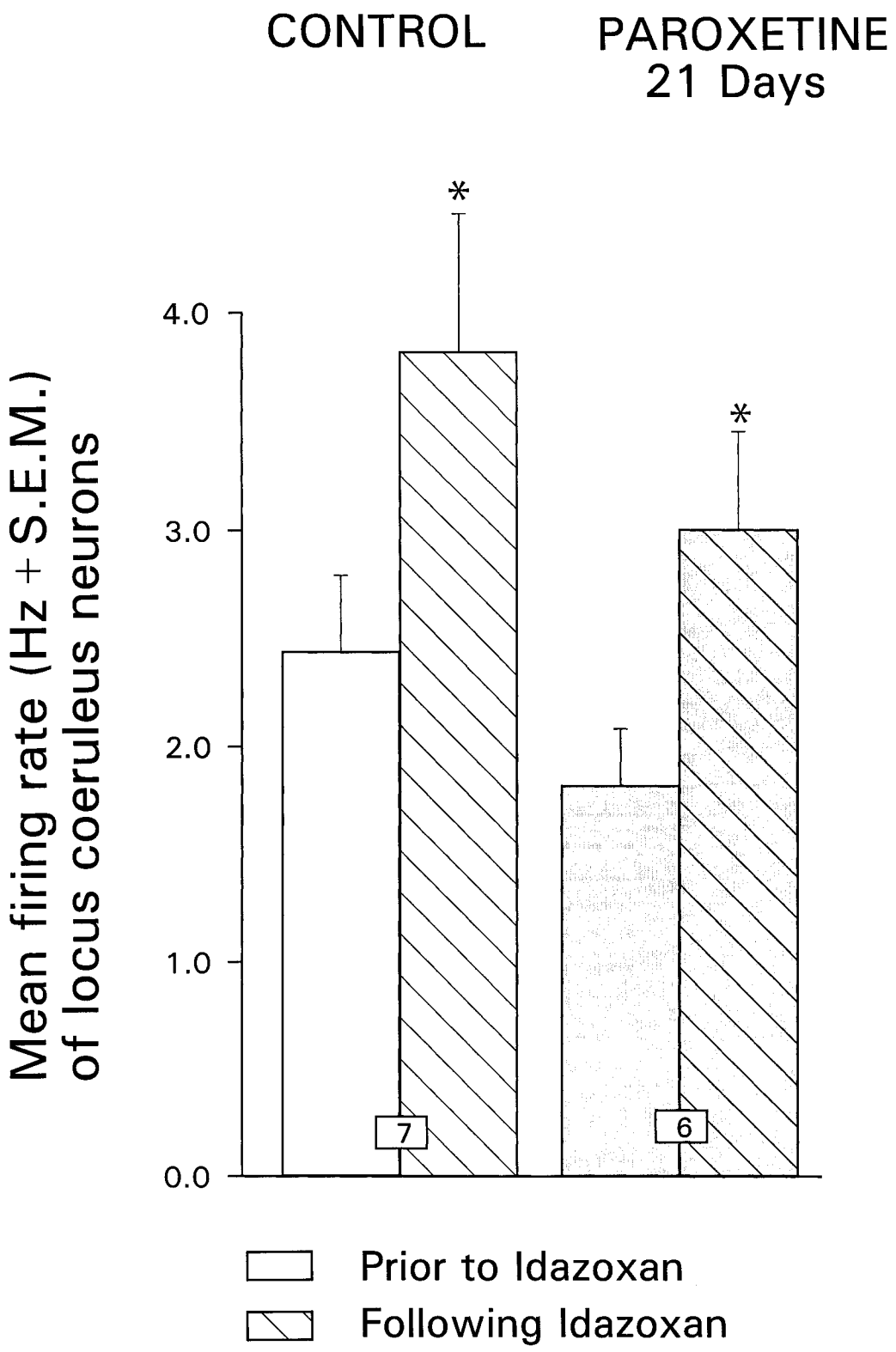
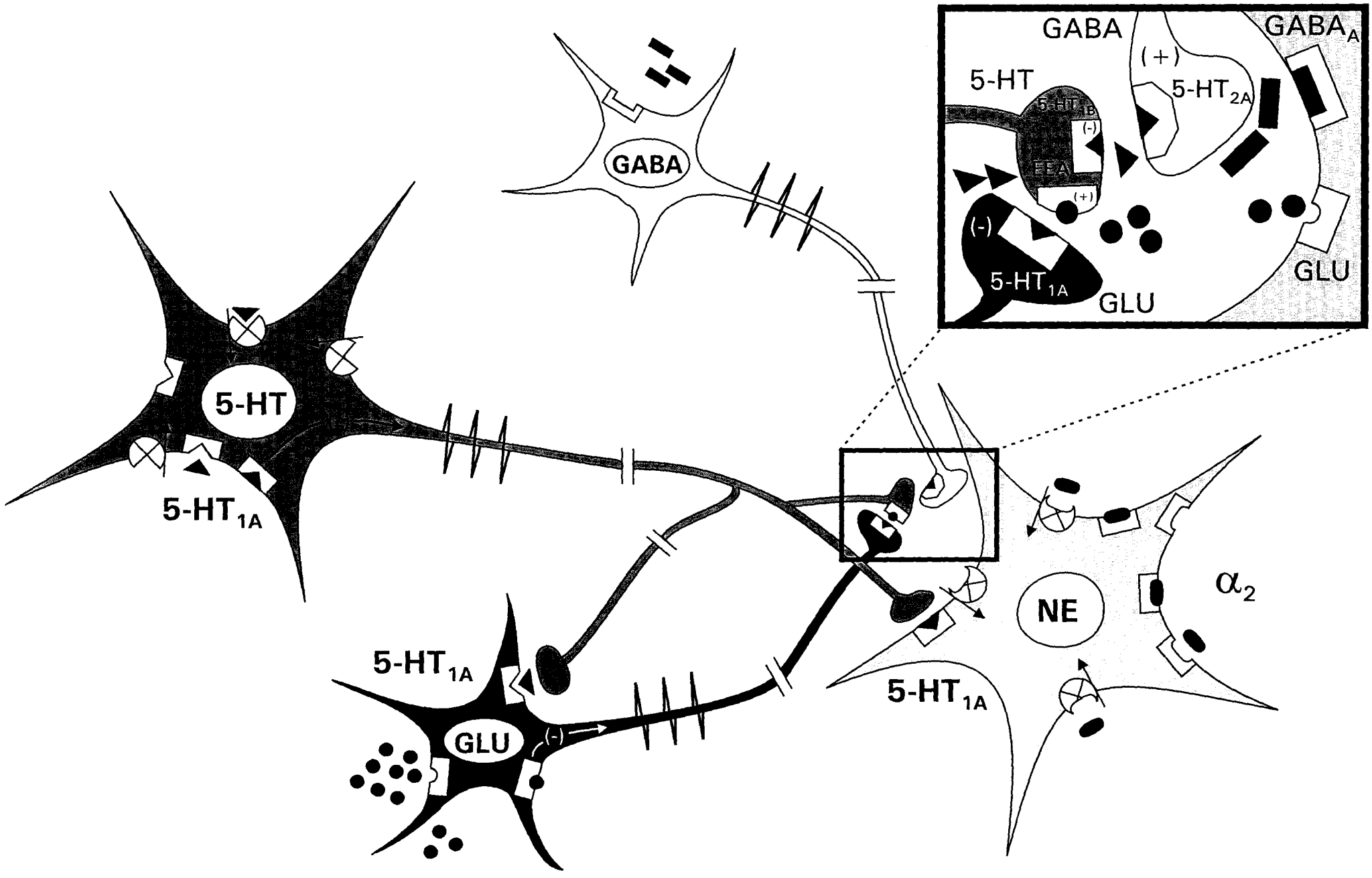

CONTROL
PAROXETINE
21 Days
Mean firing rate (Hz + S.E.M.)
of locus coeruleus neurons
4.0
3.0
2.0
1.0
0.0
*
*
7
6
Prior to Idazoxan
Following Idazoxan

Figure IX.


GABA
GABA$_A$
(+)
5-HT
5-HT$_{2A}$
5-HT$_{1B}$
(-)
(+)
(-)
5-HT$_{1A}$
GLU
GLU
GABA
5-HT
5-HT$_{1A}$
5-HT$_{1A}$
GLU
(-)
NE
α_2
5-HT$_{1A}$

Chapter V: Fourth Article

PREFATORY REMARKS

The attenuation on LC firing activity reported after a prolonged SSRI administration mediates its effects via a desensitization of 5-HT_{1A} receptors, in the presence of 5-HT reuptake blockade, to overactivate 5-HT_{2A} receptors. A complex neuronal circuitry accounting for these phenomena and the 5-HT receptor effects on LC activity was speculatively proposed in the previous chapter. This circuitry implicates that alterations in glutamate and GABA levels in this nucleus mediate the 5-HT receptor effects on LC activity. We decided to microiontophoretically eject antagonists directed against receptors in the circuitry while systemically injecting 5-HT_{1A} and 5-HT_2 receptor ligands. Thus, by locally blocking key receptors in the LC postulated to be mediating the 5-HT receptor effects on NA activity, the response to these ligands should be abolished. This next chapter further attempted to corroborate the proposed circuitry mediating the 5-HT receptor effects and SSRI induced attenuation on NA activity.

This article entitled "Serotonin $_{1A}$ receptor ligands act on norepinephrine neuron firing through excitatory amino acid and $GABA_A$ receptors: a microiontophoretic study in the rat locus coeruleus" by myself and Pierre Blier was accepted "As Is" with an impact rating of 8 out of 10 in Synapse on 7/29/01.

SEROTONIN $_{1A}$ RECEPTOR LIGANDS ACT ON NOREPINEPHRINE NEURON FIRING THROUGH EXCITATORY AMINO ACID AND GABA$_A$ RECEPTORS: A MICROIONTOPHORETIC STUDY IN THE RAT LOCUS COERULEUS

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Short Running Title: EEA/GABA$_A$ receptors and 5-HT-NE interactions

KEYWORDS: 5-HT$_{1A}$; 5-HT$_{2A}$; GABA; kainate: glutamate; anxiety disorders, major depression.

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ABSTRACT

It was previously shown that the excitatory effect of the 5-HT_{1A} agonist 8-OH-DPAT on firing activity of locus coeruleus (LC) norepinephrine (NE) neurons and the inhibitory action of the 5-HT_{1A} antagonist WAY 100,635 are dependent on the presence of 5-HT neurons, whereas the inhibitory action of the 5-HT_2 agonist DOI is not. Using *in vivo* extracellular unitary recordings performed in anesthetized rats, iontophoretic applications of the excitatory amino acid antagonist kynurenate attenuated the enhancement produced by glutamate and kainate. In contrast, GABA applications decreased the firing activity of NE neurons which was attenuated by the $GABA_A$ receptor antagonist bicuculline. 8-OH-DPAT (10 – 60 $\mu g\ kg^{-1}$, i.v.) produced a dose-dependent enhancement in the firing activity of NE neurons that was abolished in the presence of kynurenate application. The selective 5-HT_{1A} receptor antagonist WAY 100,635 (100 $\mu g\ kg^{-1}$, i.v.) suppressed NE firing which was reversed by the selective 5-HT_{2A} antagonist MDL 100,907 (200 $\mu g\ kg^{-1}$, i.v.). In the presence of bicuculline, the inhibitory effect of WAY 100,635 was blunted. These results suggest that WAY 100,635 attenuates NE neuron firing by blocking inhibitory 5-HT_{1A} receptors on glutamatergic neurons, thereby enhancing glutamate release and activating excitatory amino acid receptors, possibly kainate, on 5-HT terminals. The ensuing increased 5-HT release would then act on excitatory 5-HT_{2A} receptors on GABA neurons that would ultimately mediate the inhibition of NE neurons. The prevention of the excitatory action of 8-OH-DPAT on NE neuron firing by kynurenate is also consistent with this neurocircuitry.

INTRODUCTION

Most antidepressant agents target the serotonin (5-HT) and/or norepinephrine (NE) system. Furthermore, all antidepressant drugs alter the function of these neurons by interfering with monoamine oxidase, their receptors, or their reuptake transporters thereby markedly enhancing monoamine levels after prolonged administration (see Blier et al., 2000). Recently, it was reported that sustained treatments with selective 5-HT reuptake inhibitors (SSRIs) progressively decrease the spontaneous firing activity of locus coeruleus (LC) NE neurons in the rat, becoming significant after 14-days (Szabo et al., 1999, 2000; Szabo and Blier, 2000a) and was later confirmed by Grant and Weiss (2001). This observation is consistent with the report by Freuo et al., (1999) showing that the regional cerebral blood flow in the LC in rats treated with SSRIs is decreased only after long-term administration. The attenuation of LC firing activity reported during long-term SSRI administration can be partially attributed to an overactivation of postsynaptic 5-HT_{2A} receptors mediating an inhibitory effect on NE activity (Szabo and Blier, 2000a). Also, the increase on LC firing activity produced with the 5-HT_{1A} receptor agonist 8-OH-DPAT is abolished upon sustained SSRI treatment and, interestingly, with NE reuptake inhibitor (NRI) treatments as well (Szabo et al., 2000; Szabo and Blier, 2000a, b, c).

5-HT_{1A} and 5-HT_{2A} receptors regulate LC firing activity (Gorea and Adrien, 1988; Piercey et al., 1994; Chiang and Aston-Jones, 1993; Szabo et al., 2000), albeit in opposite directions. During a long-term SSRI treatment, the former are desensitized and the latter receptors remain normosensitive and are overactivated. This would produce a net inhibitory effect by the 5-HT system on LC firing with a temporal delay

congruent with the beneficial action of SSRIs in anxiety and affective disorders (Blier and de Montigny, 1999; Blier, 2000). The effect of the activation and the blockade of 5-HT_{1A} receptors on NE neuron firing is dependent on the presence of 5-HT neurons whereas the activation of 5-HT_{2A} receptors is not (Haddjeri et al., 1997; Szabo and Blier, 2000a). These observations, when combined with recent results showing the suppressant effect of the selective 5-HT_{1A} receptor antagonist WAY 100,635 on NE neuron firing is reversed by an injection of the selective 5-HT_{2A} receptor antagonist MDL 100,907 (Szabo and Blier, 2000a), lead us to propose a neuronal circuitry accounting for how these receptor subtypes and long-term SSRI treatment produce their effects on LC firing activity (figure 1; Szabo and Blier, 2000a). Furthermore, this speculative circuitry implies that activation of excitatory 5-HT_{2A} receptors on GABA terminals in the LC imparts an inhibitory action on NE neuron firing largely mediated via $GABA_A$ receptors on these neurons (Chiang and Aston-Jones, 1993). Given that 5-HT terminals containing EEA receptors synapse on NE neurons and terminals in the LC (Van Bockstaele, 2000), and activation of 5-HT_{1A} receptors on glutamate terminals decreased glutamate release in the LC (Bobker and Williams, 1989), the present studies were undertaken to determined whether the 5-HT_{1A} receptor-mediated effects on NE neuron firing activity are attenuated by EEA antagonism with kynurenate iontophoresed in the LC. In addition, it was assessed whether the inhibitory effects of systemically injected WAY 100,635, which is dependent on intact 5-HT neurons, were mediated via $GABA_A$ receptor activation in the LC by ejecting the $GABA_A$ antagonist bicuculline directly onto NE neurons by iontophoresis (figure 1). Before carrying out

these experiments, it was ensured that kynurenate and bicuculline could indeed block EEA and $GABA_A$ receptors under the experimental conditions used.

Material and Methods

Animals

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g. Rats were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and *water ad libitum*). Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, rats were inserted a catheter in a lateral tail vein for systemic i.v. injection of drugs. All experiments were performed in compliance with NIH guidelines and the Canadian Council on Animal Care.

Electrophysiological experiments

Extracellular unitary recording and microiontophoresis of drugs onto NE neurons of the LC were conducted with five-barrelled micropipettes, pulled conventionally with the tips broken to a diameter of 9 to12 μm under microscopic control. The central and a side barrel, used for recording and automatic current balancing, respectively, was filled with a 2 M NaCl solution. Two side barrels in each electrode always contained kynurenic acid (100 mM in 200 mM NaCl, pH 7.5) and bicuculline methiodide (3 mM in 200 mM NaCl, pH 4). Furthermore, it is important to mention that all pipettes contained

these solutions because of the effects they may exert on the spontaneous firing activity of LC neurons due to leakage from the pipette (Aston-Jones, et al., 1991). The remaining side barrel was filled either with monosodium glutamate (200 mM in 200 mM NaCl, pH 8.5), kainate (10 mM in 200 mM NaCl, pH 8.8), or GABA (200 mM in 200 mM NaCl, pH 4.1). All of the barrels were within the impedance range of -20 to -80 MΩ. Alkaline drug solutions were ejected as anions and retained with a +10 nA current between ejections. GABA and bicuculline solutions being acidic were ejected as cations and retained with a -10 nA current. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for LC neuron recordings. They were recorded with micropipettes lowered at -0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active NE neurons of the LC were identified using the following criteria: regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to a nociceptive pinch of the contralateral hind paw. NE neurons were recorded for at least 1 min to establish their basal firing rate.

Dose-response Curves

Dose-response curves for the alteration of NE neuron firing activity were obtained for systemic (i.v.) administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT in the absence and presence of iontophoretically applied kynurenate. After systemic injection of 8-OH-DPAT, the selective 5-HT_{1A} receptor anatagonist WAY 100,635 was administered in order to assess its effects on LC NE neuron firing and/or to reverse the

increase in firing activity produced by the previously injected compound. Changes in the firing activity are expressed as percentages of the baseline firing rate and were measured after systemic drug administration. Dose-response curves were constructed for 8-OH-DPAT where it was the first dose injected to each rat to generate the curves and estimates of effective doses 50 (ED_{50}).

Drugs

The following drugs were used: monosodium glutamate, kainate acid, kynurenic acid, GABA, bicuculline methiodide, 8-OH-DPAT, WAY 100,635, DOI, and idazoxan (RBI, Natick, MA, U.S.A.); MDL 100,907 (Hoechst Marion Roussel, Cincinnati, OH, USA). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were dissolved in distilled water and injected in about 0.1 ml volumes.

Statistical comparisons

All results were expressed as mean (± S.E.M.) of single neuron values. Statistical comparisons of microiontophoretically applied agents on LC firing with different currents in rats in the presence and absence of its antagonist were carried out using one-way analysis of variance (ANOVA) on ranks. The difference for each of the current ejections on LC firing was compared between groups to effects produced in the absence or presence of its respective antagonist with a Student's *t*-test. Correlation coefficients (*r* values) for the dose-response relationship observed on the LC were

calculated using simple linear/curvilinear regression analysis. The SEM for the ED_{50} values for the LC were calculated by regression analysis, with the Y value of 50 used as the regressor. Difference between the two regressions in the rats injected with 8-OH-DPAT in the presence and absence of kynurenate were assessed by comparing their ED_{50} values using the confidence intervals method. The 95% confidence limit was determined from the Student's *t* distribution. The differences between the effects of WAY 100,635 and MDL 100,907 on LC NE neuron firing activity in the absence and presence of bicuculline application was assessed with a one-way and one-way repeated measure ANOVA, respectively. All post hoc multiple comparisons were carried out with the Student- Newman-Keuls method.

RESULTS

Effect of iontophoretically applied kynurenate on LC NE neuron firing and antagonism of excitatory responses to glutamate and kainate ejections.

As summarized in table 1 and illustrated in figures 2 and 3, the firing activity of LC NE neurons in the absence and during kynurenate application did not differ with respect to each other in the group of rats receiving glutamate or kainate ejections (p = 0.69 and p = 0.75, respectively). Furthermore, the basal firing activity of all LC NE neurons recorded with five barrelled pipettes (n = 50), while nothing was iontophoresed, was not statistically different from the spontaneous firing of LC NE neurons recorded with single barrelled pipettes (n = 90; table 1). The firing activity of NE neurons in the absence and presence of kynurenate applications in groups of rats receiving glutamate or kainate ejections did not significantly differ from the firing activity of all LC NE neurons recorded in rats with a five-barrelled pipette (p = 0.35 and p = 0.39, respectively). Kynurenate ejected with a -10 nA current, by itself, produced a similar and statistically significant 27% decrease on basal LC NE neuron firing in rats receiving glutamate and kainate (p = 0.013 and p = 0.008, respectively). Microiontophoretic applications of glutamate enhanced LC NE neuron firing activity which was attenuated in the presence of the excitatory amino acid (EEA) antagonist kynurenate always ejected at a -10 nA current, an example of which is provided in figure 2A. The increase on NE neuron firing activity by -2, -4, and -8 nA ejections of glutamate in the absence and presence of kynurenate were current-dependent (p = 0.001 and p = 0.005, respectively). Importantly, kynurenate was able to significantly attenuate these

responses by 77% (p = 0.010), 77% (p = 0.004), and 69% (p = 0.005) when comparing between groups for each current (figure 2). Similarly, microiontophoretic applications of kainate enhanced LC NE neuron firing activity and these responses were attenuated in the presence of kynurenate, an example of which is provided in figure 3A. This increase on NE neuron firing activity produced by -1, -2, and -4 nA ejections of kainate in the absence and presence of kynurenate were current-dependent ($p < 0.001$ and p = 0.016, respectively). Also, these kainate responses under kynurenate ejection were significantly attenuated by 88% (p = 0.005), 79% (p = 0.021), and 92% (p = 0.005) when compared to the effects on LC firing in the absence of kynurenate within each current (figure 3).

Effect of iontophoretically applied bicuculline on LC NE neuron firing and antagonism of inhibitory responses to GABA ejections.

The firing activity of NE neurons before and during bicuculline application did not differ in comparison to the firing activity of groups of rats receiving glutamate (p = 0.59 and p = 0.17, respectively) or kainate applications (p = 0.23 and p = 0.17, respectively; table 1). Furthermore, these values obtained from rats receiving GABA applications in the absence and presence of bicuculline did not differ statistically from neurons recorded with five-barrelled pipettes when nothing was iontophoresed (p = 0.29 and p = 0.62, respectively; table 1). Bicuculline ejected by itself significantly increased the basal firing activity of NE neurons by 23 % (p = 0.001). Microiontophoretic applications of GABA inhibited LC NE neuron firing activity and these responses were attenuated by a 10 nA ejection current of the selective $GABA_A$ receptor antagonist bicuculline, an

example of which is provided in figure 4A. The decrease of NE neuron firing activity produced by 5, 10, and 20 nA ejections of GABA in the presence and absence of bicuculline were current-dependent ($p = 0.003$ and $p = 0.008$, respectively). Importantly, bicuculline applications significantly attenuated these responses by 81% ($p < 0.001$), 73% ($p < 0.001$), and 52% ($p = 0.001$), when compared to the effects on LC firing in the absence of bicuculline for each current (figure 4).

Effect of intravenous injection of 8-OH-DPAT on LC NE neurons during iontophoretic application of kynurenate

The firing activity of LC NE neurons in the presence of kynurenate before the injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT did not significantly differ from that of LC NE neuron firing previously recorded with a single barrelled pipette (2.2 ± 0.3 and 2.3 ± 0.4, respectively, $p = 0.70$; Szabo and Blier, 2000a). The augmentation of NE neuron firing activity by 8-OH-DPAT was dose-dependent, as previously reported (Piercey et al., 1994). The enhancing effect of 8-OH-DPAT and inhibitory effects of the selective 5-HT_{1A} receptor antagonist WAY 100,635 ($n = 5$) on NE neuron firing were abolished by the application of kynurenate (figure 5). Interestingly, this lack of effect observed with 8-OH-DPAT on LC firing under kynurenate application was noted even at doses of up to 100 $\mu g/kg^{-1}$ (Figure 6). Furthermore, injection of the selective 5-HT_{2A} antagonist MDL 100,907 after WAY 100,635 did not alter the firing activity of LC NE neurons under kynurenate application ($n = 3$ rats; figure 5B).

Effect of intravenous injection of WAY 100,635 on NE neuron firing activity under iontophoretic bicuculline ejection

The i.v. administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 produced an attenuation on the firing activity of LC NE neurons which was fully reversed by a subsequent injection of the selective 5-HT_{2A} receptor antagonist MDL 100,907, an example of which is provided in figure 7A. However, under bicuculline application, the inhibitory effect of WAY 100,635 was significantly attenuated, but a subsequent injection of MDL 100,907 was still able to reverse firing activity (figure 7B). When comparing the effects of WAY 100,635 obtained in rats under the presence and absence of bicuculline application, the inhibitory effect of this agent was blunted, yielding only a 24 % decrease on LC firing as opposed to a 91% attenuation ($p<0.05$ for both; figure 8). Furthermore, the relative increase observed with a subsequent administration with MDL 100,907 was smaller in the presence of bicuculline.

DISCUSSION

The results presented herein showed that the increase in firing activity of LC NE neurons produced by iontophoretic applications of glutamate and kainate is attenuated in the presence of the EEA antagonist kynurenate (figure 2 and 3), as previously reported (Aston-Jones et al., 1991; Ennis et al., 1992; Charléty et al., 1993). Despite the observation that kainate produced a greater enhancement on LC NE neuron firing than glutamate, consistent with other studies (Charléty et al., 1991; Aston-Jones et al., 1991; Rassmussen et al., 1996), kynurenate was more effective to attenuate the excitatory action of kainate than that of glutamate (Ennis et al., 1992). On the other hand, iontophoretic applications of GABA decreased NE neuron firing, and this inhibition was attenuated by the concomittant ejection of the selective $GABA_A$ receptor antagonist bicuculline (figure 4), as previously reported by Ennis and Aston-Jones (1989b).

Due to reports that the basal firing activity of LC NE neurons is altered when recording with multibarrelled pipettes containing the two antagonists utilized in this study, probably because of their leakage from the electrode (Chouvet et al., 1988; Aston-Jones et al., 1991; Ennis et al., 1992; Charléty et al., 1993), it was mandatory to first assess the firing activity of LC neurons under the present conditions. The spontaneous firing activity of LC NE neurons recorded with five-barrelled pipettes in comparison to that previously obtained with single barrel electrodes did not differ significantly from each other (table 1). The explanation as to why altered basal firing activities on NE neurons with these iontophoretic agents were not encountered, as opposed to the abovementioned work, may be due to the presence of both the $GABA_A$ and EEA antagonists in the pipettes at all times. Their opposite actions on firing activity

thus most likely cancelled each other out (see figures 2, 3, and 4). Consequently, the effects of 5-HT ligand injections on LC firing during iontophoretic applications of antagonists can reliably be compared to results previously obtained with these agents in rats recorded with a single barrel electrode without being confounded by an alteration of basal LC firing (Gorea and Adrien, 1988; Piercey et al., 1994; Haddjeri et al., 1997; Szabo et al., 2000; Szabo and Blier, 2000a).

The effects of 5-HT_{1A} receptor ligands on LC NE neuron firing are dependent on the presence of 5-HT neurons (Szabo and Blier 2000a; Haddjeri et al., 1997). However, 5-HT_{1A} binding density in the LC is unaffected by lesioning 5-HT neurons with a 5,7-DHT treatment (Weissman-Nanopoulous, 1985), further indicating that these receptors are not 5-HT autoreceptors, although 50% of the 5-HT input to the LC is from the dorsal raphe (Kaehler et al., 1999). Nevertheless, 5-HT exerts a potent effect on EEA-mediated responses on LC neurons (Shiekhattar and Aston-Jones, 1993), with the most robust interaction being between 5-HT and kainate (Charléty et al., 1993). Furthermore, perfusion of the AMPA/kainate receptor antagonist DNQX in the LC reduces 5-HT release in this nucleus via a tetrototoxin insensitive mechanism and suggests a tonic activation of these receptors (Singewald et al., 1998). When combined with elegant anatomical evidence produced by Van Bockstaele (2000) demonstrating that 5-HT terminals, some of which are endowed with kainate receptors, impinge directly or on other terminals which synapse on NE neurons in the LC, it thus appears that 5-HT_{1A} ligands do not act primarily on these neurons, but possibly upstream on EEA containing neurons via a 5-HT dependent neuronal circuitry (figure 1).

Both the excitatory effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT and the inhibitory effect of the selective 5-HT_{1A} antagonist WAY 100,635 were abolished by the application of kynurenate (figures 5 and 6). These 5-HT_{1A} receptor ligands therefore produce their effects via the activation of EEA receptors within the LC. Since, 5-HT_{1A} receptor ligands iontophoresed on LC NE neurons produce no effect on spontaneous firing (Gorea et al., 1991), this receptor subtype may thus not be located on NE neurons, consistent with the observation that these neurons do not contain 5-HT_{1A} receptor mRNA (Pompeiano et al., 1992). It is therefore possible that 5-HT_{1A} receptors in the LC are localized on axon terminals of glutamate afferents to this nucleus. This possibility is consistent with *in vitro* work demonstrating that the activation of presynaptic 5-HT_{1A} receptors on glutamate afferents decreased the release of glutamate in brainstem slices containing the LC (Bobker and Williams, 1989). On the other hand, 5-HT_{1A} receptors have also been identified in the rostral ventral medulla (RVM; Helke et al., 1997). In addition, 5-HT_{1A} receptor agonists when bath perfused on rat brain slices containing RVM neurons, lacking immunoreactivity for tryptophan and tyrosine hydroxylases, inhibit the firing of these neurons (Piguet et al., 2000). Because the nucleus paragigantocellularis in this brain region possesses glutamate neurons that send afferents to the LC and exert a major excitatory influence on NE neuron firing (Ennis et al., 1988), it is possible that activation of inhibitory 5-HT_{1A} receptors on RVM neurons could attenuate the release of EEAs in the LC. Thus, the alteration of EEA release in the LC by 5-HT_{1A} ligands is requisite to produce the effects on NE firing, and may be mediated through 5-HT_{1A} receptors located on a glutamate neuron (Figure 1).

Systemic injection of the 5-HT_2 receptor agonist DOI produces a decrease in LC NE neurons which is prevented by a prior injection of the 5-HT_{2A} antagonists MDL 100,907 and YM992, thus indicating that this 5-HT_2 agonist acts on LC activity via 5-HT_{2A} receptors (Szabo et al., 2000; Szabo and Blier, 2000a; 2000b). Although 5-HT_{2A} receptor immunoreactivity has been documented in the LC (Lopez-Gimenez et al., 1999; 2001), these receptors are probably not on NE neurons as a mRNA hybridization signal for this receptor subtype is not present in the LC (Pompeiano et al., 1994). Indeed, the direct application of 5-HT_2 receptor agonists and antagonist by microiontophoresis onto LC neurons does not modify the firing rate of NE neurons (Charléty et al., 1993; Gorea et al., 1991). Therefore, the 5-HT_{2A} receptors controlling LC firing activity cannot be on NE neurons. Interestingly, the prepositus hypoglossi nucleus (PrH) possesses GABA neurons, which were shown to bind antibodies directed against the 5-HT_{2A} receptors (Fay and Kubin, 2000), and imparts a major inhibitory influence on LC NE neuron firing (Ennis and Aston-Jones, 1989a). Furthermore, the integrity of this nucleus is necessary to produce 5-HT_2-mediated effects on LC NE firing (Gorea et al., 1991). Also, the inhibitory response to systemic DOI and 5-HT_2 receptor agonist and antagonists are not altered to a great extent in 5,7-DHT and PCPA treated rats, respectively (Szabo and Blier, 2000a; Gorea et al., 1991). However, these 5-HT_{2A} receptors are not located in the PrH because iontophoretic application of DOI in this nucleus does not alter LC NE firing activity (Gorea et al., 1991). Finally, the inhibitory effect of DOI is abolished by application of the selective $GABA_A$ receptor antagonist bicuculline in the LC (Chiang and Aston-Jones, 1993). Taken together, these results indicate that 5-HT_2 receptor agonists mediate their inhibitory effects on LC firing via

excitatory 5-HT_{2A} receptors probably on GABA terminals, originating from the PrH, which leads to an increase of the degree of activation of $GABA_A$ receptor activation and ultimately to a decrease in NE neuron firing (figure 1).

The firing activity of LC NE neurons is almost completely suppressed by an injection of the selective 5-HT_{1A} receptor antagonist WAY 100,635 which is dependent on the presence of 5-HT neurons, and it is fully reversed by a subsequent injection of the selective 5-HT_{2A} receptor antagonist MDL 100,907 (figure 8). This indicates that WAY 100,635 decreases NE firing through a postsynaptic 5-HT_{2A} receptor (Haddjeri et al., 1997; Szabo and Blier, 2000a). This 5-HT_{1A} receptor blockade and the ensuing postulated increase in glutamate release required to activate EEA receptors, and possibly kainate receptors on 5-HT terminals, would presumably lead to augmented 5-HT release (figure 1). Such an enhanced 5-HT release produced by WAY 100,635 would overactivate 5-HT_{2A} receptors in the LC, augment the release of GABA onto NE neurons, and finally decrease firing via $GABA_A$ receptor activation (figure 1). Previous results showing a suppression of firing activity of NE neurons by (-) mirtazapine, which is a preferential antagonist for the α_2-adrenergic heteroceptors on 5-HT terminals (Haddjeri et al., 1996), are also consistent with this neurocircuitry. This inhibition of firing by (-) mirtazapine is dependent on the presence of 5-HT neurons, as is the case for WAY 100,635. Therefore, by blocking this presynaptic element normally decreasing 5-HT release, (-) mirtazapine is expected to produce a marked enhancement of synaptic 5-HT level within the LC. This would overactivate the 5-HT_{2A} receptors on GABA

terminals, thereby increasing GABA release and attenuate NE firing via inhibitory $GABA_A$ receptors (figure 1).

In conclusion, activation of 5-HT_{1A} and 5-HT_2 receptors exert opposite effect on LC firing. The effects of the 5-HT_{1A} receptor ligands are dependent on intact 5-HT neurons and on EEA receptors to mediate their effects on LC NE firing. Nevertheless, 5-HT_{1A} receptor ligands and the 5-HT_2 agonist DOI likely exert their effect on LC NE firing ultimately by altering the activation of 5-HT_{2A} receptors and the tonic activation of $GABA_A$ receptors on LC NE neurons (figure 4). Since long-term SSRIs administration attenuates spontaneous LC NE firing, this would presumably be due to an increase of 5-HT in the LC leading to an overactivation of 5-HT_{2A} receptors and a similar augmentation of GABA released onto NE neurons as described above. This decrease of LC NE firing produced by SSRIs occurs with a time-course that is congruent to the delayed onset of action of SSRIs in the treatment of anxiety and affective disorders (Szabo et al., 1999; Blier et al., 2000). Indeed, all antidepressants tested thus far abolish the 5-HT_{1A} receptor response of LC NE neurons after prolonged administration. Consequently, according to the circuitry proposed herein, most antidepressants would produce an increase in GABA release in this nucleus after prolonged administration, thus contributing to attenuate NE neuron firing. This greater inhibitory influence on LC NE neurons by antidepressants, leading to a suppressed firing activity, may be relevant to their delayed anxiolytic effect. It is thus interesting to consider this possible mechanism of action of antidepressants in the perspective of the immediate facilitatory action of benzodiazepines on $GABA_A$ receptor function and their rapid anxiolytic effect.

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LEGENDS TO FIGURES

Figure 1. Speculative neuroanatomical and neurochemical bases for the interactions between 5-HT neurons and LC NE neurons. This diagram was prepared on the basis of the results obtained by several groups of investigators as well as data generated in our laboratory (see Szabo and Blier, 2000a).

Figure 2. Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of glutamate in the absence and presence of kynurenate (A). Histograms representing the percent increase in NE neuron firing activity from microiontophoretic applications of glutamate in the absence and presence of kynurenate. The number of neurons tested is given at the bottom of each column. * $P < 0.05$, Student *t*-test were used to analyze differences for each current between the mean increase on LC NE neuron firing to iontophoretic applications of glutamate in the absence and presence of kynurenate.

Figure 3. Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of kainate in the absence and presence of kynurenate (A). Histograms representing the percent increase in NE neuron firing activity from microiontophoretic applications of cumulative current ejections of kainate in the absence and presence of kynurenate. The number of neurons tested is given at the bottom of each column. * $P < 0.05$, Student *t*-test were used to analyze differences for each

current between the mean increase on LC NE neuron firing to iontophoretic applications of kainate in the absence and presence of kynurenate.

Figure 4. Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of GABA in the absence and presence of bicuculline (A). Histograms representing the percent decrease in NE neuron firing activity from microiontophoretic applications of cumulative current ejections of GABA in the absence and presence of bicuculline. The number of neurons tested is given at the bottom of each column. * $P < 0.05$, Student *t*-test were used to analyze differences for each current between the mean decrease on LC NE neuron firing to iontophoretic applications of GABA in the absence and presence of bicuculline.

Figure 5. Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of glutamate, the excitatory effects of intravenous administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT, the subsequent injection of the selective 5-HT_{1A} receptor antagonist WAY 100,635 producing a halt in the firing activity, and a final injection of the selective 5-HT_{2A} receptor antagonist MDL 100,907 bringing back the firing activity to basal levels (A). Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of glutamate and the lack of a response to the sequential intravenous administration of 8-OH-DPAT, WAY I00,635, and MDL 100,907 under kynurenate application (B). The identity of the NE neuron recorded under kynurenate ejection was assessed by showing

an augmentation of NE neuron firing activity using the injection the selective α_2-adrenoceptor antagonist idazoxan. Note the decrease in basal firing NE neuron firing activity under kynurenate application.

Figure 6. Relationship between the degree of augmentation of LC NE firing activity and doses of 8-OH-DPAT administered intravenously in absence and presence of kynurenate application. Only the initial response of a single NA neuron to the first dose of 8-OH-DPAT in each rat was used to construct the curves. Outer lines represent the standard error of the regression line. The arrows pointing to the data points on the dose-response curves correspond to the doses used in figure 5. The 8-OH-DPAT dose-response curve generated in the absence of kynurenate was previously reported in Szabo et al., 2000, and the open circles represents two sets of experiments performed herein in order to further validate the curve and solution utilized.

Figure 7. Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of GABA, the attenuation of this response under bicuculline ejection, the shutting down in firing activity produced with intravenous injection of the selective 5-HT_{2A} receptor antagonist WAY 100,635, and 5-HT_{2A} receptor antagonist MDL 100,907 re-establishing the firing activity back to baseline (A). Integrated firing rate histograms illustrating the response of a LC NE neuron to microiontophoretic applications of GABA, the blunted response to WAY 100,635 under

bicuculline ejection, and MDL 100,907 increasing the firing activity above baseline (B). Note the increase in basal NE neuron firing activity under bicuculline application.

Figure 8. Mean effects of systemic administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 and the 5-HT_{2A} receptor antagonist MDL 100,907 on the firing activity of LC NE neurons (A) and in the presence of bicuculline application (B). *P < 0.001 (paired t-test) when compared to NE neuron basal firing activity of the before WAY 100,635 drug injections. † P<0.001 (Paired t-test) when compared to NE neuron basal firing activity before MDL 100,907 drug injections. The number of neurons tested is given at the bottom of each column. The histograms obtained in control rats (A) were previously reported in Szabo and Blier, 2000a, and used herein because these experimental series were performed concurrently.

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Table 1. Firing activity of LC NE neurons recorded with five barrelled pipettes containing kynurenate and bicuculline in comparison to a single barrelled electrodes

	Mean firing rate of NE neurons (Hz)	No. of neurons recorded	No. of rats
Single-barrel	2.6 ± 0.2 †	90	6
Five-barrel pipette	3.0 ± 0.2	50	17
Kynurenate (Glutamate in retention)			
Before	3.0 ± 0.7	8	5
After	2.2 ± 0.5 *		
Kynurenate (Kainate in retention)			
Before	3.3 ± 0.4	8	6
After	2.4 ± 0.3 *		
Bicuculline (GABA in retention)			
Before	2.6 ± 0.4	8	6
After	3.2 ± 0.4 *		

*$P < 0.05$, when comparing the before and after firing rates of NE neurons, using ANOVA followed by Student-Newman-Keuls method.
† These results were recently reported in Szabo et al., 2000.

Figure I.

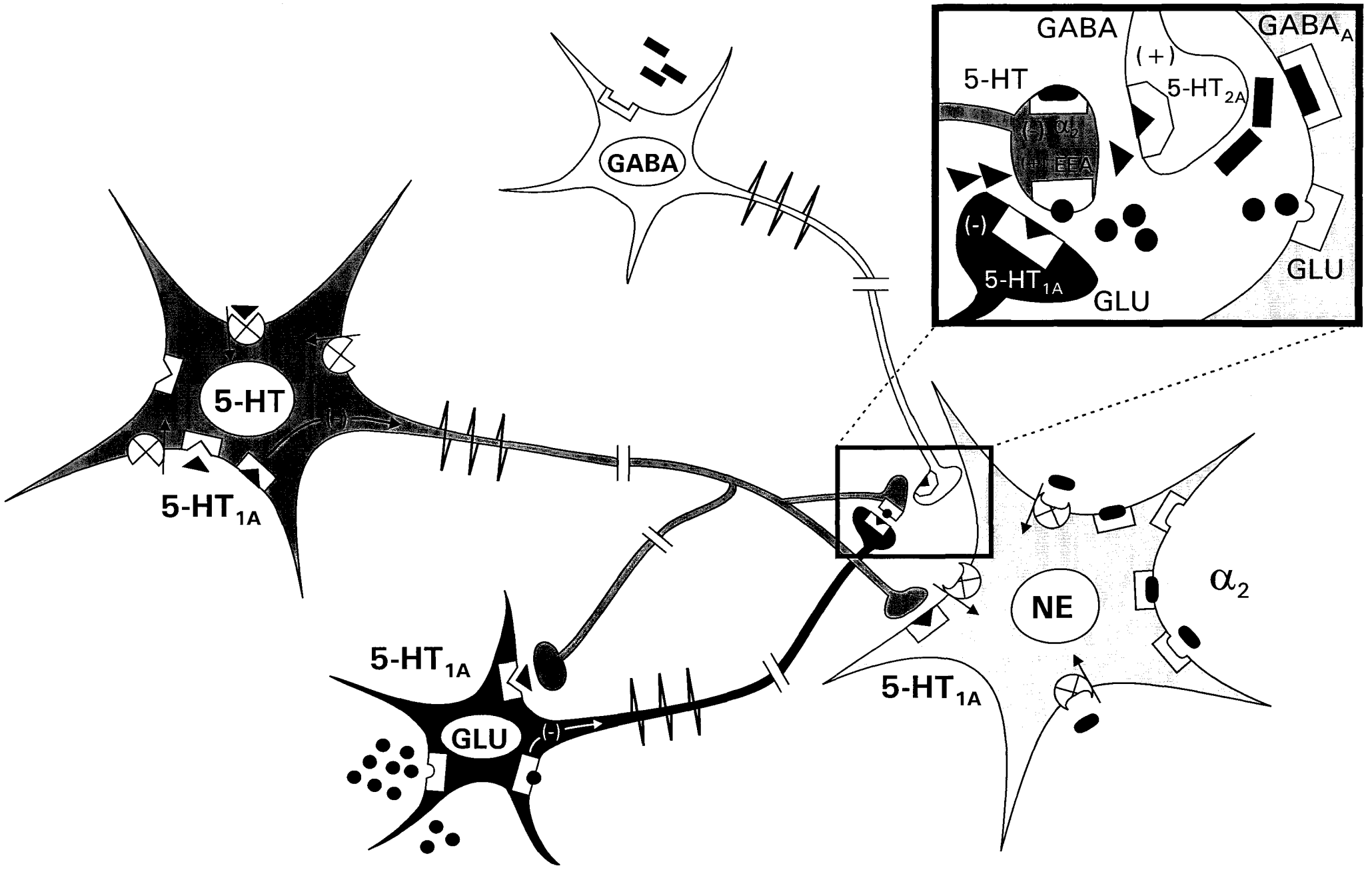
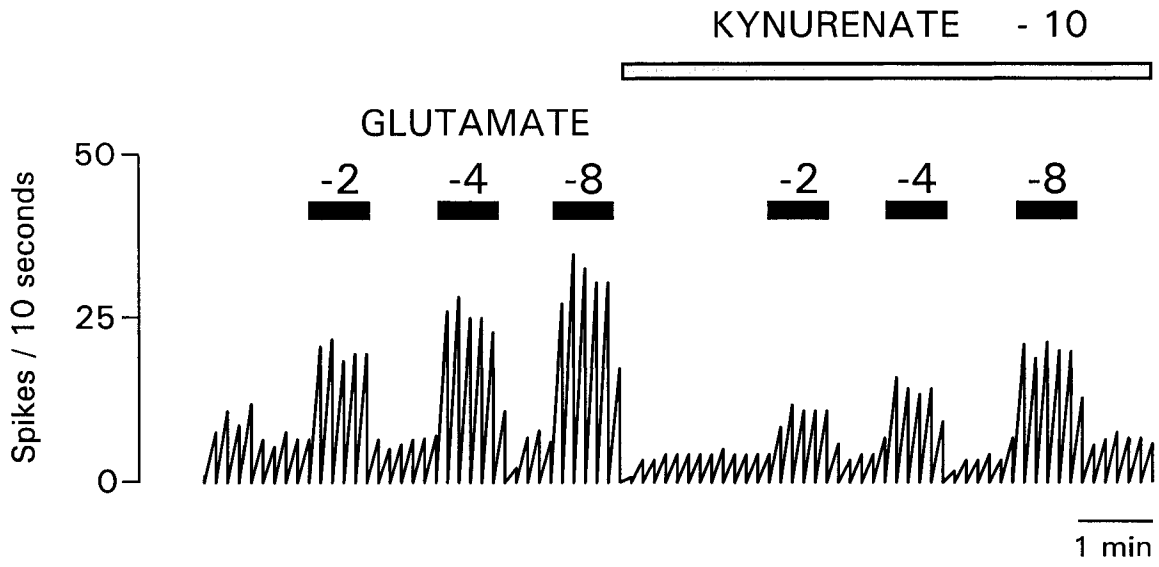

GABA
GABA$_A$
(+)
5-HT
5-HT$_{2A}$
(-) α_2
(+) EEA
(-)
5-HT$_{1A}$
GLU
GLU
GABA
5-HT
(-)
5-HT$_{1A}$
5-HT$_{1A}$
GLU
(-)
NE
α_2
5-HT$_{1A}$

Figure II.

A.


KYNURENATE - 10
GLUTAMATE
-2
-4
-8
-2
-4
-8
50
25
0
Spikes / 10 seconds
1 min

B.

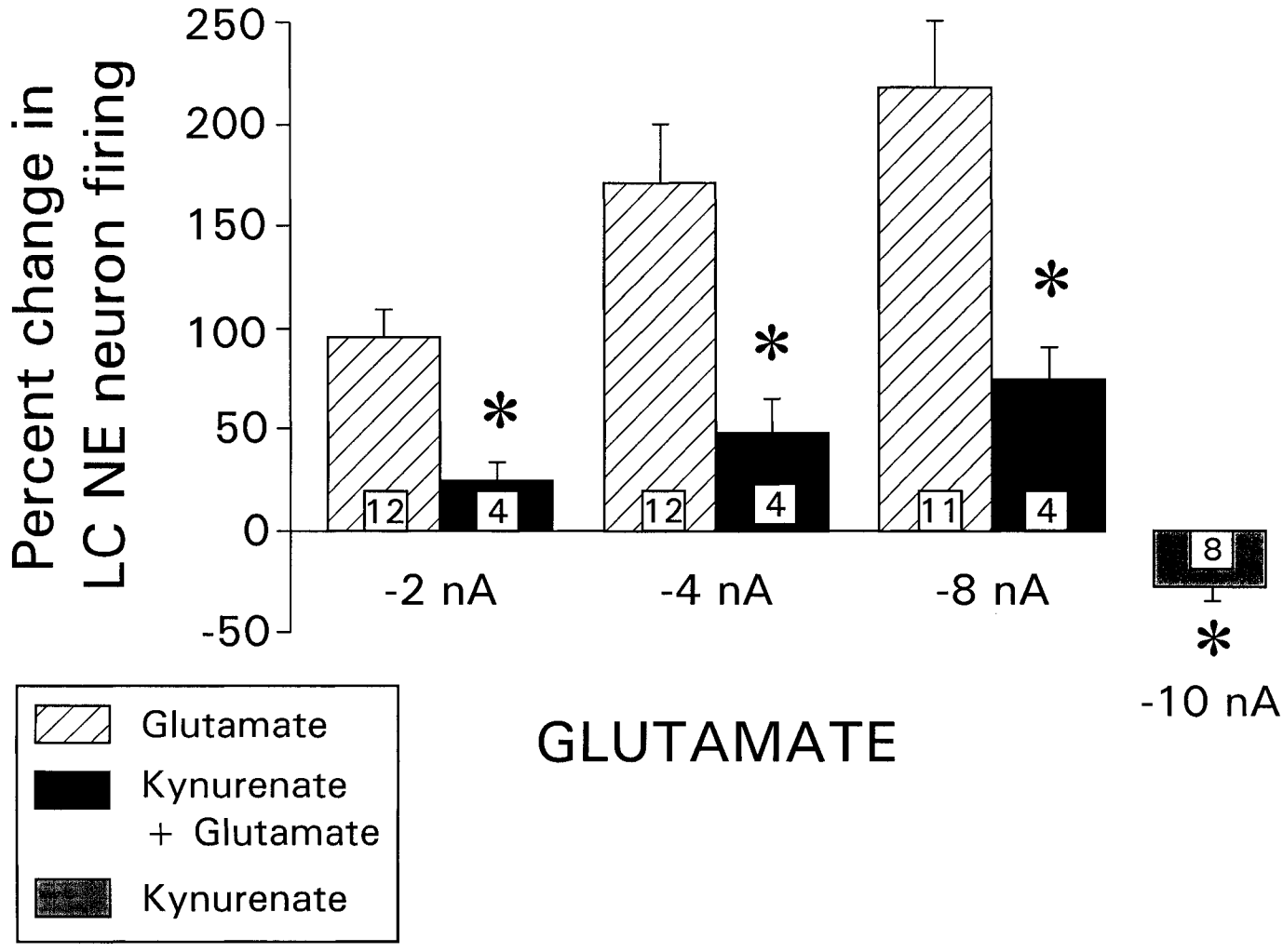
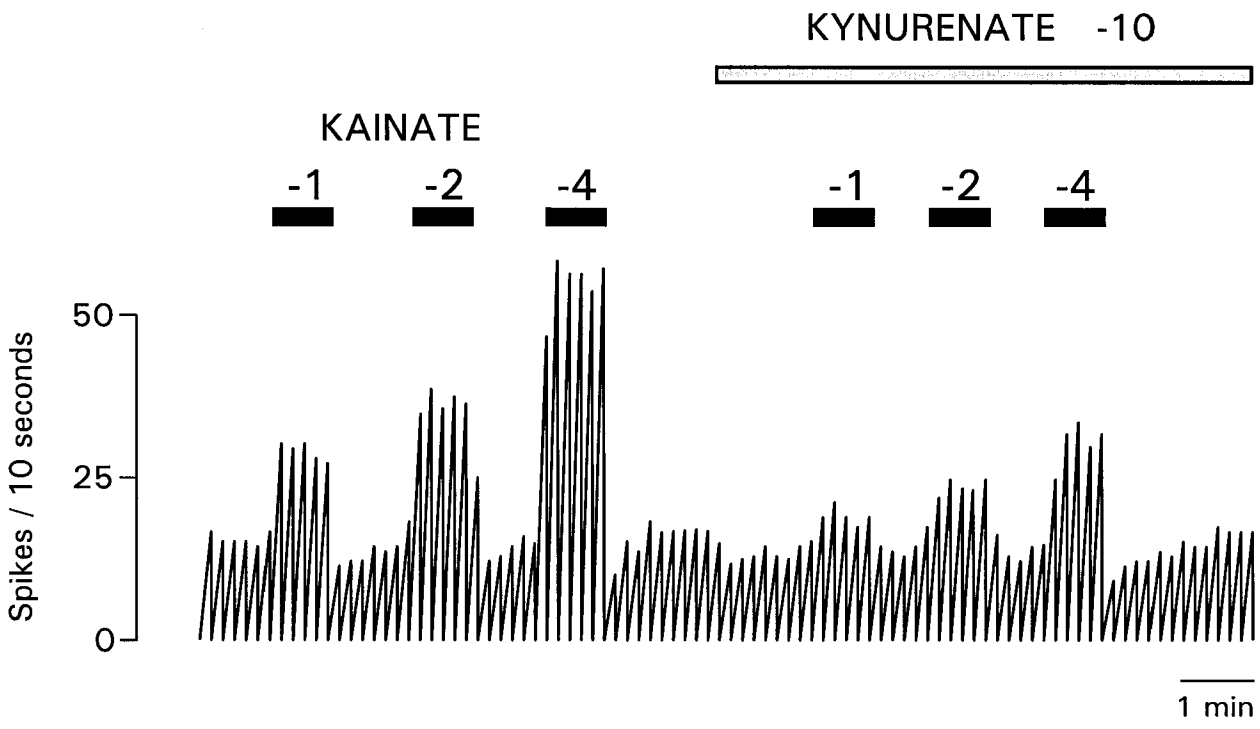

Percent change in LC NE neuron firing
250
200
150
100
50
0
-50
12
4
12
4
11
4
8
-2 nA
-4 nA
-8 nA
-10 nA
GLUTAMATE
Glutamate
Kynurenate + Glutamate
Kynurenate

Figure III.


KYNURENATE -10
KAINATE
-1
-2
-4
-1
-2
-4
50
25
0
Spikes / 10 seconds
1 min

B.

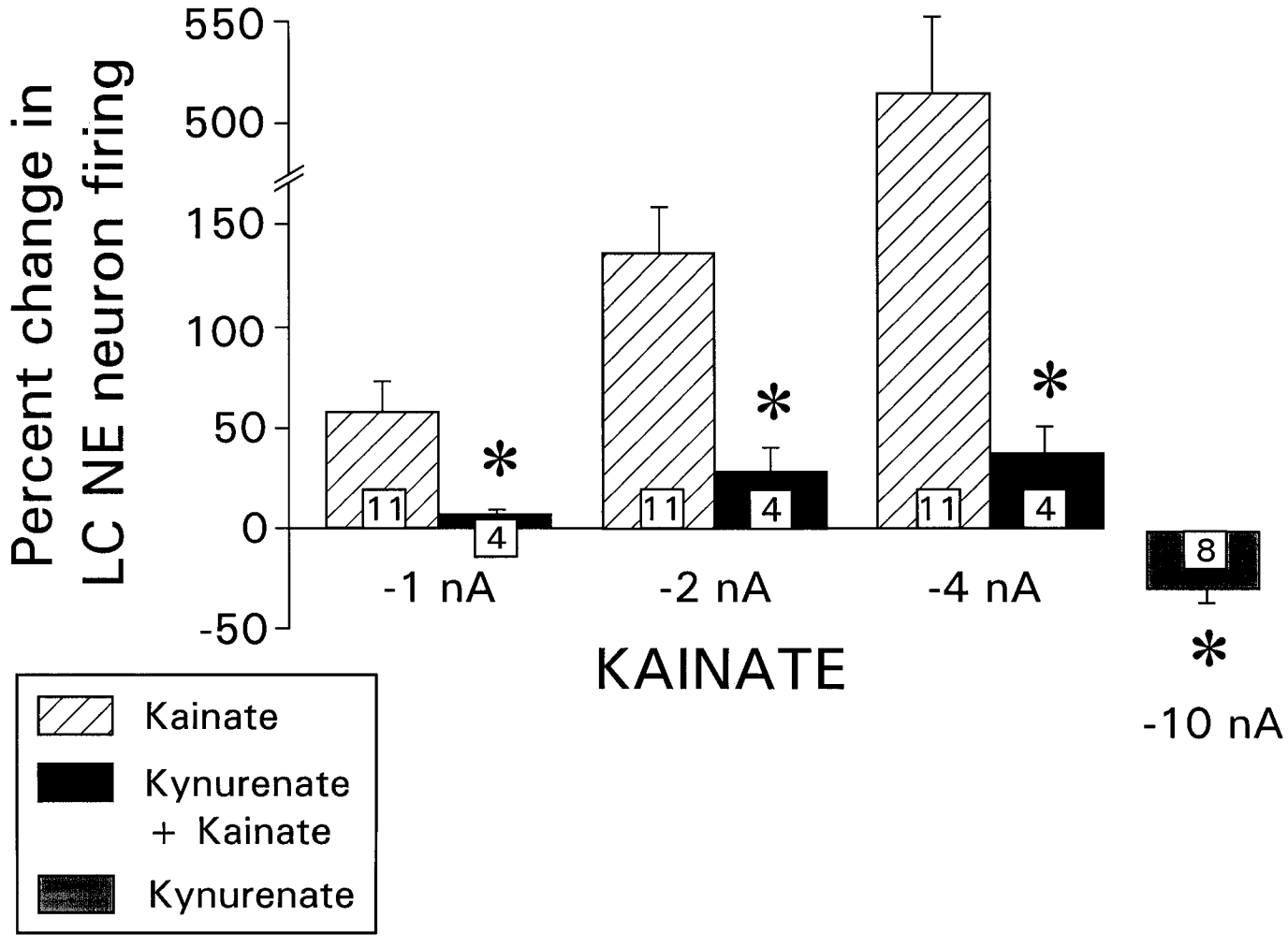
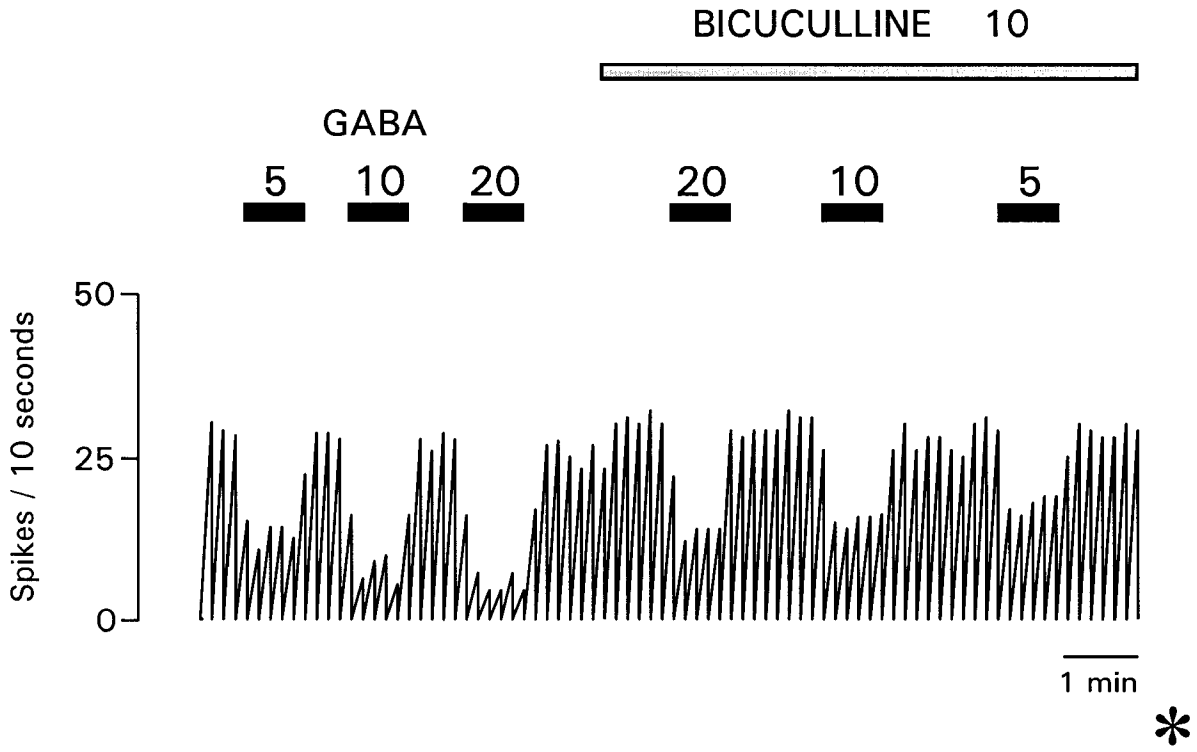

Percent change in LC NE neuron firing
550
500
150
100
50
0
-50
11
4
11
4
11
4
8
*
*
*
*
-1 nA
-2 nA
-4 nA
KAINATE
-10 nA
Kainate
Kynurenate + Kainate
Kynurenate

Figure IV.

A.


BICUCULLINE 10
GABA
5
10
20
20
10
5
50
25
0
Spikes / 10 seconds
1 min
*

B.

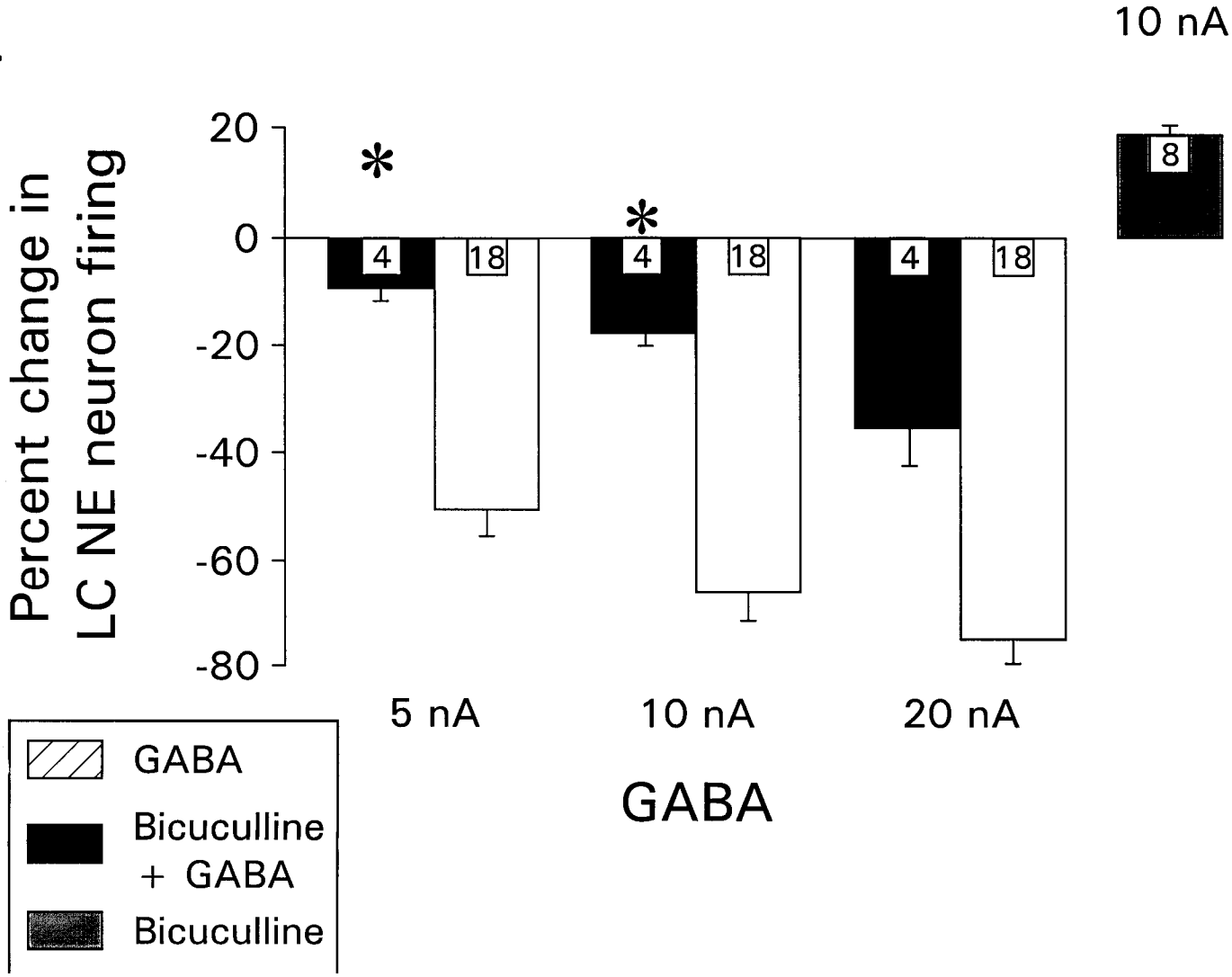
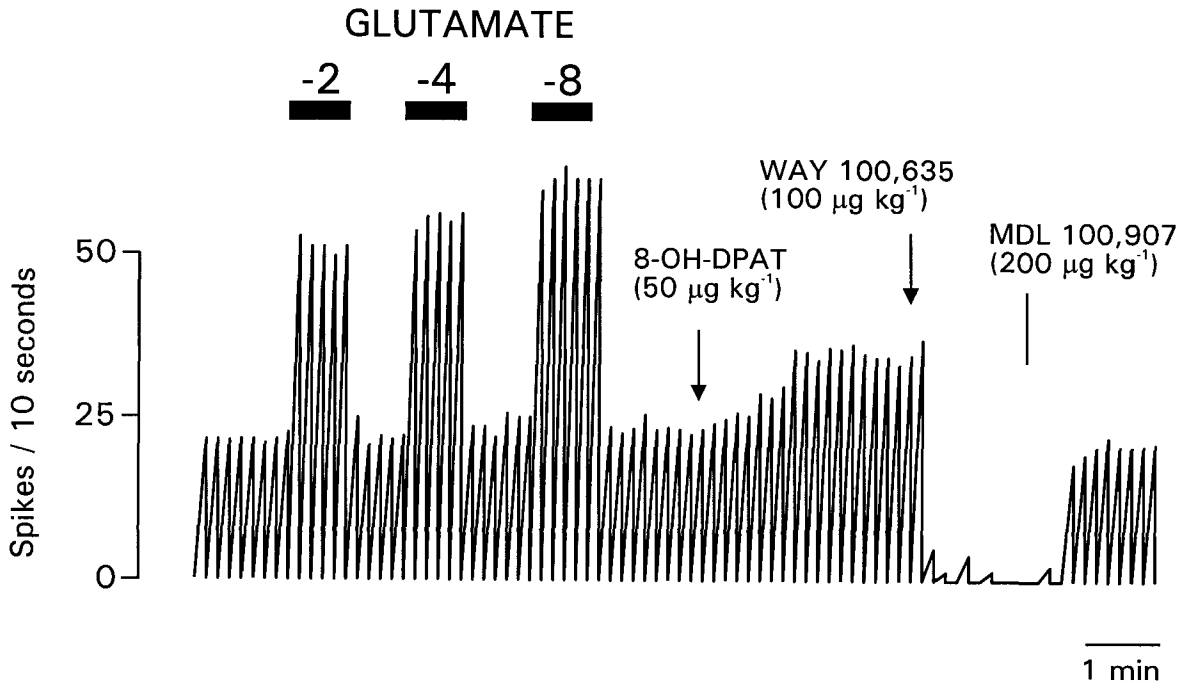

10 nA
8
20
0
-20
-40
-60
-80
Percent change in LC NE neuron firing
*
*
4
18
4
18
4
18
5 nA
10 nA
20 nA
GABA
GABA
Bicuculline + GABA
Bicuculline

Figure V.

A.


GLUTAMATE
-2
-4
-8
WAY 100,635
(100 μg kg-1)
MDL 100,907
(200 μg kg-1)
8-OH-DPAT
(50 μg kg-1)
Spikes / 10 seconds
50
25
0
1 min

B.

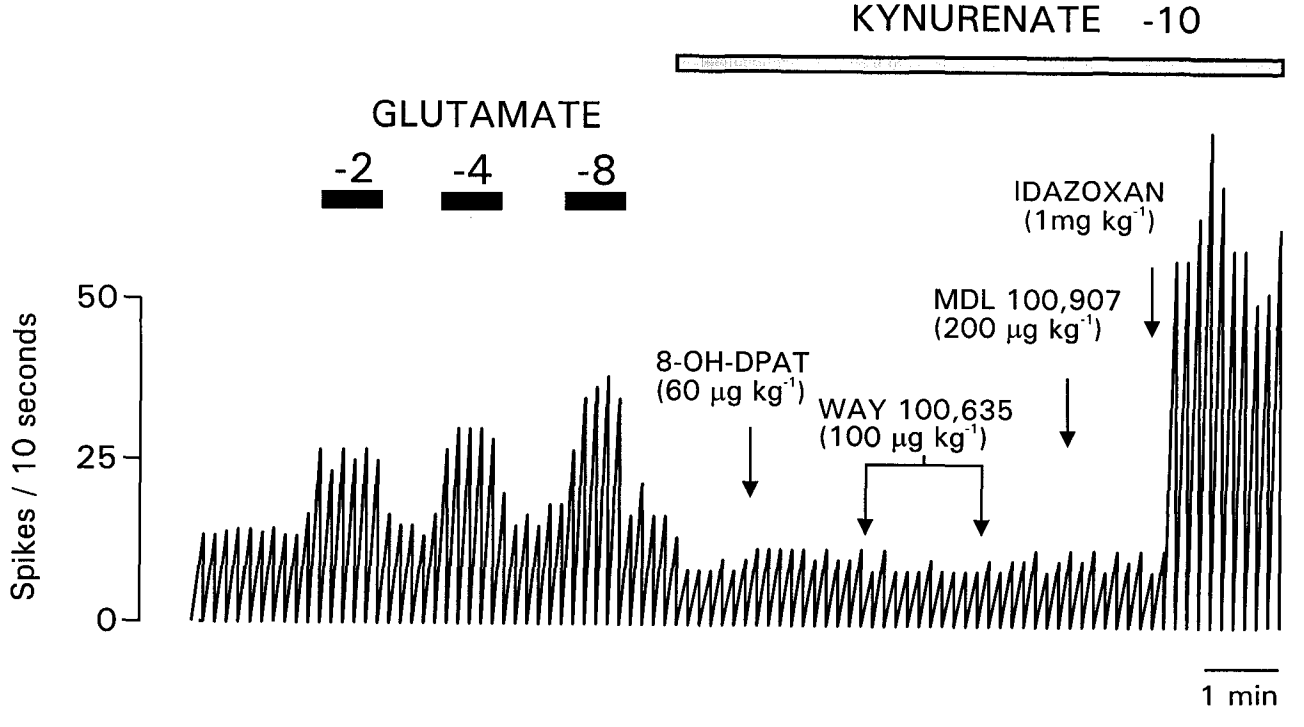

KYNURENATE -10
GLUTAMATE
-2
-4
-8
IDAZOXAN
(1mg kg-1)
MDL 100,907
(200 μg kg-1)
8-OH-DPAT
(60 μg kg-1)
WAY 100,635
(100 μg kg-1)
Spikes / 10 seconds
50
25
0
1 min

Figure VI.

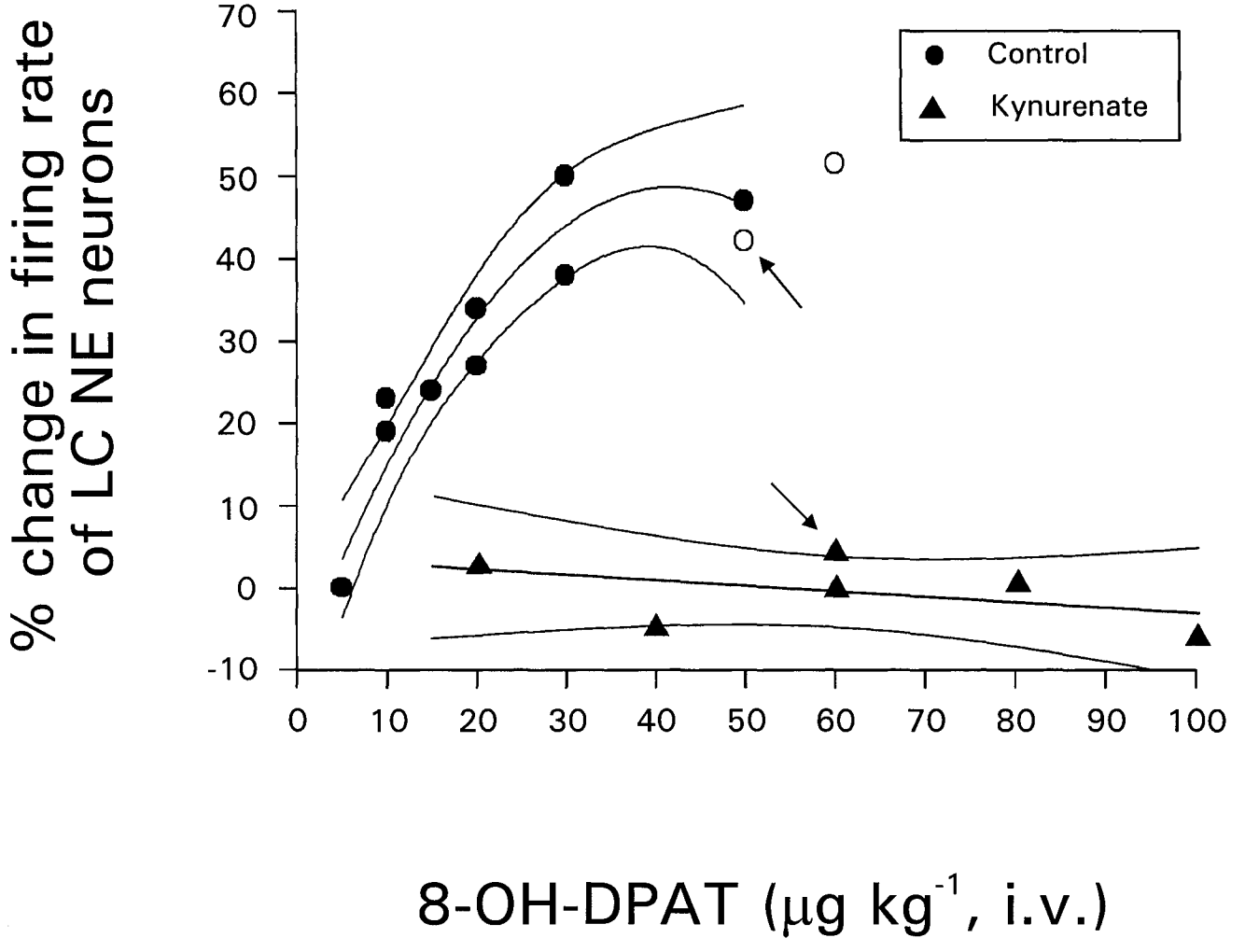
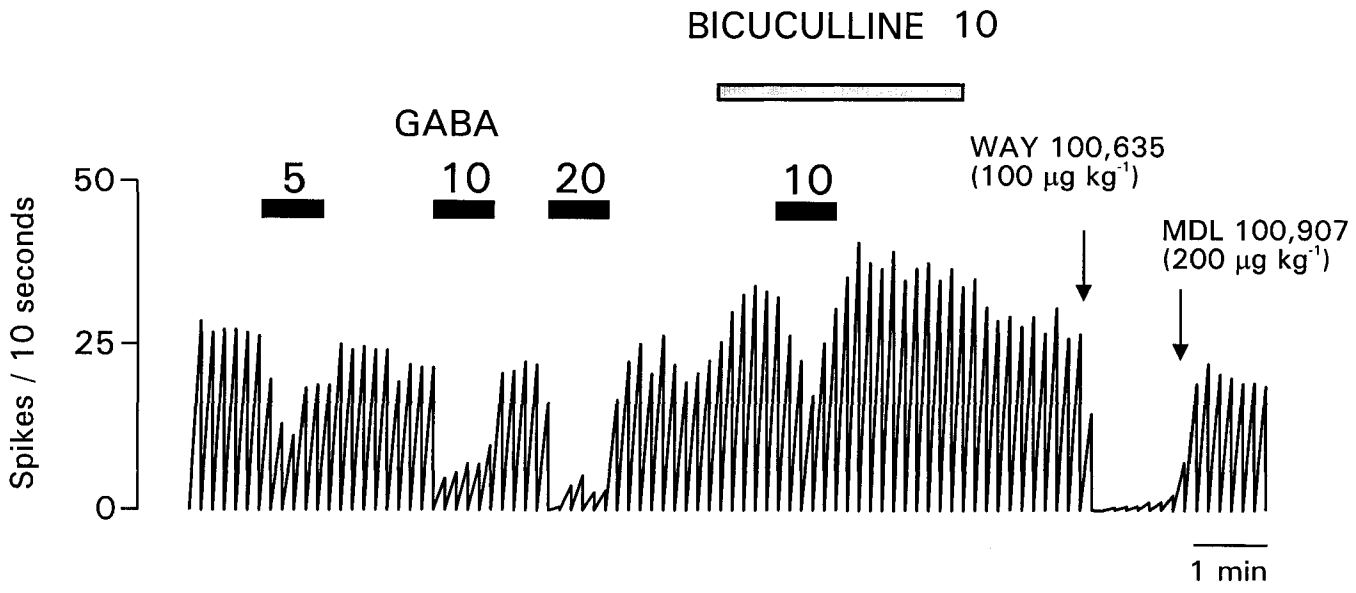

% change in firing rate of LC NE neurons
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100
Control
Kynurenate
8-OH-DPAT (μg kg-1, i.v.)

Figure VII.


BICUCULLINE 10
GABA
5
10
20
10
WAY 100,635
(100 μg kg-1)
MDL 100,907
(200 μg kg-1)
50
25
0
Spikes / 10 seconds
1 min

B.

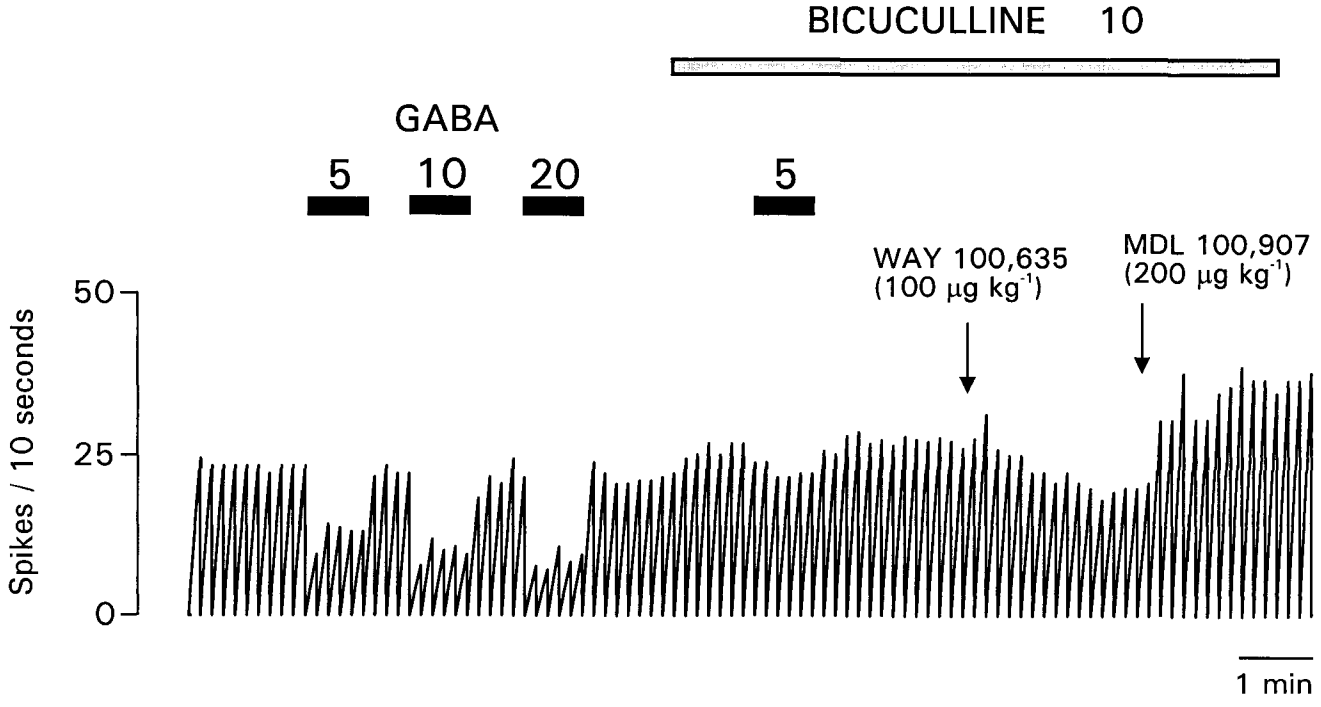
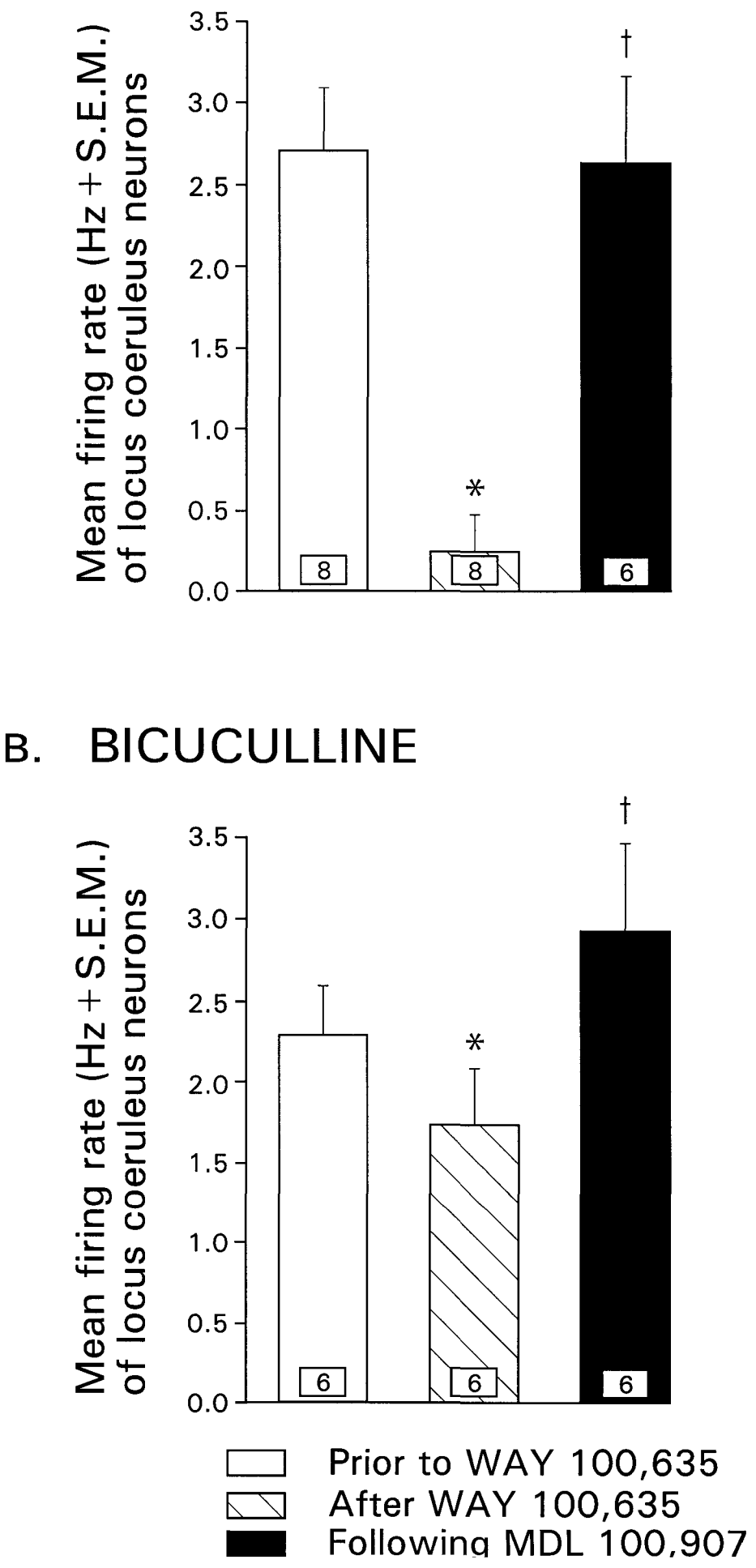

BICUCULLINE 10
GABA
5
10
20
5
WAY 100,635
(100 μg kg-1)
MDL 100,907
(200 μg kg-1)
50
25
0
Spikes / 10 seconds
1 min

Figure VIII.


A. CONTROL
Mean firing rate (Hz + S.E.M.) of locus coeruleus neurons
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
†
*
8
8
6
B. BICUCULLINE
Mean firing rate (Hz + S.E.M.) of locus coeruleus neurons
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
†
*
6
6
6
Prior to WAY 100,635
After WAY 100,635
Following MDL 100,907

CHAPTER VI: Fifth Article

PREFATORY REMARKS

The attenuated activity of LC NA neurons following a prolonged SSRI administration is due to an overactivation of 5-HT_{2A} receptors that enhance $GABA_A$ receptor activation in this nucleus. Given this, we assessed the effect of acute and sustained administration of 5-HTT and 5-HT_{2A} receptor blockade on the spontaneous firing activity of LC NA neurons with the potential antidepressant agent YM992. The effect of 8-OH-DPAT on LC activity was assessed in sustained YM992 treated rats to delineate whether these receptors become desensitized as other antidepressants tested thus far. The 5-HT_{2A} receptor antagonistic capability of YM992 was also assessed. This was accomplished by assessing if a prior injection of YM992 could attenuated a subsequent injection of DOI. The results with YM992 coupled with our circuitry will aid to elucidate the impact of blocking a key receptor implicated in mediating much of the effects of 5-HT on NA activity.

This article entitled "Effect of serotonin reuptake inhibition plus 5-HT_{2A} receptor antagonism on the firing activity of norepinephrine neurons" is a contribution by myself and Pierre Blier. Data in this manuscript was previously presented at the European College of Neuropsychopharmacology, 2000; and received a poster award.

EFFECTS OF SEROTONIN REUPTAKE INHIBITION PLUS 5-HT_{2A} RECEPTOR ANTAGONISM ON THE FIRING ACTIVITY OF NOREPINEPHRINE NEURONS[1, 2]

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Journal of Pharmaceutical and Experimental Therapeutics

Short Running Title: 5-HT Reuptake/5-HT_{2A} Receptor Blockade and NE Firing

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Non-Standard Abbreviations:
(S)-2-{{7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride (YM992); Locus Coeruleus (LC), Norepinephrine (NE), 5-Hydroxytryptamine (5-HT), Selective Serotonin Reuptake Inhibitor (SSRI)

Text Pages: 33
Tables: 1
Figures: 9
References: 45
Abstract: 259 words
Introduction: 423 words
Discussion: 1,528 words

Abstract

YM992 is a selective serotonin (5-HT) reuptake inhibitor (SSRI) and a potent 5-HT_{2A} receptor antagonist. The aim of the present study was to assess, using *in vivo* extracellular unitary recordings, the effect of acute and sustained administration of this drug (40 mg kg^{-1} day^{-1}, s.c. using osmotic minipumps) on the spontaneous firing activity of locus coeruleus (LC) norepinephrine (NE) neurons. The acute intravenous injection of YM992 (4 mg kg^{-1}) did not alter NE neuron firing activity, but blocked the inhibitory effect of a subsequent injection of the 5-HT_2 agonist DOI (40 - 100 μg kg^{-1}). A two-day treatment with YM992 decreased the firing rate of NE neurons by 66 %, whereas a partial recovery was observed after a 7-day treatment, and a complete one after 21-day treatment. The α_2-adrenoceptor antagonist idazoxan (1 mg kg^{-1}, i.v.) enhanced NE firing activity by 56% in controls and by 118% in short-term YM992 treated rats, thus putting into evidence an increased degree of activation of α_2-adrenergic autoreceptors in the treated rats. The suppressant effect of the α_2-adrenoceptor agonist clonidine was significantly decreased in long-term YM992 treated rats. The recovery of LC firing activity after long-term YM992 administration could thus be explained by a decreased sensitivity of α_2-adrenergic autoreceptors. Sustained SSRI administration leads to a gradual reduction of the firing activity of NE neurons during long-term administration, whereas YM992 produces the opposite effects. The exact biological basis for the increased synaptic availability of NE by YM992 remains to be elucidated, but this NE activity might confer additional therapeutic benefits in affective and anxiety disorders.

Keywords:antidepressant; locus coeruleus; SSRI; 5-HT_{1A} receptors; major depression; panic disorder

Introduction

The norepinephrine (NE) and the serotonin (5-HT) systems have both been implicated in anxiety and affective disorders. While the ethiopathology of these two disorders remain enigmatic, greater knowledge exists pertaining to the interactions/alterations of these monoaminergic systems during antidepressant drug treatment. It is well established that locus coeruleus (LC) NE neurons modulate the 5-HT system and evidence is accumulating for a major influence of 5-HT on the NE system (see Haddjeri et al., 1997 and Kaehler et al., 1999). The LC receives dense 5-HT projections coming from dorsal raphe and pericoerulear 5-HT neurons (Aston-Jones et al., 1991; Kaehler et al., 1999), which exert an inhibitory role (Léger and Descarries, 1978; Segal, 1979; McRae-Degueurce et al., 1985). This is supported by the observation that lesioning 5-HT neurons with a 5-HT neurotoxin produces a marked elevation of firing rate of NE neurons (Haddjeri et al., 1997). Previous research indicates that long-term, but not acute or short-term (2-day) administration of SSRIs decrease the spontaneous firing activity of LC NE neurons in the rat (Béïque et al.,1998; Szabo et al., 1999; Szabo et al., 2000a). This delayed reduction on NE neuron firing activity parallels the lag in onset of therapeutic action of antidepressant drugs in affective and anxiety disorders. Consequently, the therapeutic effect of drugs selective for the 5-HT system, like SSRIs, could thus be dependent on a modification of the efficacy of 5-HT transmission in the LC.

YM992 ((S)-2-{{7-fluoro-4-indanyl)oxy]methyl]morpholine monohydrochloride) is a novel SSRI agent (K_i= 21 nM) which enhances 5-HT neurotransmission after long-term (21-day) administration and possesses 5-HT_{2A} antagonistic properties (K_i= 86 nM;

Dong et al., 1999;Takeuchi et al, 1997). In addition to blocking 5-HT reuptake, YM992 may contribute to superior antidepressant and antipanic activities by immediately blocking the 5-HT_{2A} receptors. Indeed, 5-HT is believed to exert a tonic inhibitory action on the firing activity of NE neurons via a 5-HT_{2A} receptor (Haddjeri et al., 1997). Changes in NE function in various brain areas by antidepressant drugs may play a crucial role in controlling 5-HT output and NE/5-HT interactions and may thus be ultimately relevant to antidepressant efficacy, as well to their side effect profile.

The present studies were designed to characterize the effects of acute administration of YM992 on the spontaneous firing activity of LC NE neurons and verify its 5-HT_{2A} receptor antagonistic potential in this brain region. Sustained treatment regimens with YM992 were carried out in rats to assess whether their effect on the spontaneous firing activity of LC NE neurons differed from that previously obtained with SSRIs devoid of 5-HT_{2A} receptor antagonism.

Methods

Animals and Sustained Treatment

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water ad libitum). Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instuments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, rats were inserted with a catheter in a lateral tail vein for systemic i.v. injection of drugs.

In sustained treatment regimens, rats were anaesthetized with halothane containing a 2 to 1 O_2/N_2O mixture for subcutaneous implantation of osmotic minipumps (ALZA, Palo Alto, CA). The rats were tested with the minipumps in place in order to mimic the clinical condition whereby patients present an antidepressant response while taking their medication. Rats were treated with YM992 (40 mg kg^{-1} day^{-1}) or the saline vehicle for either 2, 7, or 21 days delivered by osmotic minipumps.

Electrophysiological experiments

Extracellular unitary recording of NE neurons were conducted with single-barrelled glass micropipettes preloaded with fiberglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1-3 μm and filled

with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for NE neurons recordings. NE neurons were recorded with micropipettes lowered at -0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active NE neurons of the LC were identified using the following criteria: regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. NE neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of LC NE neurons in the treated animals, four to five electrode descents were carried out through this nucleus in control and YM992 treated rats.

Dose effect curves for the alteration of NE neuron firing activity were obtained for systemic (i.v.) administration of YM992 and clonidine in untreated rats. After systemic injection of YM992, the preferential 5-HT_{2A} receptor antagonist DOI (Aulakh et al., 1995; Mazzola-Pomietto et al., 1995; Yamada et al., 1995) was administered in order to functionally assess the 5-HT_{2A} receptor blocking capability of YM992. In rats treated for 21-days with YM992, 8-OH-DPAT and clonidine were systemically administered and alterations in NE firing activity was assessed. Changes in the firing activity are expressed as percentages of the baseline firing rate and were measured after systemic drug administration. Dose-response curves were constructed for 8-OH-DPAT, DOI, and clonidine. However, in experiments where YM992 was systemically administered,

only one dose of DOI preceded by the YM992 pre-injection in each rat was used to generate an effective dose 50 (ED_{50}).

Drugs

The following drugs were used: YM992 from Yamanouchi Pharmaceutical Co. (Ibaraki, Japan), MDL 100,907 from Marion Merrell Dow Inc. (Cincinnati, OH, U.S.A.), 5,7-dihydroxytryptamine (5,7-DHT), creatinine sulphate from Sigma Chemical (St. Louis, MO, U.S.A.), 8-OH-DPAT, DOI, clonidine, ritanserin, and idazoxan from RBI (Natick, MA, U.S.A.). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water while ritanserin was dissolved dropwise using acetic acid and then titrated with distilled water to the appropriate concentration.

Statistical comparisons

All results were expressed as mean (± S.E.M.) of single neuron values. Statistical comparisons of the number of NE neurons recorded per descent into the locus coeruleus and spontaneous firing activity obtained in treated and control rats were carried out using Kruskal-Wallis one-way analysis of variance on ranks. Dunn's multiple comparison test was used to assess the difference between controls and treated groups. Analysis of the effects of idazoxan on NE neuron firing activity before and after injection in control and YM992 treated rats was performed with a Student's t test. Correlational coefficients (*r* values) for the dose-response relationship observed in the

LC were calculated using simple linear regression analysis. The SEM for the ED_{50} values were calculated by regression analysis, with the Y value of 50 used as the regressor. Difference between the two regressions were assessed by comparing their ED_{50} values using the confidence intervals method, when indicated. The 95% confidence limit was determined from the Student's *t* distribution.

Results

Effect of acute YM992 administration on the firing activity of NE neurons and its ability to block the inhibitory action of DOI

Acute administration of the SSRI paroxetine or fluoxetine does not alter the firing activity of LC neurons (Béïque, et al., 1999). However, due to the unique capacity that YM992 possesses in selectively blocking both 5-HT reuptake and 5-HT_{2A} receptors, YM992 was injected while recording NE neurons to assess the effect of 5-HT_{2A} receptor blockade in the presence of 5-HT reuptake inhibition. Inspite of 5-HT_{2A} antagonism, acute systemic administration of YM992 at a dose (4 mg kg^{-1}) previously reported to completely inhibit dorsal raphe 5-HT neuron firing activity (Dong et al., 1999), did not alter the firing activity of NE neurons (figure 1). Identical to results previously obtained with the selective 5-HT_{2A} antagonist MDL 100,907 (Kehne et al.,1996; Szabo et al., 2000b), a prior administration of YM992 (4 mg kg^{-1}, i.v.) blocked the suppressant effect of a subsequent injection of DOI (30 - 120 μg kg^{-1}, i.v.; figure 2). After DOI injections, the selective α_2-adrenoceptor agonist or antagonist clonidine or idazoxan, respectively, were injected whenever possible in order to demonstrate the NE nature of neuron tested (see figure 1).

Effect of sustained YM992 administration on the firing activity of LC NE neurons

Five systematic electrode descents into the LC nucleus were carried out in rats treated with YM992 (40 mg kg^{-1} day^{-1}) for 2, 7, or 21 days as well as in their respective

controls. An example of each is provided in figure 3. Since control groups treated with saline for varying durations did not differ from each other with respect to LC spontaneous firing activity, these data were therefore merged to make up a single control group (range of firing: 1.2 to 4.1 Hz). Short-term (2-day) administration of YM992 resulted in a significant 66% decrease (range of firing: 0.2 to 1.9 Hz) in the spontaneous firing activity of NE neurons when compared to controls. There was a partial recovery of LC firing activity after a 7-day YM992 treatment and when compared to control values, the decrease was then of 43% (range of firing: 0.3 to 2.8 Hz). Long-term (21-days) YM992 administration lead to a complete recovery (range of firing: 0.3 to 5.3 Hz) in the spontaneous firing activity of LC neurons, as indicated by a non-significant difference compared to the control rat values (figure 4). Analysis of the number of spontaneously active neurons in YM992 treated rats and control rats did not reveal significant differences (table 1).

Assessment of the responsiveness of the somatodendritic α_2-adrenoceptors on NE neuron firing activity in sustained YM992 treated rats.

The α_2-adrenoceptor located on the cell body of LC NE neurons is important in the negative feedback regulation of the firing activity of these neurons (Freedman et al., 1984; Mateo et al., 1998). Systemic injection of the selective α_2-adrenoceptor antagonist idazoxan (1 mg kg^{-1}, i.v.) enhanced to a greater degree the firing activity of attenuated NE neurons in rats treated with YM992 for 2-days (118%) as compared to the control treated group (56%; n = 5 for each group). Examples of such experiments

are provided in figure 5. The selective α_2-adrenoceptor agonist clonidine decreases the firing activity of NE neurons (Svensson et al., 1975; Adams et al., 1988; Mongeau et al., 1998). Using a dose which interrupts the firing activity of NE neurons in control rats, clonidine (10 $\mu g\ kg^{-1}$) produced a lesser effect in a 21-day YM992 treated rat as illustrated in figure 6. In addition, idazoxan (1 $mg\ kg^{-1}$, i.v.) was able to increase the firing activity after a previous injection of clonidine in control and YM992 treated rats (*n* = 7 for each group). Upon inspection of the clonidine dose-response curves, that for the YM992-treated group was significantly shifted to the right in comparison to the control curve (figure 7).

Effect of intravenous administration of 8-OH-DPAT on the firing activity of NE neurons in YM992-treated rats

The prototypical 5-HT_{1A} agonist 8-OH-DPAT augments the spontaneous firing activity of NE neurons (Piercey et al., 1993; Szabo et al., 2000a). However, a 21-day treatment with citalopram abolishes the excitatory effect of 8-OH-DPAT on NE neuron firing activity, thus indicating that drugs which selectively block 5-HT reuptake desensitize various populations 5-HT_{1A} receptors. Systemic injection of 8-OH-DPAT produced a dose-dependent increase in the firing activity of NE neurons (figure 8A) and yielded an ED_{50} of 15 $\mu g\ kg^{-1}$ (figure 9). As predicted, rats treated with 40 $mg\ kg^{-1}\ day^{-1}$ of YM992 for 21 days did not present any incremental effect to 8-OH-DPAT injection (figure 8B). The enhancing action of 8-OH-DPAT (30 - 120 $\mu g\ kg^{-1}$, i.v.) on NE neuronal

firing activity in 21-day YM992 treated rats was abolished, even at extremely high doses (figure 9).

Discussion

The present study revealed that the SSRI/5-HT_2 antagonist YM992 failed to alter the firing activity of NE neurons but abolished the suppressant effect of a subsequent injection of the 5-HT_2 receptor agonist DOI. Although DOI has affinity for 5-HT_{2A} and 5-HT_{2C} receptors, its action on NE neurons is likely mediated via the 5-HT_{2A} receptor subtype because the selective 5-HT_{2A} antagonist MDL 100,907 antagonizes it as well (Szabo et al, 2000b). Based on these results, YM992 (4 mg kg^{-1}, i.v.) is an effective 5-HT_{2A} receptor antagonist able to block the inhibitory effects of DOI in the LC, even at doses up to 120 μg kg^{-1} (figure 2). In contrast, YM992 does not alter the responsiveness of medial prefrontal cortex neurons to DOI, whereas the 5-$HT_{2A/2C}$ receptor antagonist ritanserin was effective (Dong et al., 1999). This apparent discrepancy in the 5-HT_{2A} receptor antagonistic capability of YM992 may be explained by the heterogeneous pharmacological profile of 5-HT_2 receptors (El Mansari and Blier, 1997 and Bergqvist et al., 1999). For instance, ritanserin and YM992 reverse the inhibitory effects of DOI (Rasmussen and Aghajanian, 1986; Chiang et al., 1993; Szabo et al., 2000a) while the former drug also produces a small (16%) increase in LC firing activity (VanderMaelen et al., 1992). These effects of ritanserin are presumably mediated through 5-HT_{2A} and 5-HT_{2C} receptor antagonism, respectively. Because YM992 did not block the effects of DOI in the medial prefrontal cortex, the effects of the latter drug may thus be mediated by 5-HT_{2C} receptors. However, the possibility of an edited form of the 5-HT_2 receptor has been proposed and cannot at present be ruled out

(Fitzgerald et al., 1999; Rueter et al., 2000). Thus, the 5-HT_2 receptors which mediate the effect(s) of DOI vary considerably with respect to different brain regions.

The lack of acute effect of YM992 on NE neuron firing activity is consistent with similar results obtained with SSRIs (Béïque et al.,1999). Although, when rats were treated with YM992 (40 mg kg^{-1} day^{-1}) for a period of 2 days, the firing activity of NE neurons was markedly decreased (figure 3 and figure 4). This effect of YM992 on LC firing activity was unexpected as it differed from that observed in rats treated for 2 days with paroxetine and citalopram, antidepressants which selectively block 5-HT reuptake and leave LC firing activity unaltered (Szabo et al., 1999). Interestingly, this decrease in LC firing activity after 2-day YM992 administration (66%) was in the range of that reported with NE reuptake blocking agents desipramine (10 mg/kg/day; *ca* 70%) and reboxetine (2.5 mg/kg/day; *ca* 68%; Szabo et al., 2000a; Szabo et al., 2000c). In light of this, 2-day YM992 treated rats were challenged with the α_2-adrenoceptor antagonist idazoxan (1 mg kg^{-1}, i.v.), and the firing activity of NE neurons increased (118%), that is within the physiological control firing range. In control rats, a more modest (56%) increase in firing activity resulted. It thus appears that a 2-day YM992 administration increases NE synaptic availability, which consequently overactivates α_2-autoreceptors located on LC neurons to produce a suppression on NE neuronal firing activity. It would be expected that if the decrease in NE neuron firing activity resulted from an action different from an increase in NE availability, the relative effect of idazoxan would be blunted due to attenuated NE release resulting from suppressed NE neuron firing. Recently, Takeuchi et al., 2000, reported an increase of NE in dialysates collected from

the rat frontal cortex with YM992, citalopram plus MDL 100,907, but not with citalopram or MDL 100,907 alone. These findings are in accord with electrophysiological data demonstrating that 5-HT agents such as MDL 100,907 and SSRIs, when given alone fail to alter NE firing activity upon acute administration (Béïque et al.,1999). Also, the increase in extracellular NE produced with YM992 is consistent with the effects on firing activity observed with desipramine (Thomas et al., 1991; Perry and Fuller, 1997; Mateo, et al., 1998) and reboxetine (Sachetii et al., 1999), although it is certainly not achieved by blocking NE reuptake as is the case with the latter two drugs. This striking difference in the effect of 2-day YM992 and SSRI treatments on LC firing activity seems to be solely attributed to 5-HT_{2A} receptor antagonism in the presence of 5-HT reuptake blockade. The exact neurobiological basis for this peculiar action of YM992 remains to be elucidated.

When treatment duration with YM992 was further increased to 7 and 21 days, the firing rate of NE neurons increased and reached the control range (table 1 and figure 4). In 21-day YM992 treated rats, the dose-response curve for the α_2-adrenoceptor agonist clonidine was significantly shifted to the right with an ED_{100} six times greater than obtained in control animals (figure 7). Prolongation of YM992 treatment from 2 to 21 days therefore desensitize somatodentritic α_2-adrenoceptors presumably due to an increase in NE concentration, ultimately resulting in normalization of LC firing activity. This effect is different from that observed in desipramine or reboxetine treated animals where the firing rate of NE neurons was still attenuated and the inhibitory response to clonidine was either normal or slightly decreased after a 14- to 21-day treatment

(Lacroix et al., 1990; Szabo et al., 2000c). Presumably, the firing rate of the NE neurons recovered in the presence of YM992 because this drug does not block NE reuptake, obviously a major mechanism by which NE is removed from the extracellular milieu, particularly in the LC which contains the highest density of NE transporters. It still remains puzzling why this adaptative desensitization does not occur to a significant extent in the presence of NE reuptake and MAO inhibition (Blier and de Montigny, 1985; Lacroix et al., 1991; Szabo et al, 2000c).

In addition to its indirect adrenergic effects, YM992 abolished the incremental action of the selective 5-HT_{1A} receptor agonist 8-OH-DPAT on LC firing activity, similar to antidepressants selective for 5-HT or NE reuptake blockade (Szabo et al., 2000a; Szabo et al., 2000c). Indeed, the desensitization of somatodendritic 5-HT_{1A} autoreceptors has been a proposed mechanism of action of SSRIs (Blier and de Montigny, 1994). This phenomenon has been well documented for the desensitization of 5-HT_{1A} autoreceptors which impart a negative feedback influence on 5-HT neuron firing activity following long-term SSRI treatment including YM992, but not for drugs which blocks NE reuptake such as desipramine (Blier & de Montigny, 1980; Blier & de Montigny, 1994; Dong et al., 1999) or reboxetine (Szabo et al., 2000c). As with other SSRIs, YM992 induces an attenuation and normalization in 5-HT neuron firing activity after 2- and 21-day treatments, respectively, which is attributed to the initial overactivation of somatodendritic 5-HT_{1A} receptors and subsequent desensitization of these receptors in the latter treatment group (Dong et al., 1999). In addition, it also appears that drugs which block 5-HT or NE reuptake produce a desensitization of 5-HT_{1A} receptors which control LC firing activity. This phenomenon is common to all

major classes of antidepressant drugs tested thus far and may reflect an important finding with respect to the treatment of anxiety and affective disorders. These 5-HT_{1A} receptors are probably not the 5-HT_{1A} autoreceptor controlling 5-HT neuron firing activity. For instance, the selective 5-HT_{1A} antagonist WAY 100,635 at a dose of 0.1 mg kg^{-1}, i.v., does not alter the firing rate of 5-HT neurons, but shuts off that of LC neurons. In 5-HT lesioned rats, this inhibitory effect of WAY 100,635 on NE neuron firing rate is abolished. It therefore appears that an intact 5-HT system is also necessary to produce the augmentation effect of 8-OH-DPAT on LC firing activity (Szabo et al., 2000b). Further research will be needed to determine the exact location of the 5-HT_{1A} receptor which, for example, could be located on projection neurons feeding onto a 5-HT terminal (Szabo et al., 2000b).

YM992 behaves as an SSRI on dorsal raphe 5-HT neurons during acute and sustained treatments (Dong et al., 1999). Unlike SSRIs, YM992 decreases NE firing activity upon a sustained 2-day administration which is normalized after 21-days. This rapid decrease in NE firing activity may aid in alleviating the initial exacerbation of panic disorder symptoms generally observed in panic disorder patients with usual starting doses of SSRIs for major depression (Westenberg et al., 1996). Indeed, the enhancement of NE neuron firing and release achieved with the α_2-adrenoceptor antagonist yohimbine can produce anxiety in health volunteers and tigger panic attacks in patients with panic disorder (Charney et al., 1984). In contrast to SSRIs, which can decrease the firing activity of NE by as much as 50% upon long-term administration (Szabo et al., 1999), YM992 may guard against a lethargic effect sometimes produced

with SSRIs by keeping a normal NE tone (Blier, 2000). Consequently, this normalization of NE neuron firing activity, together with 5-HT_{2A} receptor antagonism, may also prove beneficial in preventing the sexual dysfunctions which commonly plague SSRIs. The normal firing activity of LC and DR neurons in the presence of increased 5-HT and NE synaptic availability may contribute to a decreased side-effect profile and an increase in efficacy as reported with drugs that increase both 5-HT and NE concentrations (Danish University Antidepressant group,1986; Danish University Antidepressant group, 1990; Einarson et al., 1997; Poirier & Boyer, 1999; Silverstone & Ravindran, 1999).

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Legends to the Figures

Figure 1. Integrated firing rate histogram of single locus coeruleus NE neurons illustrating the effects of intravenous administration of various compounds on spontaneous discharge frequency. The preferential 5-HT_{2A} agonist DOI produced an attenuation in the firing activity of a LC NE neuron in a naive rat while a subsequent injection of the 5-$HT_{2A/2C}$ antagonist of ritanserin reversed that effect (A). In (B), the intravenous administration of the SSRI/5-HT_{2A} antagonist YM992 and a subsequent injection of DOI failed to produce an alteration in neuron firing rate (n = 5)(B). Note that a final injection of the α_2-adrenoceptor agonist clonidine or of the α_2-adrenoceptor antagonist idazoxan produced the expected effects normally observed in naive animals. The dotted lines in between neurons indicate approximately a 15-minute time laps.

Figure 2. Relationship between the degree of suppression of LC NE firing activity and doses of the 5-HT_2 agonist DOI administered intravenously in controls and YM992 pre-injected rats. Only the initial response of a single NE neuron to the first dose of DOI in one rat was used to construct the curves. Outer lines represent the standard error of the regression line.

Figure 3. Integrated firing rate histograms of LC NE neurons, recorded in single electrode descents in the locus coeruleus showing their spontaneous firing activity in control (A) and in rats treated with 40 mg kg^{-1} day^{-1} of YM992 for 2- (B), 7- (C), and 21-

days (D). The dotted lines in between neurons indicate approximately a 5-minute time lap. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded. The time base applies to all four traces.

Figure 4. Effects of 2-, 7- and 21-day YM992 treatments (40 mg kg^{-1} day^{-1}; n = 3 rats for each treatment group) on the firing activity of LC neurons. The horizontal rectangle represents the range (SEM x 2) of the mean firing activity of neurons recorded in control rats. *P < 0.05 (Dunn's Method) when compared to the control value. The number of neurons recorded is displayed in each box.

Figure 5. Integrated firing rate histograms of locus coeruleus NE neurons illustrating the effects of intravenous administration of the selective α_2-adrenoceptor antagonist idazoxan augmenting the firing activity in a control rat (n = 7)(A) and a YM992 treated rat (n = 6)(B). Note that after a prior injection of idazoxan, the 5-HT_2 agonist DOI decreased firing, the selective 5-HT_{1A} agonist 8-OH-DPAT was able to enhance firing and the selective 5-HT_{1A} WAY100635 shut down firing activity of this NE neuron.

Figure 6. Integrated firing rate histograms of LC NE neurons illustrating the effect of intravenous administration of the selective α_2-adrenoceptor agonist clonidine in suppressing firing activity. In (A), subsequent injections of the selective 5-HT_{1A} agonist 8-OH-DPAT partly restored the firing activity. In (B), idazoxan completely reversed the

inhibitory effect of clonidine (n = 7). Note that the effect of clonidine is blunted in the YM992 treated rat when compared to the control.

Figure 7. Relationship between the degree of suppression of LC NE firing activity and doses of clonidine administered intravenously in controls and YM992 treated rats. Only the initial response of a single NE neuron to the first dose of clonidine in every rat was used to construct the curves. Outer lines represent the standard error of the regression line.

Figure 8. Integrated firing rate histogram of locus coeruleus NE neurons illustrating the enhancing effect of intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT on firing activity in a control rat (A) and in a rat treated with YM992 (40 mg kg^{-1} day^{-1}; n = 6)(B). The identity of the NE neuron recorded in the YM992 treated rat was assessed by showing the enhancing effect on firing activity of a subsequent intravenous administration of the α_2-adrenoceptor agonist idazoxan on NE firing activity (n = 3).

Figure 9. Relationship between the degree of augmentation of locus coeruleus NE firing activity and doses of 8-OH-DPAT administered intravenously in controls and YM992 treated rats. Only the initial response of a single NE neuron to the first dose of 8-OH-DPAT in every rat was used to construct the curves. Outer lines represent the standard error of the regression line.

Table 1. Firing activity of Locus Coeruleus noradrenergic neurons in controls and treated rats

		Average number of noradrenergic neurons per descent	No. of descents
Control		3.6 ± 0.4	14
YM992	(40 mg/kg/day)		
	2 days	3.0 ± 0.5	20
	7 days	4.2 ± 0.6	12
	21 days	3.6 ± 0.5	18

P= 0.258, NO STATISTICAL DIFFERENCE among treatment groups using Kruskal-Wallis One-Way Analysis of Variance on Ranks

Figure I.

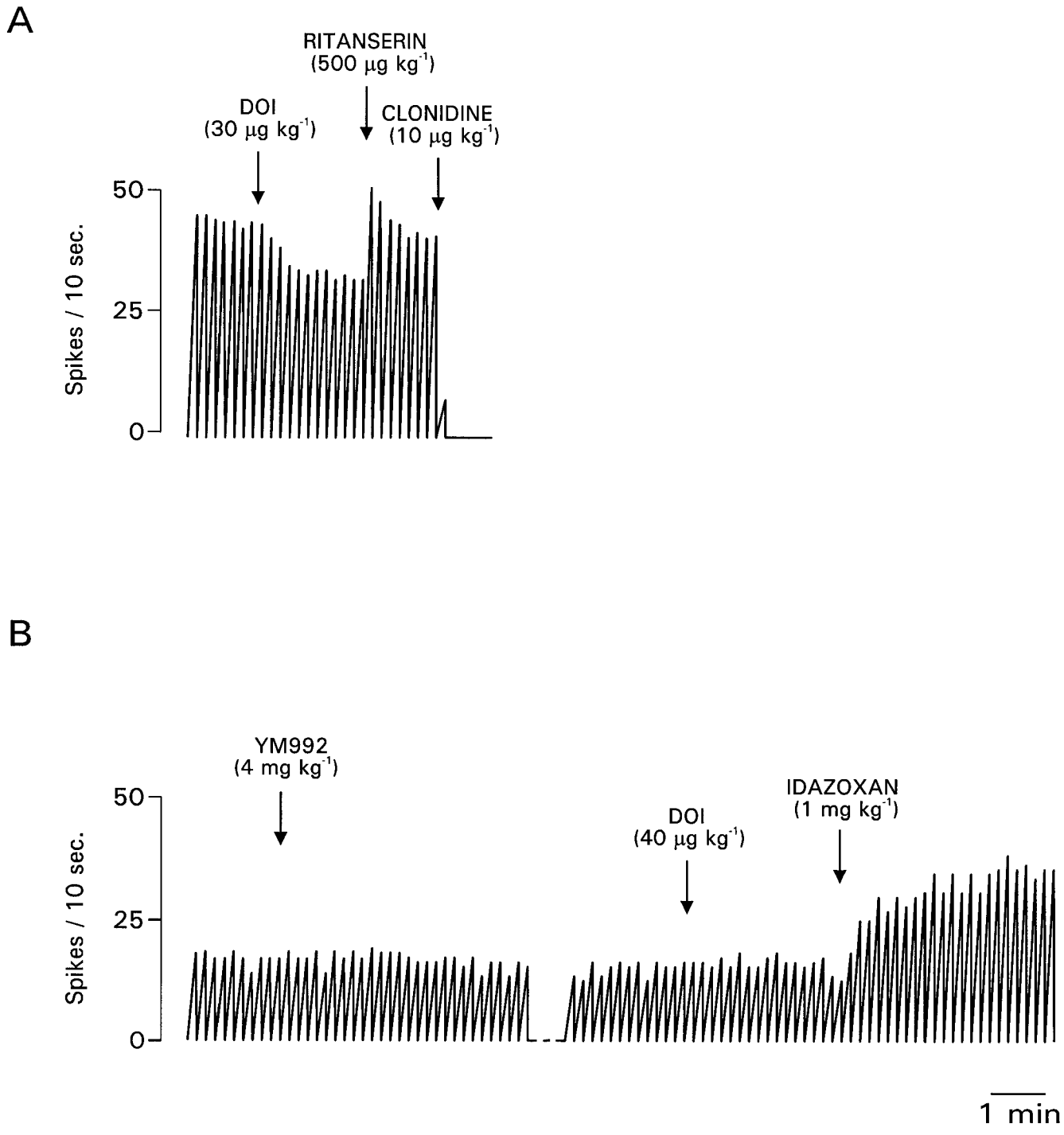

A
RITANSERIN
(500 μg kg-1)
DOI
(30 μg kg-1)
CLONIDINE
(10 μg kg-1)
50
25
0
Spikes / 10 sec.
B
YM992
(4 mg kg-1)
DOI
(40 μg kg-1)
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure II.

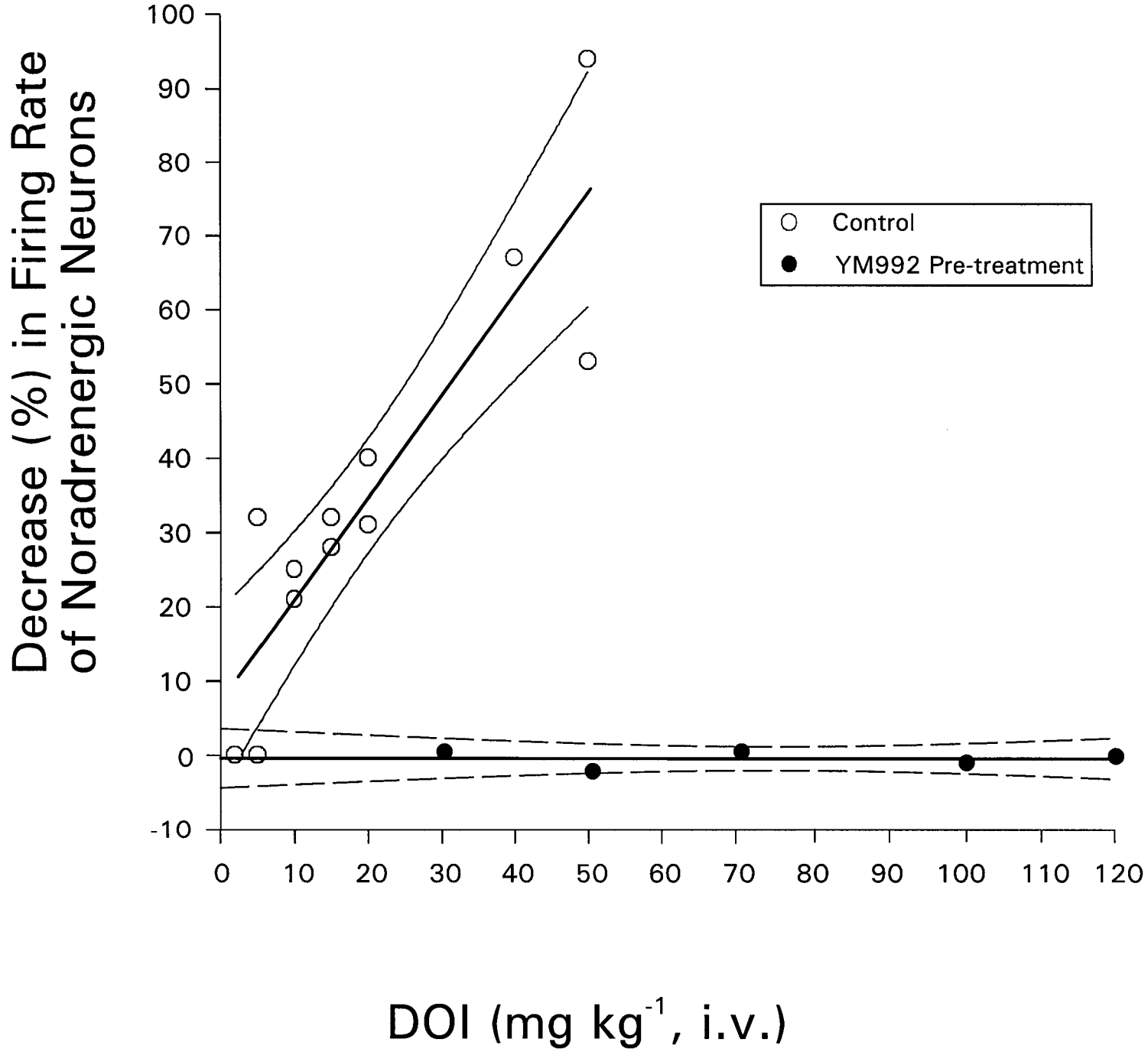

Decrease (%) in Firing Rate of Noradrenergic Neurons
100
90
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
Control
YM992 Pre-treatment
DOI (mg kg-1, i.v.)

Figure III.

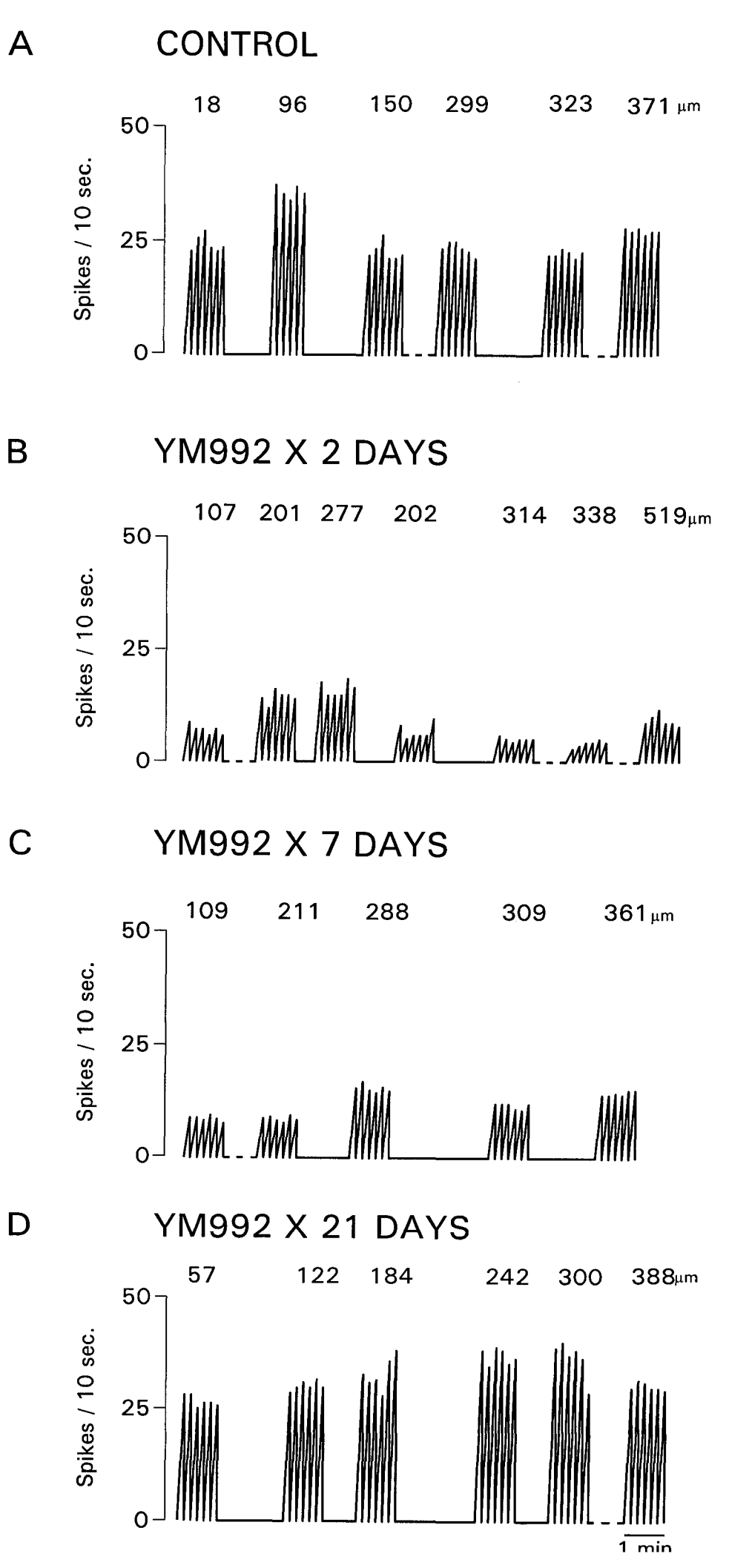

A
CONTROL
18
96
150
299
323
371 µm
50
25
0
Spikes / 10 sec.
B
YM992 X 2 DAYS
107
201
277
202
314
338
519µm
50
25
0
Spikes / 10 sec.
C
YM992 X 7 DAYS
109
211
288
309
361 µm
50
25
0
Spikes / 10 sec.
D
YM992 X 21 DAYS
57
122
184
242
300
388µm
50
25
0
Spikes / 10 sec.
1 min

Figure IV.

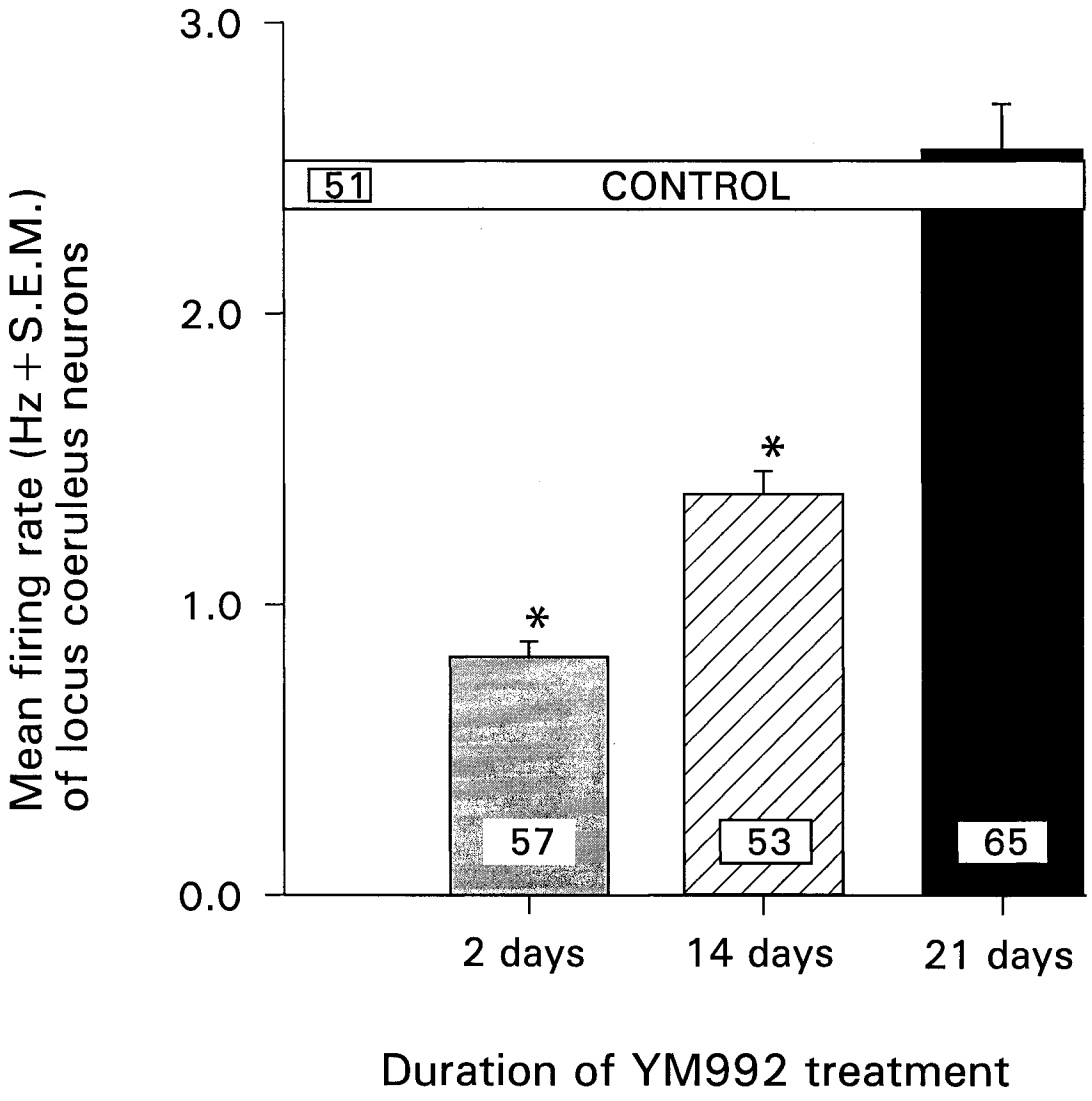

3.0
2.0
1.0
0.0
Mean firing rate (Hz + S.E.M.) of locus coeruleus neurons
51
CONTROL
*
*
57
53
65
2 days
14 days
21 days
Duration of YM992 treatment

Figure V.

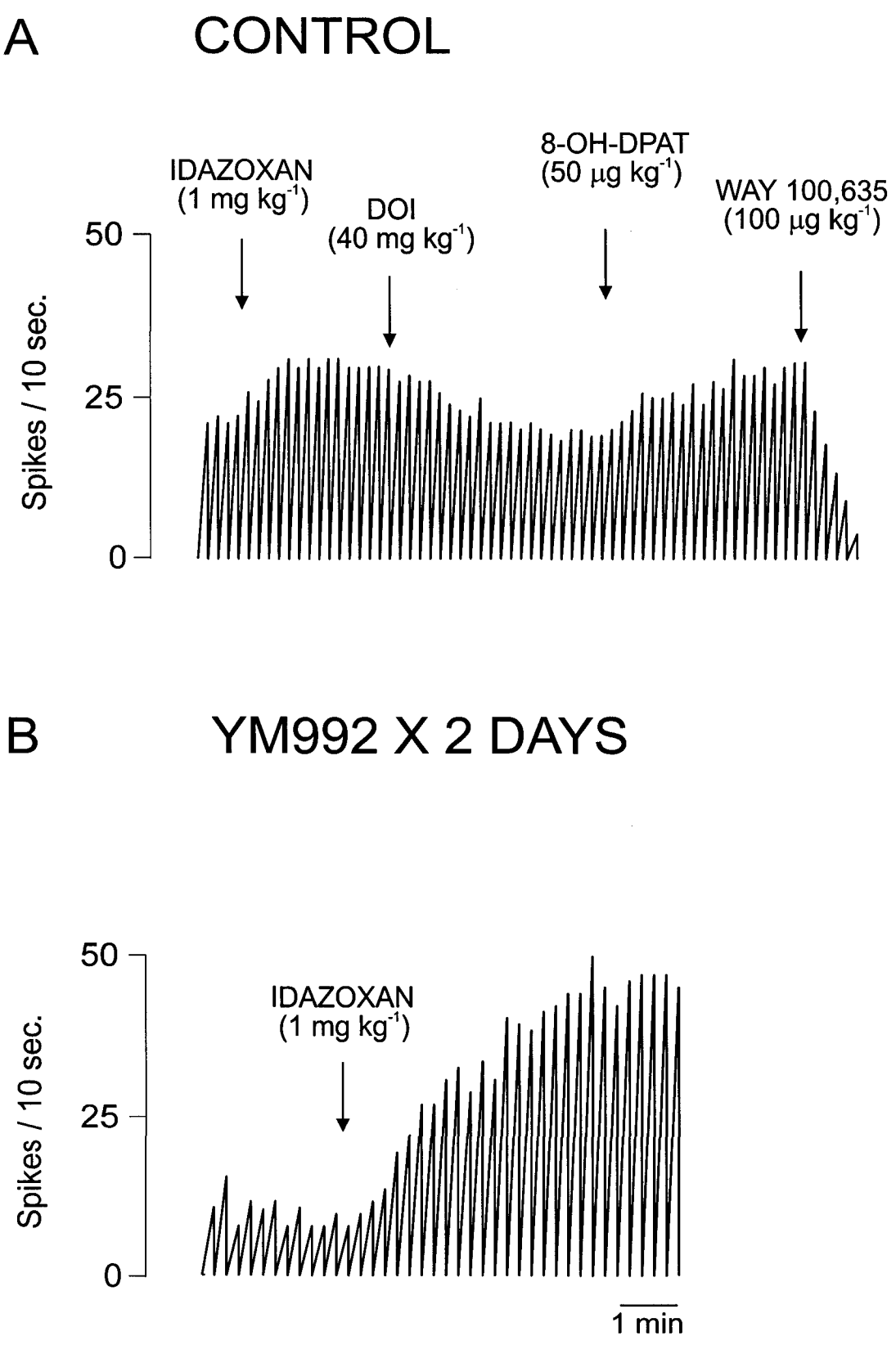
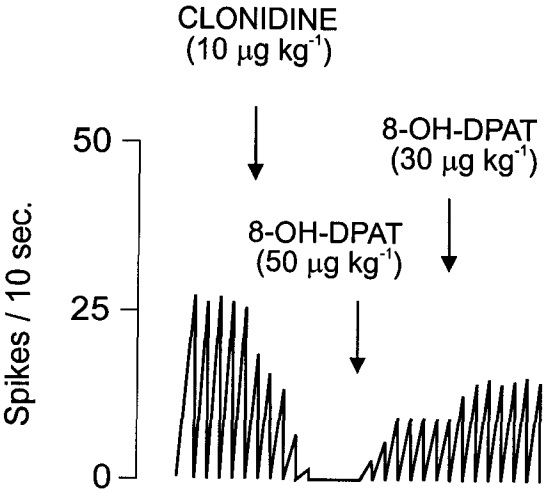

A
CONTROL
IDAZOXAN
(1 mg kg-1)
DOI
(40 mg kg-1)
8-OH-DPAT
(50 µg kg-1)
WAY 100,635
(100 µg kg-1)
50
25
0
Spikes / 10 sec.
B
YM992 X 2 DAYS
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure VI.

A

CONTROL



B

YM992 X 21 DAYS

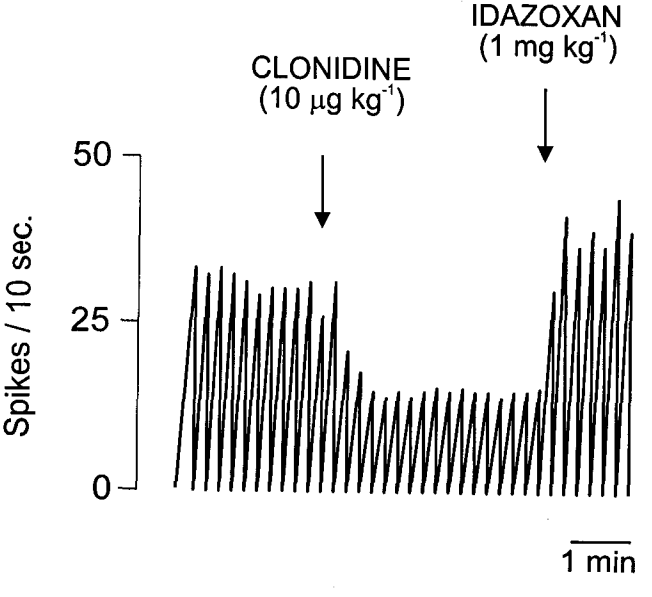


Figure VII.

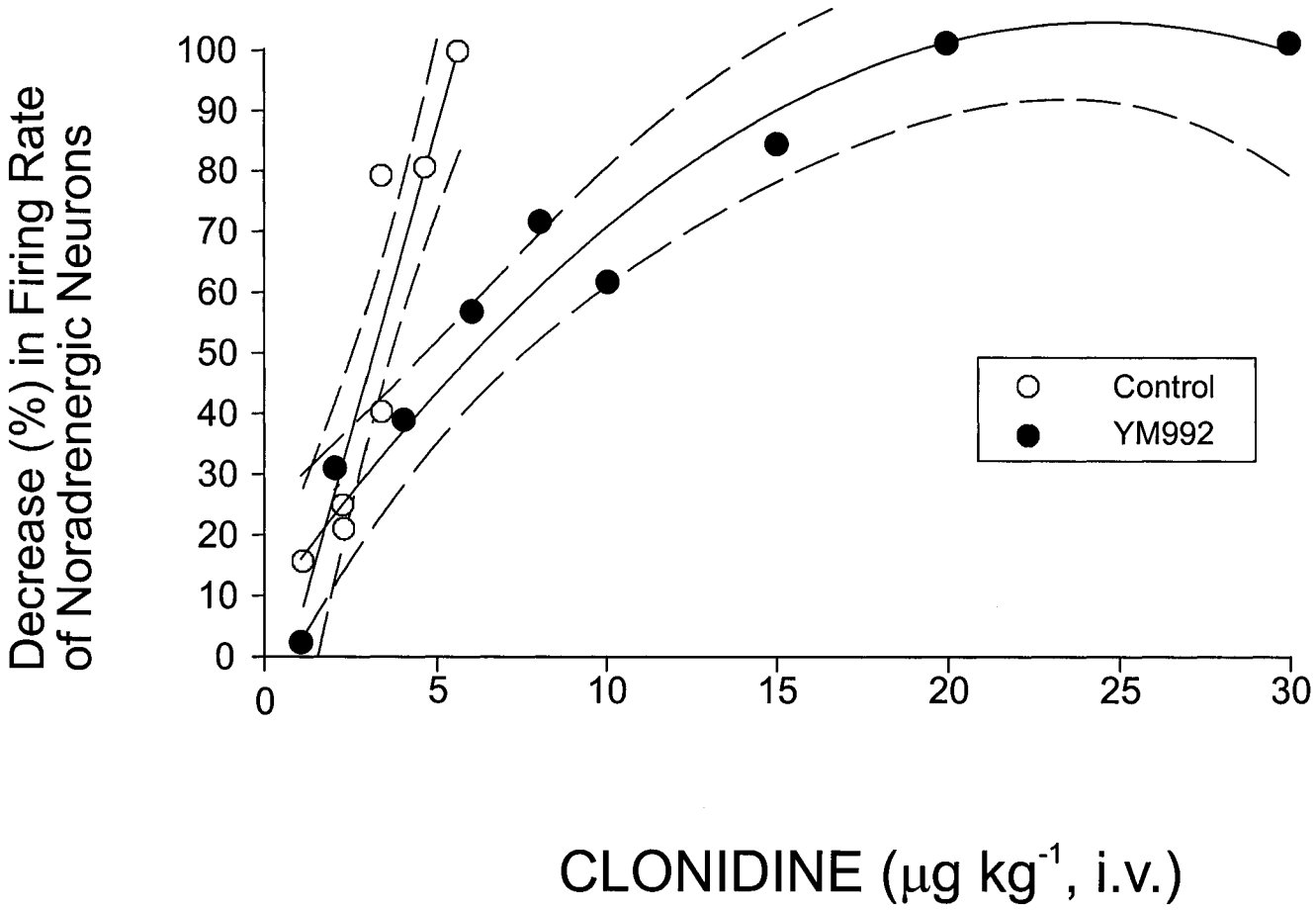

Decrease (%) in Firing Rate of Noradrenergic Neurons
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Control
YM992
CLONIDINE (μg kg-1, i.v.)

Figure VIII.

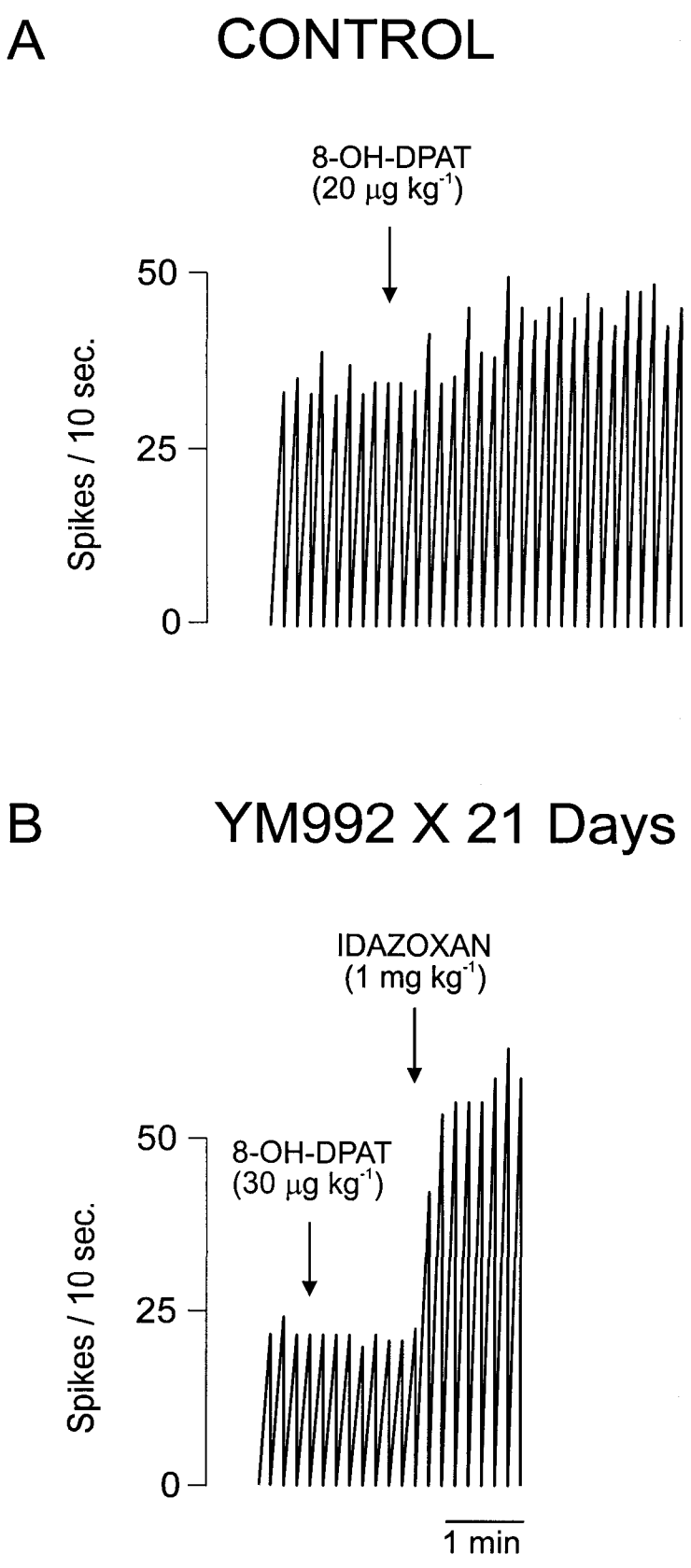
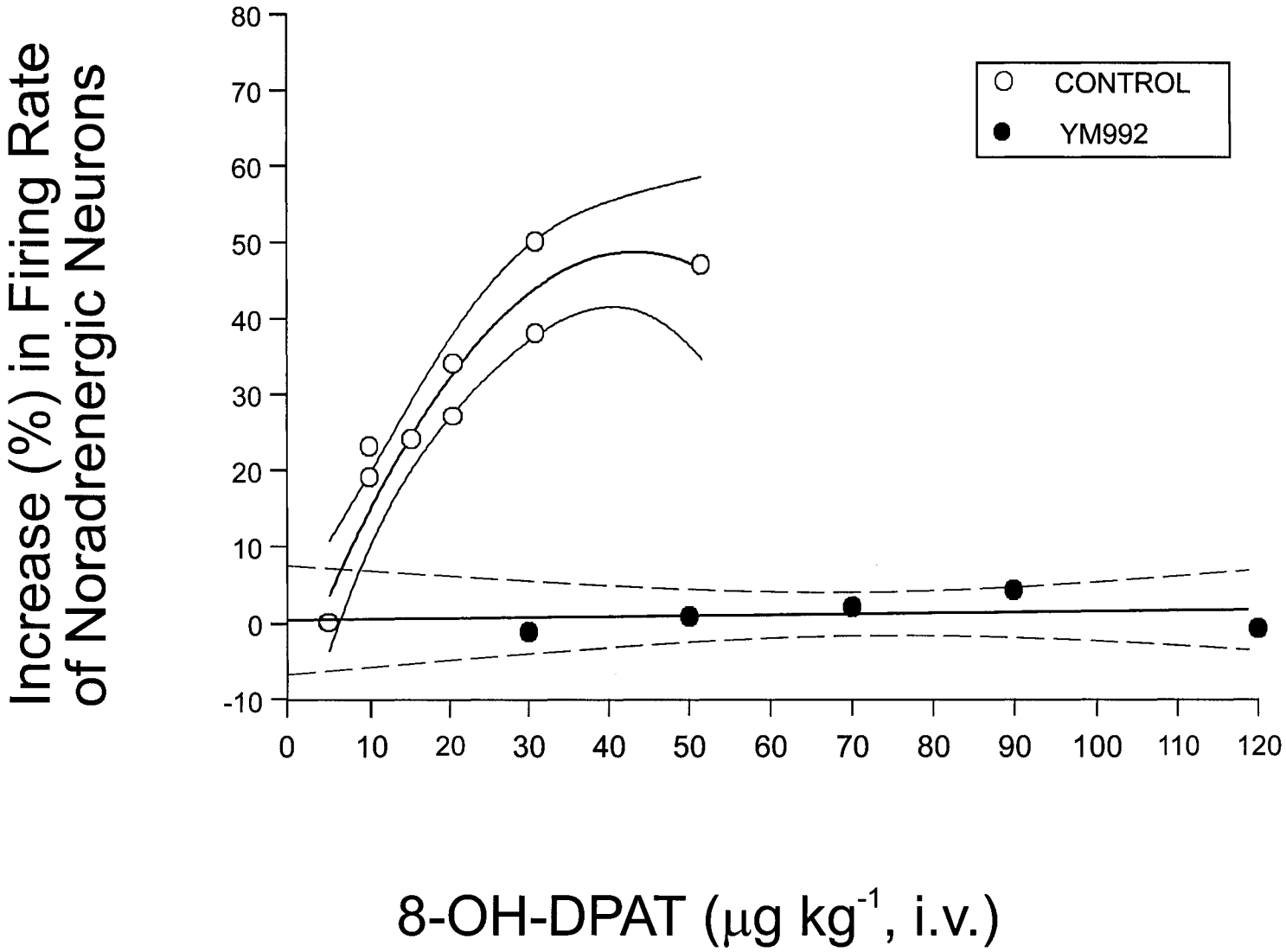

A
CONTROL
8-OH-DPAT
(20 μg kg-1)
50
25
0
Spikes / 10 sec.
B
YM992 X 21 Days
IDAZOXAN
(1 mg kg-1)
8-OH-DPAT
(30 μg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure IX.


Increase (%) in Firing Rate of Noradrenergic Neurons
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
CONTROL
YM992
8-OH-DPAT (μg kg-1, i.v.)

Chapter VII: Sixth Article

PREFATORY REMARKS

A major drawback to TCAs is their side-effect profile. This has been postulated to be due to due to their chemical structure and the inhibition of acetylcholine receptors. On the other hand, the chemical moiety of this antidepressant class has been linked to some of the beneficial effects of this agent. Reboxetine is a novel antidepressant agent, in a class of its own, and is able to block the reuptake of NA transport without possessing the TCA moiety. Reboxetine is a safe and effective in the treatment of anxiety and affective disorders. We decided to assess if acute and sustained treatments with reboxetine would induce similar effects on the firing activity of 5-HT and NA neurons as that observed with the TCA desipramine. Furthermore, in rats treated with reboxetine for 21-days, the 5-HT receptor mediated effects presented on NA and 5-HT firing presented in Chapter III were assessed for commonalities between SSRIs and TCAs that may be an important in mediating the antidepressant response.

This article entitled "Effect of the selective noradrenergic reuptake inhibitor reboxetine on the activity of noradrenaline and serotonin neurons." By myself and Pierre Blier was published in the Eur J Neurosci. (2001, vol 13, pp. 2077-87). A reprint of this article is located at he end of this thesis.

EFFECT OF THE SELECTIVE NORADRENERGIC REUPTAKE INHIBITOR REBOXETINE ON THE FIRING ACTIVITY OF NORADRENALINE AND SEROTONIN NEURONS

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Summary

Reboxetine is an antidepressant with a selective noradrenergic (NA) reuptake blocking property and it does not belong to the family of tricyclic antidepressant agents. The effects of acute and sustained administration of reboxetine on the firing activity of locus coeruleus (LC) NA neurons and dorsal raphe (DR) 5-HT neurons were assessed using *in vivo* extracellular unitary recording in chloral hydrate anesthetized rats. Reboxetine (0.1 - 1.25 mg kg^{-1}, i.v.) dose-dependently decreased the firing activity of NA neurons (ED_{50} = 480 ± 14 μg kg^{-1}). A 2-day treatment with reboxetine at 1.25, 2.5, 5, or 10 mg kg^{-1} day^{-1} (using osmotic minipumps implanted subcutaneously) produced significant decreases of 52%, 68%, 81%, and 83%, respectively, of NA firing activity. When the reboxetine treatment (2.5 mg kg^{-1} day^{-1}) duration was prolonged to 7 days, a 66 % decrease in NA firing activity was observed which further decreased to 80% after 21 days of treatment. In contrast, 5-HT neuron firing rate remained unaltered following short- and long-term reboxetine treatments. The suppressant effect of the α_2-adrenoceptor agonist clonidine on the firing activity of NA neurons was unchanged in long-term reboxetine treated rats, but blunted on that of 5-HT neurons. The enhancement of NA firing activity by the 5-HT_{1A} agonist 8-OH-DPAT was abolished in long-term reboxetine treated rats whereas the inhibitory effect of the 5-HT_2 agonist DOI was attenuated by about three-fold. In conclusion, sustained NA reuptake blockade by reboxetine lead to profound alterations in the function of NA neurons and of 5-HT receptors modulating their firing activity.

Introduction

Alterations in central noradrenaline (NA) and serotonin (5-HT) function have been implicated in the pathophysiology of anxiety and affective disorders (Thase and Howland, 1995). Since the advent of the selective 5-HT reuptake inhibitors (SSRIs), the NA hypothesis related to the diathesis of anxiety and affective disorders have been overshadowed by 5-HT, mainly because of the absence of non-tricyclic drugs selective for NA reuptake. Agents which block the reuptake of NA and/or 5-HT do so within hours of administration, however a therapeutic response is not achieved in major depressive or panic disorder patients until about two to three weeks of sustained administration. It was recently reported that sustained administration of SSRIs produces a progressive decrease on the firing activity of locus coeruleus (LC) NA neurons in the rat brain, which parallels the retarded onset of action of antidepressants in major depression and panic disorder patients (Szabo et al., 1999; 2000). Thus, antidepressant drugs "selective" for one system may be producing an alteration in another neuronal system involved in mediating the therapeutic and/or side effect profiles.

The importance of the 5-HT and NA systems as well as their reciprocal interactions should be taken into account when considering antidepressant treatments as these may ultimately dictate their effectiveness, or lack thereof. Located on the cell bodies and terminals of NA neurons, α_2-adrenergic autoreceptors induce an inhibitory action on NA neurons firing activity and release, respectively (Svensson et al, 1975; Curet and de Montigny, 1989). In the presence of a NA reuptake blocker, α_2-adrenergic autoreceptors become overactivated via increased concentrations of endogenous NA attenuating the firing activity of LC NA neurons (Lacroix et al., 1991; Kasamo et al.,

1996; Mongeau et al., 1998; Béïque et al., 2000; Szabo et al., 2000). Dorsal raphe 5-HT neurons receive NA projections from the LC (Loizou, 1969; Anderson et al., 1977; Baraban and Aghajanian, 1980; Clement et al., 1992). These NA projections modulate the activity of 5-HT neurons in the dorsal raphe nucleus via excitatory α_1-adrenoceptors (Baraban and Aghajanian, 1980). In turn, NA neurons of the LC receive dense 5-HT projections of which 50% arises from the dorsal raphe (Kaehler et al., 1999) and others most probably coming from pericoerulear 5-HT neurons (Aston-Jones et al., 1991), which exert an inhibitory role.

Reboxetine is a non-tricyclic antidepressant drug that potently and selectively inhibits NA uptake (Wong et al., 2000). Given the major importance of such reciprocal interactions between NA and 5-HT neurons (Haddjeri et al., 1997; Szabo et al., 1999; 2000), acute and sustained treatment regimens of reboxetine were carried out in rats to assess whether their effect on the spontaneous firing activity of NA and 5-HT neurons differed as compared to previous studies performed with desipramine and SSRIs (Szabo et al., 2000). The responsiveness of NA and 5-HT neurons to the α_2-adrenoceptor agonist clonidine was also examined after long-term reboxetine administration. In addition, the effect of systemic administration of the 5-HT_{1A} and 5-HT_2 receptor agonists 8-OH-DPAT and DOI, respectively, on NA firing activity were assessed in long-term reboxetine treated rats to determine whether sustained NA reuptake blockade could lead to changes in 5-HT modulation of NA neuronal firing activity.

Methods

Animals

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g. Rats were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water ad libitum). Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, U.S.A.). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, rats were inserted a catheter in a lateral tail vein for systemic i.v. injection of drugs. All experiments were performed in compliance with NIH guidelines and the Canadian Council on Animal Care.

Sustained reboxetine treatments

Rats were anaesthetized with halothane containing a 2 to 1 O_2/N_2O mixture for subcutaneous implantation of osmotic minipumps (ALZA, Palo Alto, CA, U.S.A.). The rats were tested with the minipumps in place in order to mimic the clinical condition whereby patients present an antidepressant response while taking their medication. They were treated with varying doses of reboxetine (1.25, 2.5, 5, or 10 mg kg^{-1} day^{-1}) or the saline vehicle for either 2, 7, or 21 days delivered by osmotic minipumps. In calculating for the concentration of antidepressant drug solution used to effectively reach the mg kg^{-1} day^{-1} dose desired, an estimation was made for the weight of the rat

at the middle of the treatment time by approximating that the rat gains approximately 50 g per week, and this value was used to prepare the solution.

Electrophysiological experiments

Extracellular unitary recording of LC NA and DR 5-HT neurons were conducted with single-barrelled glass micropipettes preloaded with fibreglass filaments (to facilitate filling) being pulled in a conventional manner, with the tips broken back to 1-3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2 and 4 MΩ. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for LC neurons recordings. LC NA neurons were recorded with micropipettes lowered at -0.7 mm interaural and 1.1 to 1.4 mm lateral. Spontaneously active NA neurons of the LC were identified using the following criteria: regular firing rate (1-5 Hz) and positive action potential of long duration (0.8-1.2 ms) exhibiting a characteristic burst discharge in response to a nociceptive pinch of the contralateral hind paw (Aghajanian et al., 1977; Aghajanian & Vandermaelen, 1982). NA neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of NA neurons in the treated animals, four to five electrode descents were carried out through this nucleus in control and reboxetine treated rats.

For DR recordings, the pipette was positioned 1mm anterior to lambda on the midline and lowered into the DR; 5-HT neurons were usually encountered at a depth between 5.5 and 6.5 mm from the surface of the brain. 5-HT neurons were identified by their characteristic slow (0.5 - 2.5 Hz) and regular firing rate and long-duration (0.8 - 1.2 ms) positive action potential (Baraban & Aghajanian, 1980; Aghajanian &

Vandermaelen, 1982). In order to assess possible changes in the firing activity of 5-HT neurons during the course of sustained reboxetine administration, four to five electrode decents were carried out in each control and treated rat: the first 1 mm anterior to lambda on the midline, the following two 200 μm anterior and posterior to the first descent, and the last two 200 μm on either side of the first track.

Dose-response Curves

Dose-response curves for the alteration of NA and 5-HT neuron firing activity were obtained for systemic (i.v.) administration of reboxetine and the selective α_2-adrenoceptor agonist clonidine in untreated rats. After systemic injection of reboxetine or clonidine, the selective α_2-adrenoceptor antagonist idazoxan was administered in order to reverse the decrease in firing activity produced by the previously injected compound. In long-term (21 days) reboxetine and vehicle treated rats, the 5-HT_{1A} receptor agonist 8-OH-DPAT, the 5-HT_2 receptor agonist DOI, and clonidine were injected while recording the firing activity of LC NA neurons. In addition, clonidine was also systemically injected in long-term reboxetine and vehicle treated rats and the effect on 5-HT neuron firing rate were assessed. Changes in the firing activity are expressed as percentages of the baseline firing rate and were measured after systemic drug administration. Dose-response curves were constructed for 8-OH-DPAT, DOI, and clonidine in control and long-term reboxetine treated animals only using the first dose injected to each rat to generate the curves and estimates of effective doses 50 (ED_{50}).

Drugs

The following drugs were used: reboxetine (Pharmacia & UpJohn, Kalamazoo, MI, U.S.A.); desipramine HCL, DOI, 8-OH-DPAT, ritanserin, idazoxan, clonidine, and WAY 100635 (RBI, Natick, MA, U.S.A.); MDL100907 (Hoechst Marion Roussel, Cincinnati, OH, U.S.A.); and LSD (lysergic acid diethylamide; Ministry of Health and Welfare, Ottawa, Canada). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water while ritanserin was solubilized in acetic acid and then diluted with distilled water to the appropriate concentration.

Statistical comparisons

All results were expressed as mean (± S.E.M.) of single neuron values. Statistical comparisons of values obtained in treated and control rats were carried out using one-way analysis of variance on ranks. Dunnett's multiple comparison test was used to assess the difference between control and treated groups. Correlational coefficients (*r* values) for the dose-response relationship observed in the LC were calculated using simple linear/curvilinear regression analysis. The SEM for the ED_{50} values for the LC were calculated by regression analysis, with the Y value of 50 used as the regressor. Difference between the two regressions in the controls and treated rats were assessed by comparing their ED_{50} values using the confidence intervals method. The 95% confidence limit was determined from the Student's *t* distribution.

Results

Effect of the acute administration of reboxetine on the firing activity of NA neurons

A single i.v. dose of reboxetine was administered to each naive rat while recording a spontaneously active NA neuron (n = 6). Reboxetine (0.1 - 1.25 mg kg^{-1}) induced a dose-dependent decrease of the firing rate of the NA neurons (figure 1C), an example of which is provided in figure 1B. The effect of reboxetine reached maximal suppression of NA firing activity at a dose of 1.25 mg kg^{-1}. This suppressant effect was reversed by the subsequent i.v. administration of the selective α_2-adrenoceptor antagonist idazoxan (1 mg kg^{-1}, n = 6). Reboxetine displayed an ED_{50} of 480 ± 14 μg kg^{-1} (figure 1C). Desipramine injected at 500 μg kg^{-1} decreased NA firing rate of an LC neuron (64%) to a somewhat greater extent when compared to reboxetine injected at the same dose (52%; figure 1A and figure 1C).

Effect of sustained administration of reboxetine on the firing activity of LC NA neurons

On the basis of the acute experiments with reboxetine mentioned above, five systematic electrode descents into the LC nucleus were carried out in rats treated with reboxetine at various doses and time courses as well as with their respective controls. The spontaneous firing activity of NA neurons were recorded in control and reboxetine treated rats, examples of which are provided in figure 2. Control groups treated with the saline vehicle under a varying time-course resulted in no significant difference in LC

spontaneous firing activity when compared to each other, these data were therefore merged to make up a single control group (range of firing: 0.8 to 3.8 Hz). A short-term treatment (2 days) with reboxetine at 1.25, 2.5, 5, or 10 mg kg^{-1} day^{-1} produced a significant decrease of 52% (range of firing: 0.5 to 3.8 Hz), 68% (range of firing: 0.2 to 2.9 Hz), 81% (range of firing: 0.2 to 0.8 Hz), and 83% (range of firing: 0.2 to 0.8 Hz), respectively, in NA firing activity as compared to controls (figure 3). The effect of the short-term reboxetine treatment on attenuating the firing activity of LC NA neurons was dose-dependent and reached maximal levels at a dose of 5 mg kg^{-1} day^{-1}(figure 3).

Previous experiments carried out with the selective NA reuptake blocker desipramine (10 mg kg^{-1} day^{-1}) decreased the firing rate of NA neurons to the same extent after 2 and 21 days of treatment (70%; Szabo et al., 2000; data included in figure 3 for comparison). Reboxetine given at 2.5 mg kg^{-1} day^{-1} produced similar effects at the 2-day time period on the attenuation of LC neurons as desipramine (10 mg kg^{-1} day^{-1}). Rats were thus treated with this dose of reboxetine for 7 and 21 days. When treatment duration with reboxetine was increased from 2 days to 7 days, a sustained decrease of 66 % (range of firing: 0.3 to 1.6 Hz) in NA firing activity was observed which further decreased to 80% (range of firing: 0.1 to 0.9 Hz) after long-term treatment (21-day), examples of which are provided in figure 4. This greater degree of inhibition on LC firing activity during the 21-day reboxetine treatment was significantly different from those of 2- and 7-day treatment regimens ($P < 0.05$).

Effect of sustained administration of reboxetine on the firing activity of DR 5-HT neurons

Control groups treated with the saline vehicle resulted in no significant difference in DR spontaneous firing activity when compared to each other and these data were therefore merged to make up one control group (range of firing: 0.4 to 2.6 Hz). Desipramine (10 mg kg^{-1} day^{-1}) does not alter the firing activity of DR 5-HT neurons after a 2-day treatment (Mongeau et al., 1998). Systematic electrode descents into the DR of rats treated with 10 mg kg^{-1} day^{-1} for 2 days (range of firing: 0.2 to 2.0 Hz) and 2.5 mg kg^{-1} day^{-1} for 21-days of reboxetine (range of firing: 0.4 to 2.3 Hz) did not differ significantly in the mean spontaneous firing activity of 5-HT neurons when compared to each other or control rat values, examples of which are provided in figure 5.

Effect of intravenous injection of the 5-HT agonists DOI and 8-OH-DPAT on LC NA neurons in controls and reboxetine treated rats

DOI induces a suppression of NA firing activity that is reversed by a subsequent injection of the 5-$HT_{2A/2C}$ antagonist ritanserin (*n* = 3; Chiang and Aston-Jones, 1993; Szabo et al., 2000; figure 6A). It was recently reported that the inhibitory effects of DOI on LC neuron firing activity is abolished upon prior systemic administration of the selective 5-HT_{2A} antagonist MDL 100,907 (200 μg kg^{-1}), thus putting forth further evidence on 5-HT_{2A} receptors (Szabo & Blier, 2000a). In 21-day citalopram (20 mg kg^{-1} day^{-1}), but not in desipramine (10 mg kg^{-1} day^{-1}) treated rats, the response to i.v. administration of DOI is shifted approximately three-fold to the right (Szabo et al., 2000). In a rat treated with 2.5 mg kg^{-1} day^{-1} of reboxetine for 21 days, the response to DOI was blunted as compared to the control example (figure 6A and figure 6B). A subsequent injection of MDL 100,907 was able to reverse the effect of DOI (*n* = 3),

while idazoxan further increased the attenuated NA neuron firing (figure 6A and figure 6B) to physiological levels (0.8-5.3 Hz, *n* = 90; Szabo et al., 2000). A full dose-response relationship between the suppression of LC firing activity and different doses of DOI, put into evidence a significant three-fold shift to the right in the reboxetine (ED_{50} = 62 ± 5.5) treated rats as compared to controls (ED_{50} = 20 ± 0.5; figure 7).

Systemic injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT produced a dose-dependent increase in the firing activity of LC NA neurons (Piercey, et al., 1993; Szabo et al., 2000; figure 8A) and yielded an ED_{50} of 15 μg kg^{-1} (figure 9). After long-term treatment with different classes of antidepressants (desipramine or citalopram), the excitatory response on NA firing activity to 8-OH-DPAT was abolished (Szabo et al., 2000). Rats treated with 2.5 mg kg^{-1} day^{-1} of reboxetine for 21 days also abolished the enhancing effect of 8-OH-DPAT on LC NA firing activity (figure 8B). In fact, even doses of 8-OH-DPAT of up to 120 μg kg^{-1} failed to alter the firing activity of NA neurons in long-term reboxetine treated rats (figure 9). In the absence of a 8-OH-DPAT response, a subsequent injection of the 5-HT_2 receptor agonist DOI produced a suppression of firing and the selective 5-HT_{2A} receptor antagonist MDL 100,907 (*n* = 2) reversed that inhibitory effect, while a final injection of idazoxan (*n* = 3) was able to restore NA firing to the upper end of the physiological range of firing of NA neurons observed in control animals (figure 8B).

Effect of intravenous injection of clonidine on LC NA and DR 5-HT neurons in control and reboxetine treated rats

The selective α_2-adrenoceptor agonist clonidine injected systemically decreased NA neuronal firing activity, while 8-OH-DPAT subsequently enhanced it (n = 2), and a final injection of idazoxan (n = 3) reversed the effect of clonidine (figure 10A). This effect of idazoxan is consistent with previous studies showing that idazoxan is capable of reversing the inhibitory effects of clonidine on LC NA firing activity (Goldstein et al., 1983). In long-term reboxetine (2.5 mg kg^{-1} day^{-1}) treated rats, clonidine still produced the same inhibitory effect on NA neurons firing rate as in controls. The dose-response curve of clonidine in the suppression of LC firing activity was not statistically different when comparing control to reboxetine treated rats (figure 11A and figure 11B). The excitatory effect of 8-OH-DPAT was absent following the injection of clonidine and the subsequent administration of idazoxan (n = 3) increased the firing activity to the control range (figure 12B).

Systemic administration of clonidine decreases the firing activity of DR 5-HT neurons with an ED_{50} of about 5 μg kg^{-1} (Freedman & Aghajanian, 1984; Mongeau et al., 1993; Haddjeri et al., 1997). While recording a DR 5-HT neuron, clonidine at 8 μg kg^{-1} decreased the firing rate of the 5-HT neuron by approximately 70%, whereas a subsequent injection of idazoxan reversed this effect (figure 12A). In rats treated with reboxetine for 21-days, two subsequent injections of 8 μg kg^{-1} of clonidine were ineffective in decreasing 5-HT neuron firing activity (figure 12B). In the same neuron,

an additional injection of 12 μg kg^{-1} of clonidine (cumulative dose of 38 μg kg^{-1}) was able to induce a significant reduction in 5-HT neuronal firing, and administration of 10 μg kg^{-1} of the 5-HT autoreceptor agonist LSD (n = 5) shut down firing activity which was reversed by a final injection of the selective 5-HT_{1A} antagonist WAY 100,635 (n = 3) in reboxetine treated rats (figure 12B). The latter dose of LSD has consistently been found in our laboratory to be the ED_{100} value in control rats. In SSRI or 5-HT_{1A} agonist treated animals, this dose of LSD is at least increased by two-fold. The full dose-response curves for clonidine on the inhibition of 5-HT neuron firing activity in controls and reboxetine-treated rats revealed that there was a significant shift to the right in the treatment group (figure 13).

Conclusion

The results of the present study indicate that acute and sustained administration of reboxetine significantly reduced the spontaneous firing activity of LC NA neurons. The enhancement of NA concentrations in the LC via blockade of NA transporters by reboxetine produced an overactivation of cell body α_2-adrenergic autoreceptors and suppressed NA neuron firing. The exact location of these NA transporters mediating the effects on LC firing are likely on the cell body of NA neurons (Svensson et al., 1975; Mateo et al., 1998). There was, however some striking differences when comparing the acute and sustained effects of reboxetine administration to those of the selective NA reuptake inhibitor desipramine. Desipramine acutely administered suppresses the firing activity of LC neurons with an ED_{50} of about half (240 ± 54 $\mu g\ kg^{-1}$; Béïque et al., 1999) as that of reboxetine (480 ± 14 $\mu g\ kg^{-1}$, figure 1), which is consistent with *in vitro* measurement of NA reuptake inhibition by these two drugs (Wong et al., 2000). However, a suppression of NA neuron firing activity by about 70% was achieved with a quarter of a dose of reboxetine as compared to that of desipramine after a 2-day treatment (2.5 versus 10 mg $kg^{-1}\ day^{-1}$). Furthermore when treatment duration with reboxetine (2.5 mg $kg^{-1}\ day^{-1}$) and desipramine (10 mg $kg^{-1}\ day^{-1}$) was increased from 2 to 21 days (Szabo et al., 2000), LC firing activity was further reduced with reboxetine (down to 83% of the control value), but not with desipramine. A possible explanation for the marked difference in potency between acute and long-term reboxetine administration on attenuating NA firing activity is that reboxetine possesses a greater dissociation constant for the NA transporter. This possibility could be examined *in vitro* using dilution experiments rather than substrate displacement with another NA reuptake

inhibitor. Previous experiments have documented that sustained administration of desipramine (10 mg kg^{-1} day^{-1}) produces a small but significant shift to the right of the clonidine dose-response curve in the suppression of LC firing rate after a 14-day treatment (Lacroix et al., 1991). In the present study, the inhibitory effect of clonidine on NA neuronal firing rate was similar in 21-day reboxetine and saline-treated rats which would explain the lack of recovery of the firing rate of NA neurons (figure 11). Although several studies have documented the possibility that presynaptic α_2-adrenoceptors become desensitized following long-term antidepressant treatments (Crews & Smith 1978; Svensson & Usdin 1978; McMillen et al. 1980; Spyraky & Fibiger 1980; Cohen et al., 1980; Finberg & Tal, 1985; Szabo & Blier, 2000b), there are some studies that have not (Blier & de Montigny, 1985; Mateo et al., 1998).

Desipramine at a dose of 10 mg kg^{-1} day^{-1} does not alter the firing activity of DR 5-HT neurons after a 2-day treatment regimen (Mongeau et al., 1998). Reboxetine also displayed a similar lack of effect on the firing activity of DR 5-HT neurons (figure 5). These results are quite surprising because the spontaneous firing rate of 5-HT neurons is highly dependent on a tonic activation of excitatory α_1-adrenoceptors in the dorsal raphe (Baraban & Aghajanian, 1980; Vandermaelen & Aghajanian, 1983). Thus, given that 5-HT neuronal activity remained unaltered, these data suggest that either NA reuptake blockade perfectly compensated for the decreased impulse flow reaching NA terminals in the raphe, or that there was an increase of NA levels that triggered an adaptive mechanism which ultimately left unaltered the firing rate of 5-HT neurons. Indeed, acute reboxetine administration has been shown to increase the concentration of NA in the frontal cortex and hippocampus in microdialysis experiments and it may

therefore also increase NA levels in the DR (Sacchetti et al., 1999). Nevertheless, in the presence of an unaltered 5-HT firing activity, the inhibitory effects of clonidine on the firing activity of 5-HT neurons in 21-day reboxetine treated rats was attenuated, similar to results previously obtained with the monoamine oxidase inhibitor befloxatone (Haddjeri et al., 1998). This altered clonidine response on 5-HT neuron firing activity most likely reflected the already decreased NA firing rate of LC neurons on which clonidine had a proportionally smaller effect than in saline treated rats. Indeed, the mean firing activity of NA neurons was only of 0.4 Hz in 21-day reboxetine treated rats instead of 2.4 Hz in the controls (figure 3). Consequently, this attenuated responsiveness of 5-HT neurons to clonidine cannot be interpreted as a desensitization of α_2-adrenoceptors.

It was previously reported that the incremental effect of systemic administration of the 5-HT_{1A} receptor agonist on LC NA neuron firing activity is abolished by long-term desipramine (10 mg kg^{-1} day^{-1}) or citalopram (20 mg kg^{-1} day^{-1}) treatments (Szabo et al., 2000). A reboxetine treatment for 21 days also had the same effect (figure 9). It thus appears that drugs which block either 5-HT or NA transporters produce a desensitization of the 5-HT_{1A} receptors controlling LC firing activity after long-term administration. This phenomenon has been well documented for the desensitization of 5-HT_{1A} autoreceptors which impart a negative feedback influence on 5-HT neuron firing activity that occurs following long-term SSRI treatment, but not for drugs like desipramine (Blier & de Montigny, 1980; Blier & de Montigny, 1994) or reboxetine which block NA reuptake (figure 1). Unlike their postsynaptic counterparts in the hippocampus, the 5-HT_{1A} receptors which control LC firing activity desensitize after

long-term administration of SSRIs (Szabo et al., 2000). However, it seems that desensitization of the 5-HT_{1A} receptor which controls LC firing activity is common to all major classes of antidepressant drugs tested thus far and may represent an important finding with respect to the treatment of anxiety and affective disorders.

The location of this 5-HT_{1A} receptor which exerts an excitatory influence of LC firing activity is probably not the 5-HT_{1A} autoreceptor controlling DR neuron firing activity. The selective 5-HT_{1A} antagonist WAY 100,635 at a dose of 0.1 mg kg^{-1}, i.v., does not alter the firing rate of 5-HT neurons, but shuts off that of LC neurons. In 5-HT-lesioned rats, this inhibitory effect of WAY 100,635 is abolished. Furthermore, it appears that an intact 5-HT system is also necessary to produce the augmentation effect of 8-OH-DPAT on LC firing activity (Szabo & Blier, 2000a). Further research will be needed to determine the exact location of the 5-HT_{1A} receptor which, hypothetically, could be located on a projection neuron feeding onto a 5-HT terminal (Szabo & Blier, 2000a).

DOI is a non-selective 5-HT_2 receptor agonist *in vitro*, however *in vivo*, it acts as a preferential 5-HT_{2A} receptor agonist (Aulakh et al., 1995; Mazzola-Pomietto et al., 1995). It induces a dose-dependent decrease in the firing activity of LC NA neurons (Chiang and Aston-Jones, 1993; figure 7). In addition, it was reported that this suppressant effect of DOI on the firing activity of LC NA neurons is completely abolished upon prior administration of the selective 5-HT_{2A} receptor antagonist MDL 100,907 (Szabo & Blier, 2000a). When rats were treated with reboxetine for 21-days, the DOI dose-response curve on LC firing activity was significantly shifted to the right. This is different from results previously obtained with desipramine whereby no

significant difference was found between the DOI dose-response curve in long-term desipramine and control treated rats (Szabo et al., 2000). It is important to note, however, that due to the firing activity of reboxetine treated rats being decreased by 83% after long-term treatment, this may represent why the DOI dose-response appeared different. Indeed, it may be expected that 5-HT_{2A} receptor responsiveness is more difficult to assess reliably when the firing rate is so low.

Recently, it was reported that long-term administration of SSRIs is able to attenuate the firing activity of LC NA neurons (Szabo et al., 1999; 2000). This decrease in NA neuronal firing is possibly due to desensitization of 5-HT_{1A} receptors resulting in an increase in 5-HT neurotransmission in the LC impacting on 5-HT_{2A} receptors to mediate this final NA effect (Szabo & Blier, 2000a). It is nevertheless surprising that selective NA reuptake blockers like desipramine and reboxetine produce such an adaptive change of a 5-HT neuronal element which was also reported for the 5-HT_{1A} receptor inhibiting the forskolin stimulated production of cyclic AMP in the rat hippocampus (Newman & Lerer, 1988). The exact mechanism by which this occurs remains to be unveiled.

In conclusion, the present results indicate that reboxetine is a potent and selective NA reuptake blocker. However, when it was administered in a sustained fashion, its potency in suppressing the firing rate of NA neurons was enhanced, when compared to that of desipramine. Since the firing of NA neurons did not recover following long-term administration of reboxetine, it remains to be determined what adaptative changes of 5-HT and NA receptor responsiveness will be induced in

postsynaptic structures after long-term treatment and their impact on monoamine transmission. These experiments are presently underway in our laboratory.

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Abbreviations

Selective serotonin (5-HT) reuptake Inhibitor (SSRI)

Locus Coeruleus (LC)

Dorsal Raphe (DR)

Noradrenaline (NA)

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Legends to Figures

Figure 1. Integrated firing rate histogram of a LC NA neuron illustrating the effects of intravenous administration of the TCA and selective NA reuptake inhibitor desipramine producing a decrease in the firing activity, a subsequent injection the α_2-adrenoceptor antagonist idazoxan reversed the effects in a control rat (A). Integrated firing rate histogram of a LC NA neuron showing that the non-TCA selective NA reuptake inhibitor reboxetine decreases and idazoxan resurrected the firing activity of the NA neuron (B). Relationship between the degree of suppression of LC NA firing activity and doses of reboxetine administered intravenously in untreated rats. Only, the initial response of a single NA neuron to the first dose of reboxetine in each rat was used to construct the curve. The arrow pointing to the data point on the dose-response curve corresponds to the dose used in figure B. Outer lines represent the standard error of the regression line.

Figure 2. Integrated firing rate histograms of LC NA neurons, recorded in single electrode descents in the LC showing their spontaneous firing activity in control (A), 2-day reboxetine treatment (1.25 mg kg^{-1} day^{-1}) (B), 2-day reboxetine treatment (10 mg kg^{-1} day^{-1}) (C). The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

Figure 3. Effects of 2-day reboxetine (10, 5, 2.5, and 1.25 mg kg^{-1} day^{-1}; n = 3 for each dose) and desipramine (10 mg kg^{-1} day^{-1}; n = 3) treatments on the spontaneous firing

activity of LC neurons. The *shaded area* represents the range (SEM x 2) of the mean firing activity of neurons recorded in control rats (n = 3). *P < 0.05 (Dunn's Method) when compared to the control value. The number of neurons recorded is displayed in each box.

Figure 4. Integrated firing rate histograms of NA neurons, recorded in single electrode descents in the LC showing their spontaneous firing activity in control (n = 3)(A), 2-day reboxetine treatment (B), and 21-day reboxetine treatment (C). The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

Figure 5. Integrated firing rate histograms of 5-HT neurons, recorded in single electrode descents in the DR showing their spontaneous firing activity in control (A), 2-day reboxetine treatment (B), and 21-day reboxetine treatment (C). The dotted lines in between neurons indicate approximately 5-minute time laps. The number above each neuron indicates the depth at which each neuron was recorded from the ventral border of the Sylvius aqueduct.

Figure 6. Integrated firing rate histograms of LC NA neurons illustrating the effects of intravenous administration of the preferential 5-HT_{2A} agonist DOI producing suppressions of the firing activity, whereby a subsequent injection of the 5-HT_2 antagonist ritanserin reversed the effects in a control rat (A). Integrated firing rate histograms of LC NA neurons illustrating the effects of intravenous administration of the

preferential 5-HT_{2A} agonist DOI producing suppressions of the firing activity, whereby a subsequent injection of the 5-HT_{2A} antagonist MDL100,907 reversed the effects in a reboxetine (2.5 mg kg^{-1} day^{-1}) treated rat (B). The identity of the NA neuron recorded in the reboxetine treated rat was also assessed by showing the excitatory effect of a subsequent intravenous administration of the α_2-adrenoceptor antagonist idazoxan.

Figure 7. Relationship between the degree of suppression of LC NA firing activity and doses of DOI administered intravenously in controls and rats treated with reboxetine (2.5 mg kg^{-1} day^{-1}). Only, the initial response of a single NA neuron to the first dose of DOI in each rat was used to construct the curve. The arrows pointing to the data points on the dose-response curves correspond to the doses used in figure 6. Outer lines represent the standard error of the regression line and the hatched area represents the mean (SEM x 2) of the responses obtained in controls. The shift to the right of the dose-response curve was significant ($P < 0.01$).

Figure 8. Integrated firing rate histogram of a LC NA neuron illustrating the effects of intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT producing an increase in the firing activity, a subsequent injection of the α_2-adrenoceptor agonist clonidine reversed the effects in a control rat (A). Integrated firing rate histogram of a LC NA neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with reboxetine (2.5 mg kg^{-1} day^{-1}) for 21 days (B). Note that even during attenuated NA firing activity, a subsequent injection of the 5-HT_2 agonist DOI produced similar inhibition of firing when compared to controls (see figures 6 and 7). The suppressant effects of DOI on NA firing was reversed by injection of the selective 5-HT_{2A} antagonist MDL100,907. The identity of the NA neuron recorded in the reboxetine treated rat was assessed by showing the suppressant effect of a subsequent intravenous administration of the α_2-adrenoceptor antagonist idazoxan.

Figure 9. Relationship between the degree of augmentation of LC NA firing activity and doses of 8-OH-DPAT administered intravenously in controls and reboxetine treated rats. Only the initial response of a single NA neuron to the first dose of 8-OH-DPAT in each rat was used to construct the curves. The arrows pointing to the data points on the dose-response curves correspond to the doses used in figure 8. Outer lines represent the standard error of the regression line.

Figure 10. Integrated firing rate histograms of LC NA neurons illustrating the suppressant effects of intravenous administration of the selective α_2-adrenoceptor agonist clonidine producing suppressions of the firing activity, whereby a subsequent injection of the 5-HT_{1A} agonist 8-OH-DPAT increased the firing rate in a control rat (A). Integrated firing rate histograms of LC NA neurons illustrating the suppressant effects of intravenous administration of the selective α_2-adrenoceptor agonist clonidine producing suppressions of the firing activity, whereby the augmentation effect normally produced by a subsequent injection of the 5-HT_{1A} agonist 8-OH-DPAT was abolished (B). The identity of the NA neuron recorded in the control and reboxetine (2.5 mg kg^{-1} day^{-1}) treated rat was assessed by showing the excitatory effect of a subsequent intravenous administration of the α_2-adrenoceptor antagonist idazoxan.

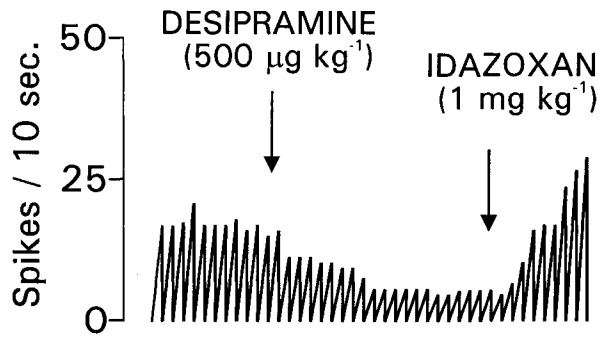
Figure 11. Relationship between the degree of suppression of LC NA firing activity and doses of clonidine administered intravenously in controls (A) and rats treated with reboxetine (2.5 mg kg^{-1} day^{-1}) for 21 days (B). Only, the initial response of a single NA neuron to the first dose of clonidine in each rat was used to construct the curve. The arrows pointing to the data points on the dose-response curves correspond to the doses used in figure 10. Outer lines represent the standard error of the regression line and the hatched area represents the mean (SEM x 2) of the responses obtained in controls.

Figure 12. Integrated firing rate histograms of 5-HT neurons of the DR nucleus showing the suppressant effects of intravenous administration of the α_2-adrenoceptor agonist clonidine, whereby a subsequent injection of the α_2-adrenoceptor antagonist idazoxan (*n* = 2)reversed the effects. Integrated firing rate histogram of a 5-HT neurons of the DR illustrating the lack of responsiveness to two intravenous injections of 8 $\mu g\ kg^{-1}$ clonidine in a rat treated with reboxetine (2.5 $mg\ kg^{-1}\ day^{-1}$) for 21 days (B). Note that a subsequent injection of 12 $\mu g\ kg^{-1}$ of clonidine decreased the firing activity of the 5-HT neuron. The identity of the 5-HT neuron recorded was assessed by showing the suppressant effect of a subsequent injection of LSD.

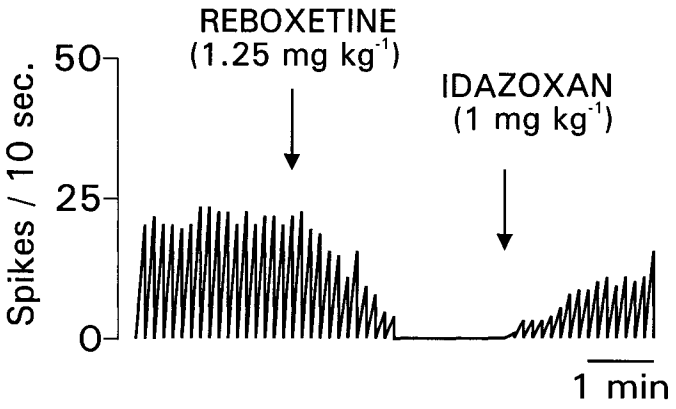
Figure 13. Relationship between the degree of suppression of DR 5-HT firing activity and doses of clonidine administered intravenously in controls and rats treated with reboxetine (2.5 $mg\ kg^{-1}\ day^{-1}$) for 21 days. Only, the initial response of a single 5-HT neuron to the first dose of clonidine in each rat was used to construct the curve. The arrows pointing to the data points on the dose-response curves correspond to the doses used in figure 12. Outer lines represent the standard error of the regression line and the hatched area represents the mean (SEM x 2) of the responses obtained in controls. The shift to the right of the dose-response curve was significant ($P<0.01$).

Figure I.

A


DESIPRAMINE
(500 μg kg-1)
IDAZOXAN
(1 mg kg-1)
Spikes / 10 sec.
50
25
0

B


REBOXETINE
(1.25 mg kg-1)
IDAZOXAN
(1 mg kg-1)
Spikes / 10 sec.
50
25
0
1 min

C

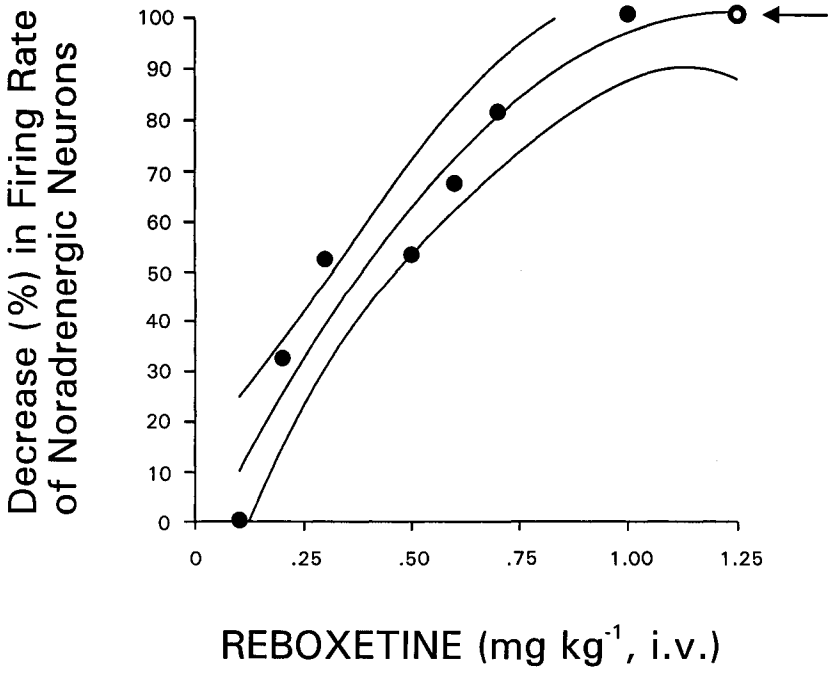

Decrease (%) in Firing Rate
of Noradrenergic Neurons
100
90
80
70
60
50
40
30
20
10
0
0
.25
.50
.75
1.00
1.25
REBOXETINE (mg kg-1, i.v.)

Figure II.

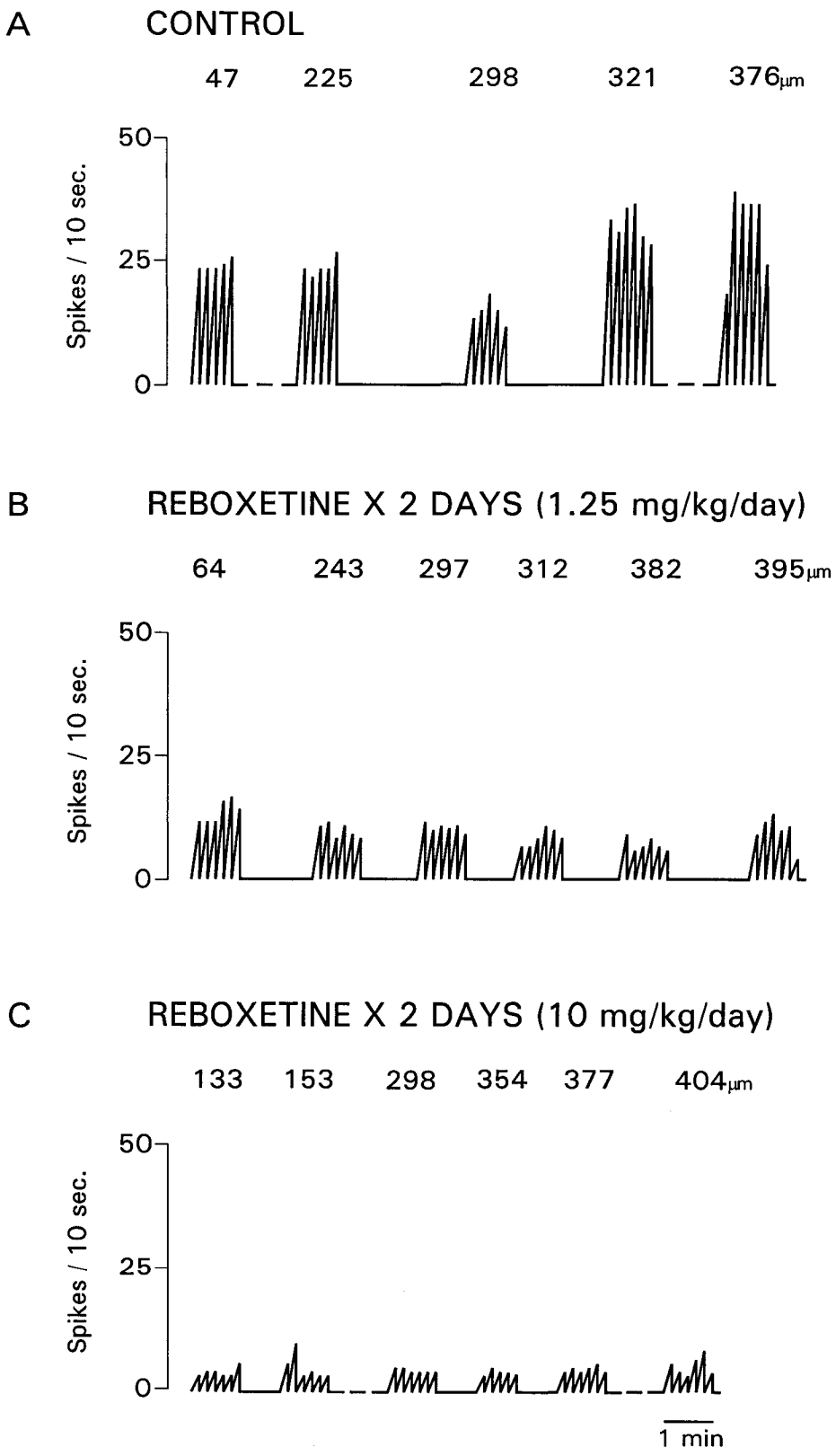

A
CONTROL
47
225
298
321
376μm
50
25
0
Spikes / 10 sec.
B
REBOXETINE X 2 DAYS (1.25 mg/kg/day)
64
243
297
312
382
395μm
50
25
0
Spikes / 10 sec.
C
REBOXETINE X 2 DAYS (10 mg/kg/day)
133
153
298
354
377
404μm
50
25
0
Spikes / 10 sec.
1 min

Figure III.

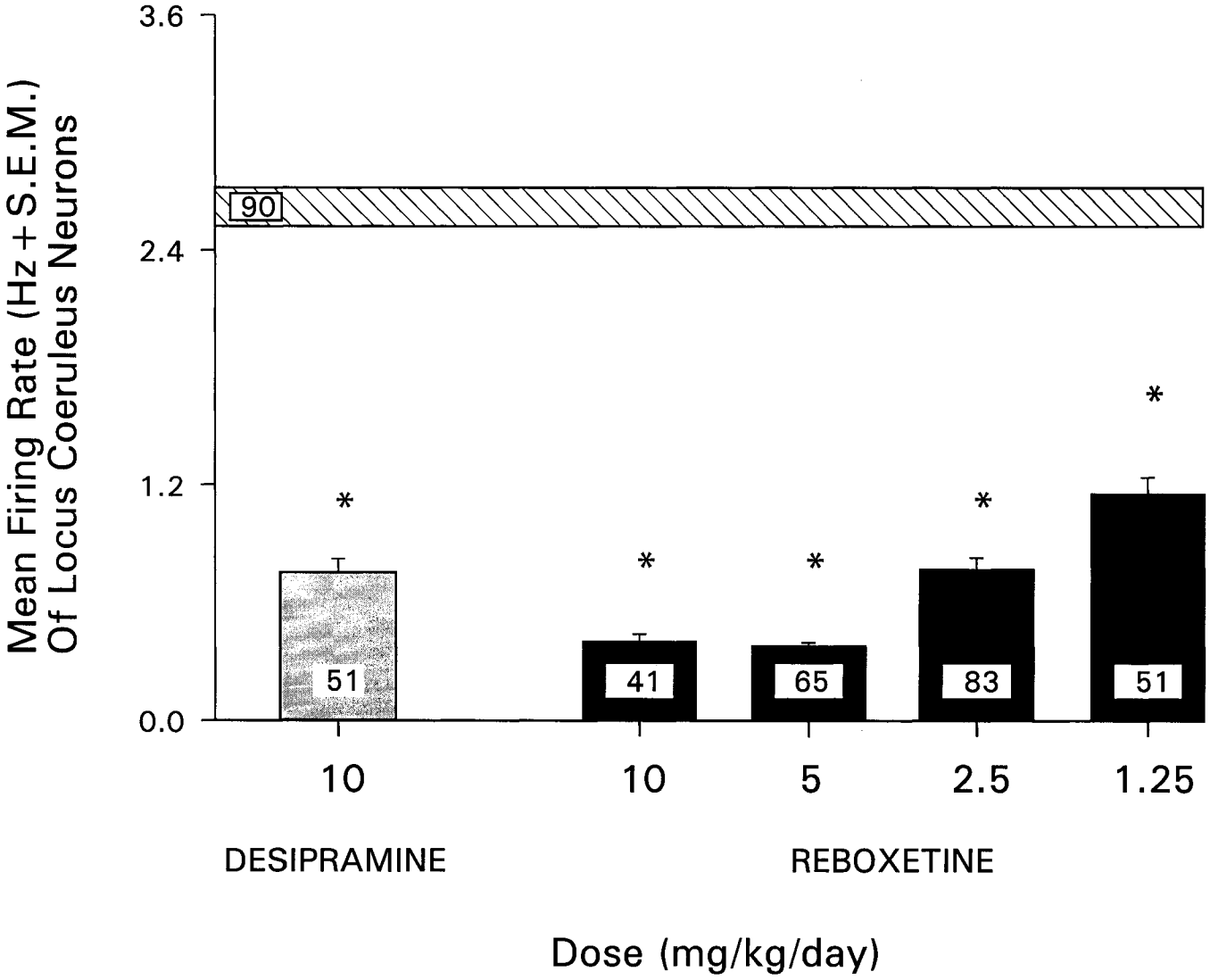

Mean Firing Rate (Hz + S.E.M.)
Of Locus Coeruleus Neurons
3.6
2.4
1.2
0.0
90
51
41
65
83
51
*
10
10
5
2.5
1.25
DESIPRAMINE
REBOXETINE
Dose (mg/kg/day)

Figure IV.

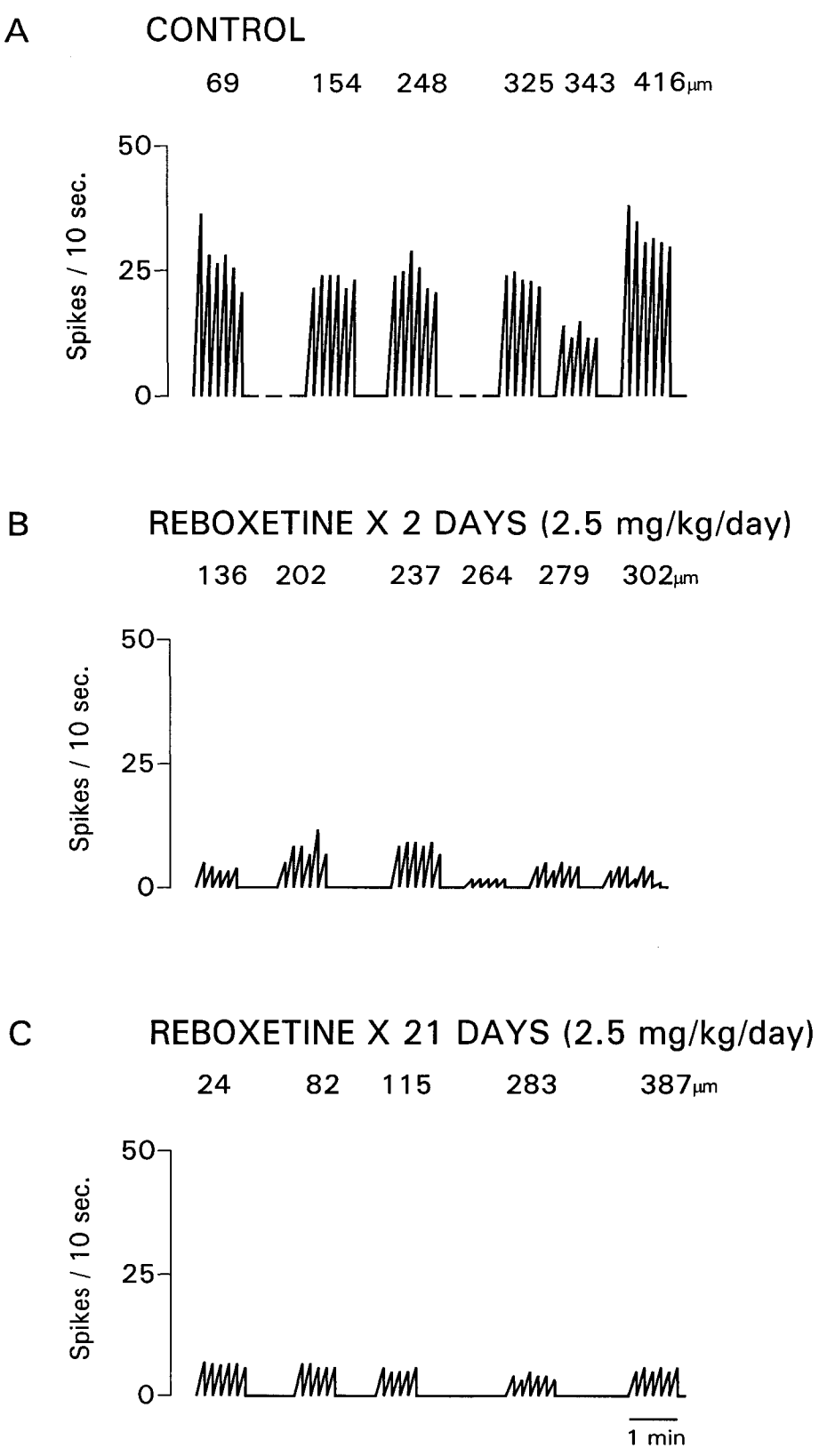

A
CONTROL
69
154
248
325
343
416μm
50
25
0
Spikes / 10 sec.
B
REBOXETINE X 2 DAYS (2.5 mg/kg/day)
136
202
237
264
279
302μm
50
25
0
Spikes / 10 sec.
C
REBOXETINE X 21 DAYS (2.5 mg/kg/day)
24
82
115
283
387μm
50
25
0
Spikes / 10 sec.
1 min

Figure V.

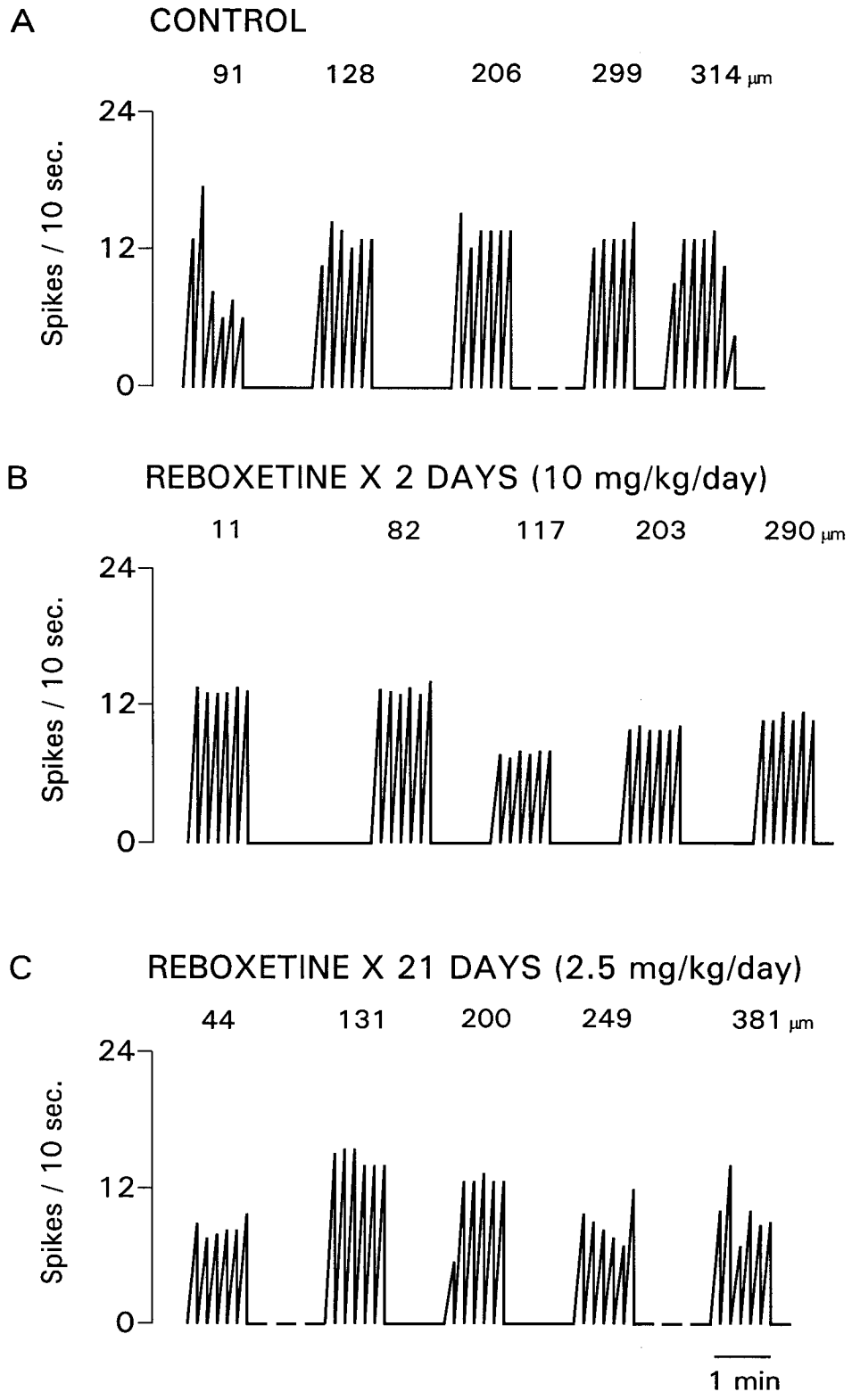

A
CONTROL
91
128
206
299
314 µm
24
12
0
Spikes / 10 sec.
B
REBOXETINE X 2 DAYS (10 mg/kg/day)
11
82
117
203
290 µm
24
12
0
Spikes / 10 sec.
C
REBOXETINE X 21 DAYS (2.5 mg/kg/day)
44
131
200
249
381 µm
24
12
0
Spikes / 10 sec.
1 min

Figure VI.

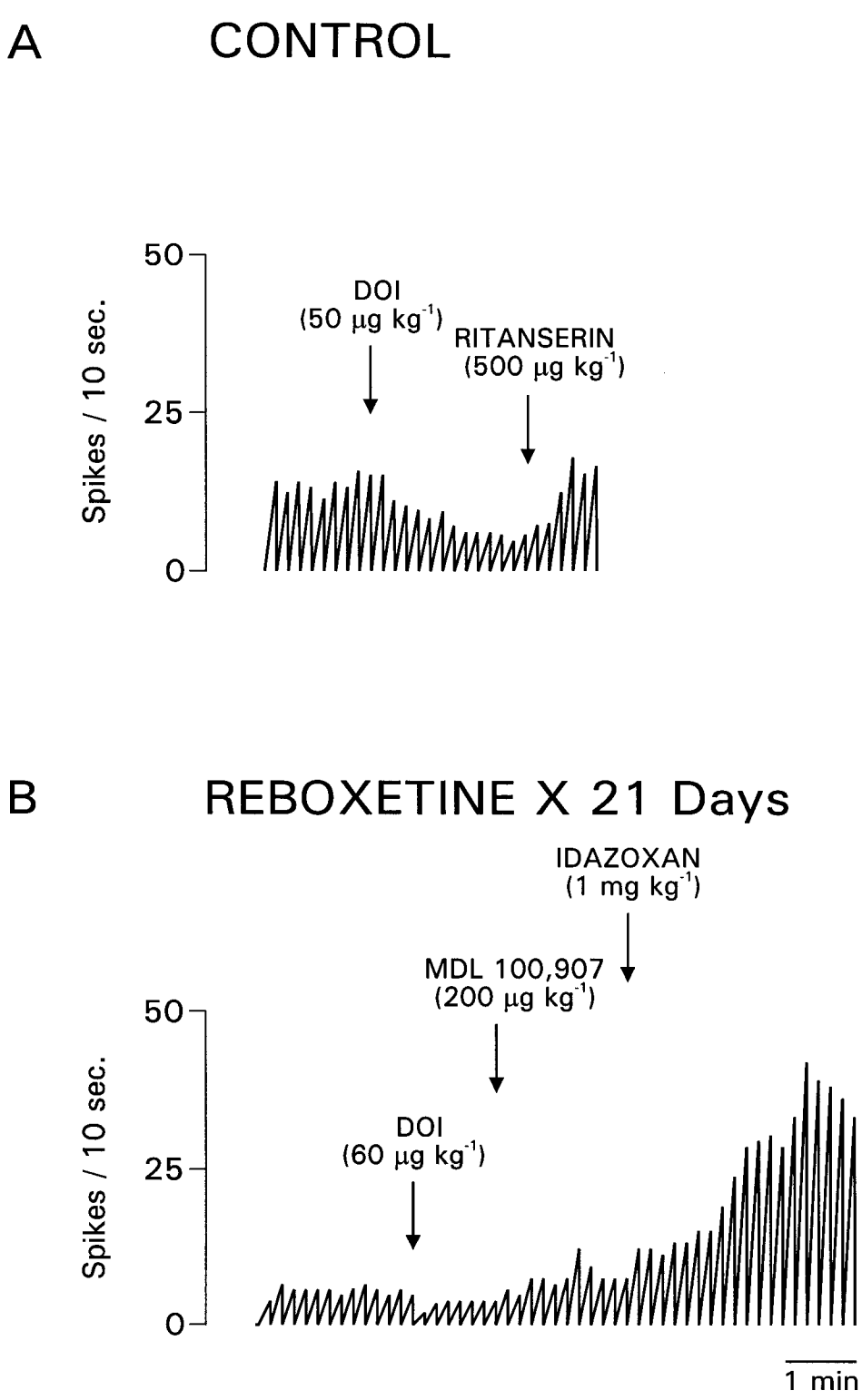

A
CONTROL
50
25
0
Spikes / 10 sec.
DOI
(50 µg kg-1)
RITANSERIN
(500 µg kg-1)
B
REBOXETINE X 21 Days
IDAZOXAN
(1 mg kg-1)
MDL 100,907
(200 µg kg-1)
DOI
(60 µg kg-1)
1 min

Figure VII.

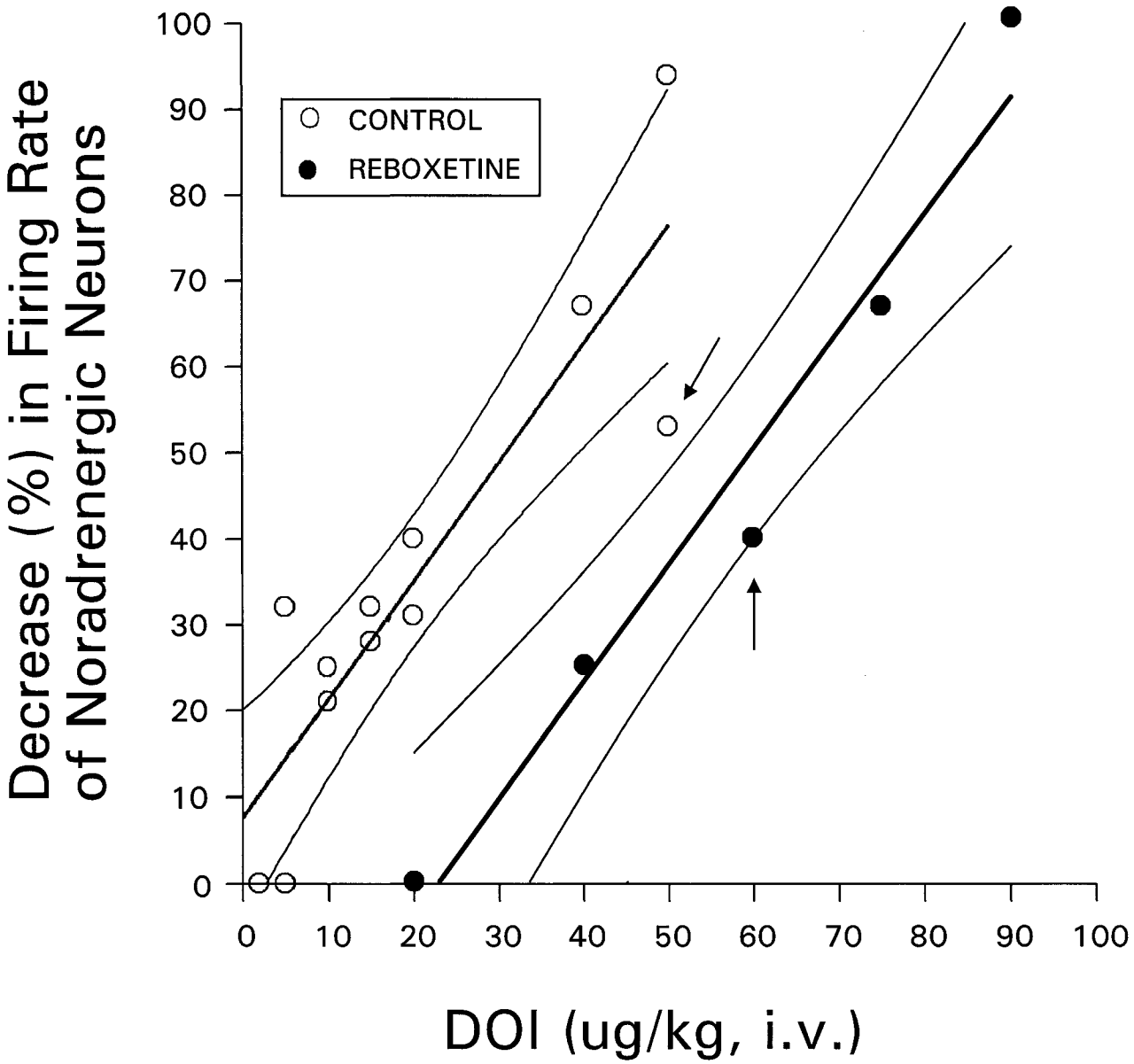

Decrease (%) in Firing Rate of Noradrenergic Neurons
CONTROL
REBOXETINE
100
90
80
70
60
50
40
30
20
10
0
0
10
20
30
40
50
60
70
80
90
100
DOI (ug/kg, i.v.)

Figure VIII.

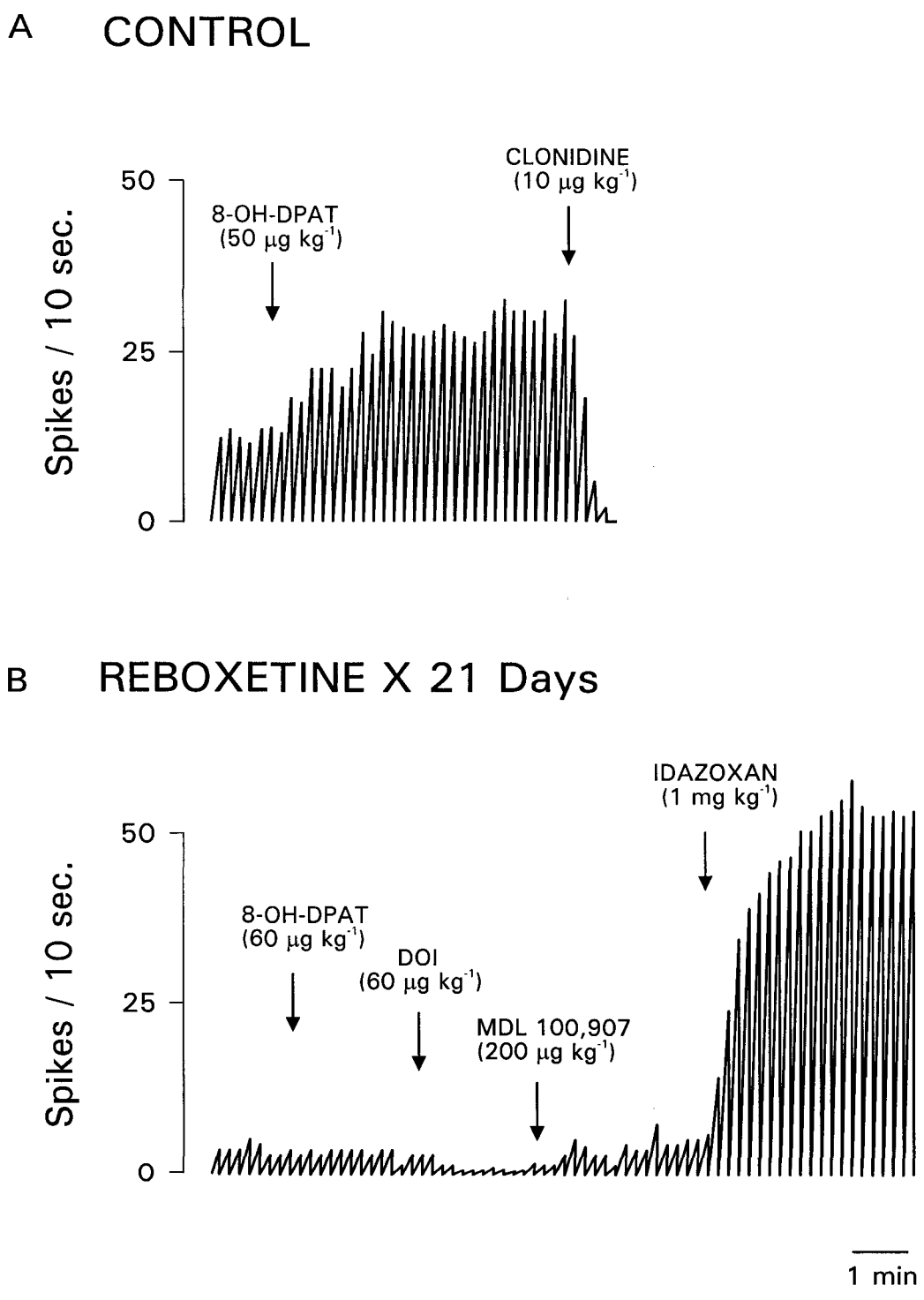

A CONTROL
CLONIDINE
(10 μg kg⁻¹)
8-OH-DPAT
(50 μg kg⁻¹)
50
25
0
Spikes / 10 sec.
B REBOXETINE X 21 Days
IDAZOXAN
(1 mg kg⁻¹)
8-OH-DPAT
(60 μg kg⁻¹)
DOI
(60 μg kg⁻¹)
MDL 100,907
(200 μg kg⁻¹)
50
25
0
Spikes / 10 sec.
1 min

Figure IX.

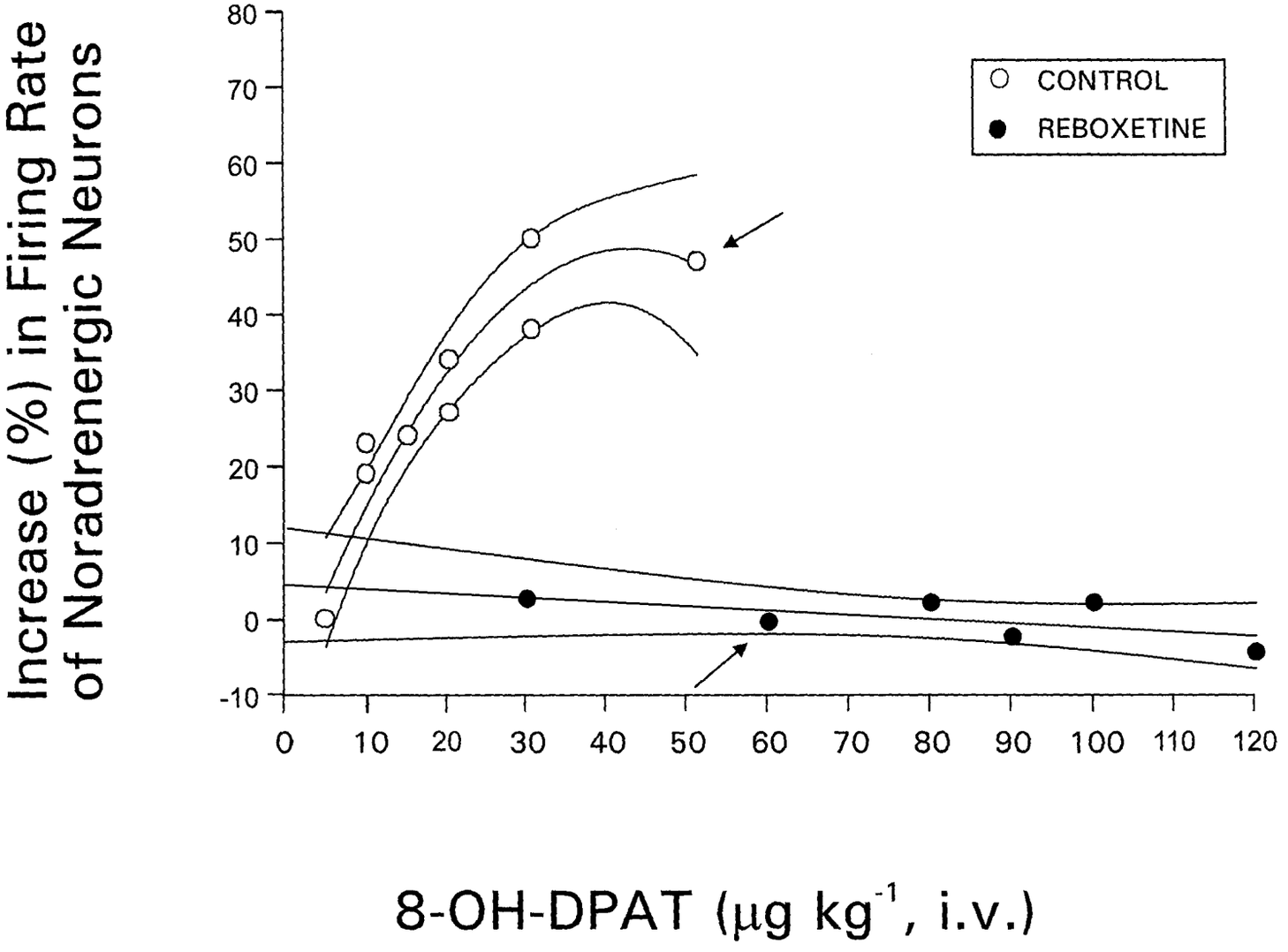

Increase (%) in Firing Rate of Noradrenergic Neurons
80
70
60
50
40
30
20
10
0
-10
0
10
20
30
40
50
60
70
80
90
100
110
120
CONTROL
REBOXETINE
8-OH-DPAT (μg kg-1, i.v.)

Figure X.

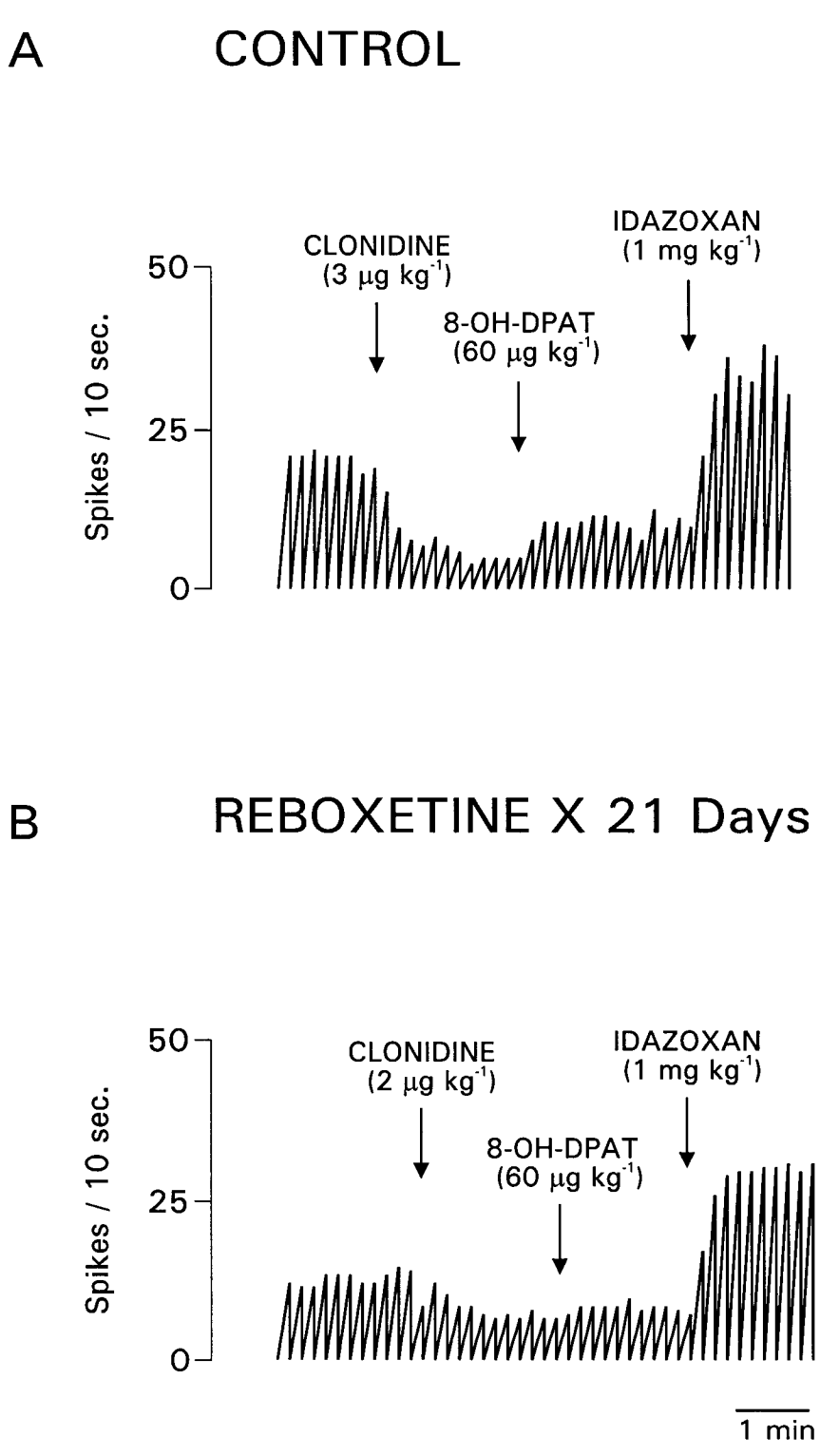
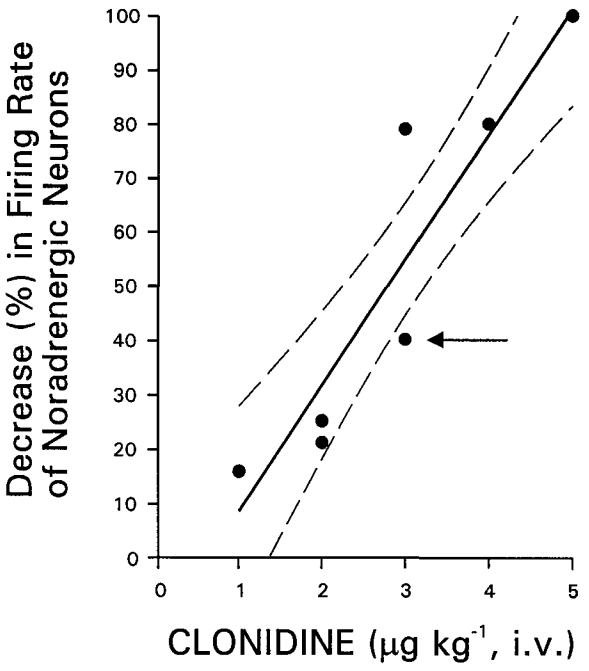

A
CONTROL
CLONIDINE
(3 μg kg-1)
8-OH-DPAT
(60 μg kg-1)
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
B
REBOXETINE X 21 Days
CLONIDINE
(2 μg kg-1)
8-OH-DPAT
(60 μg kg-1)
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
1 min

Figure XI.

A CONTROL



B REBOXETINE X 21 Days

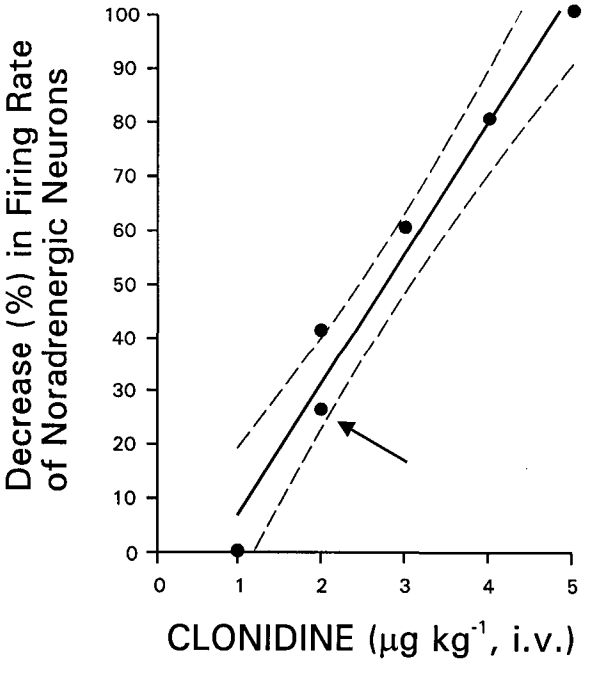


Figure XII.

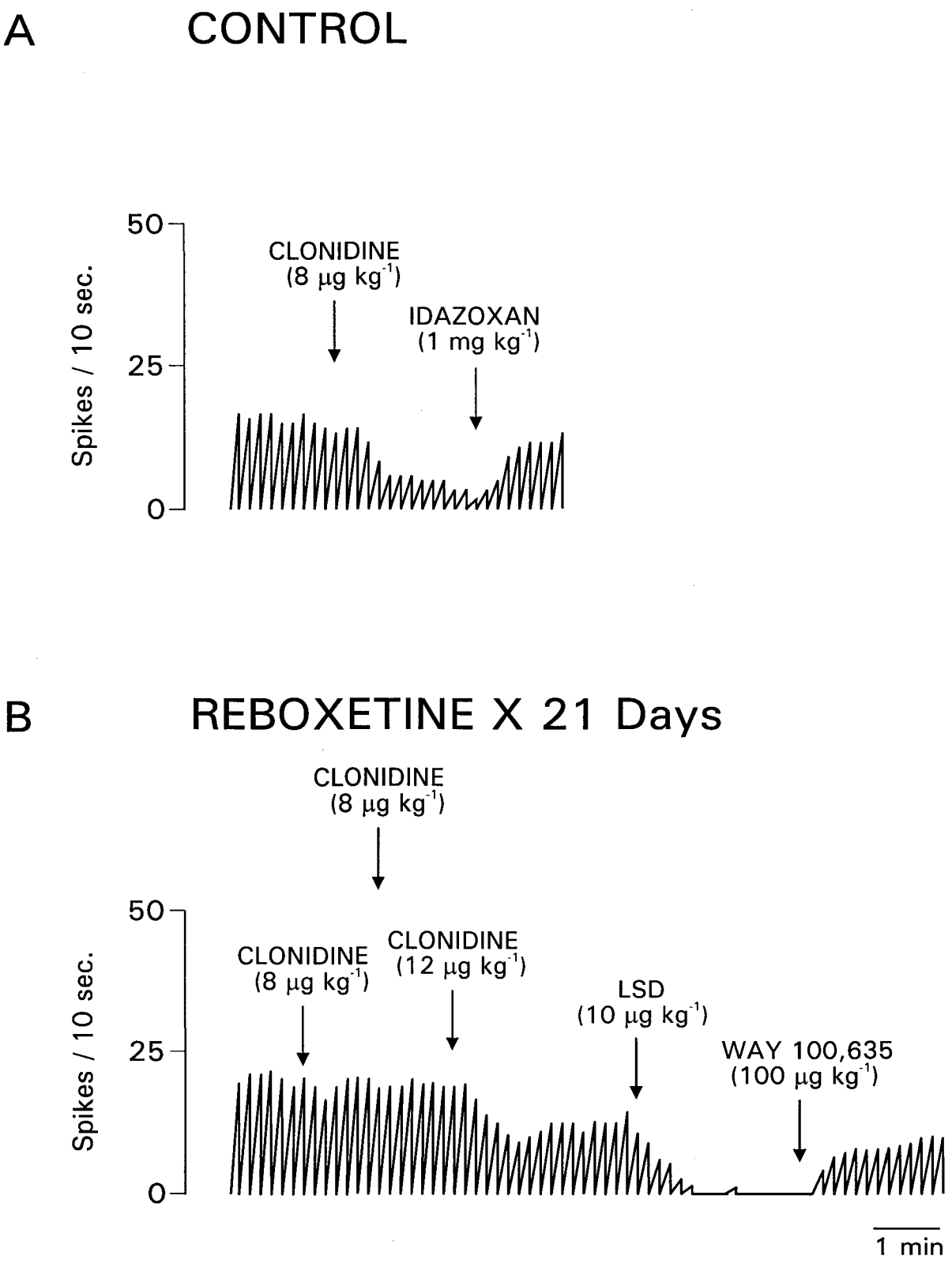
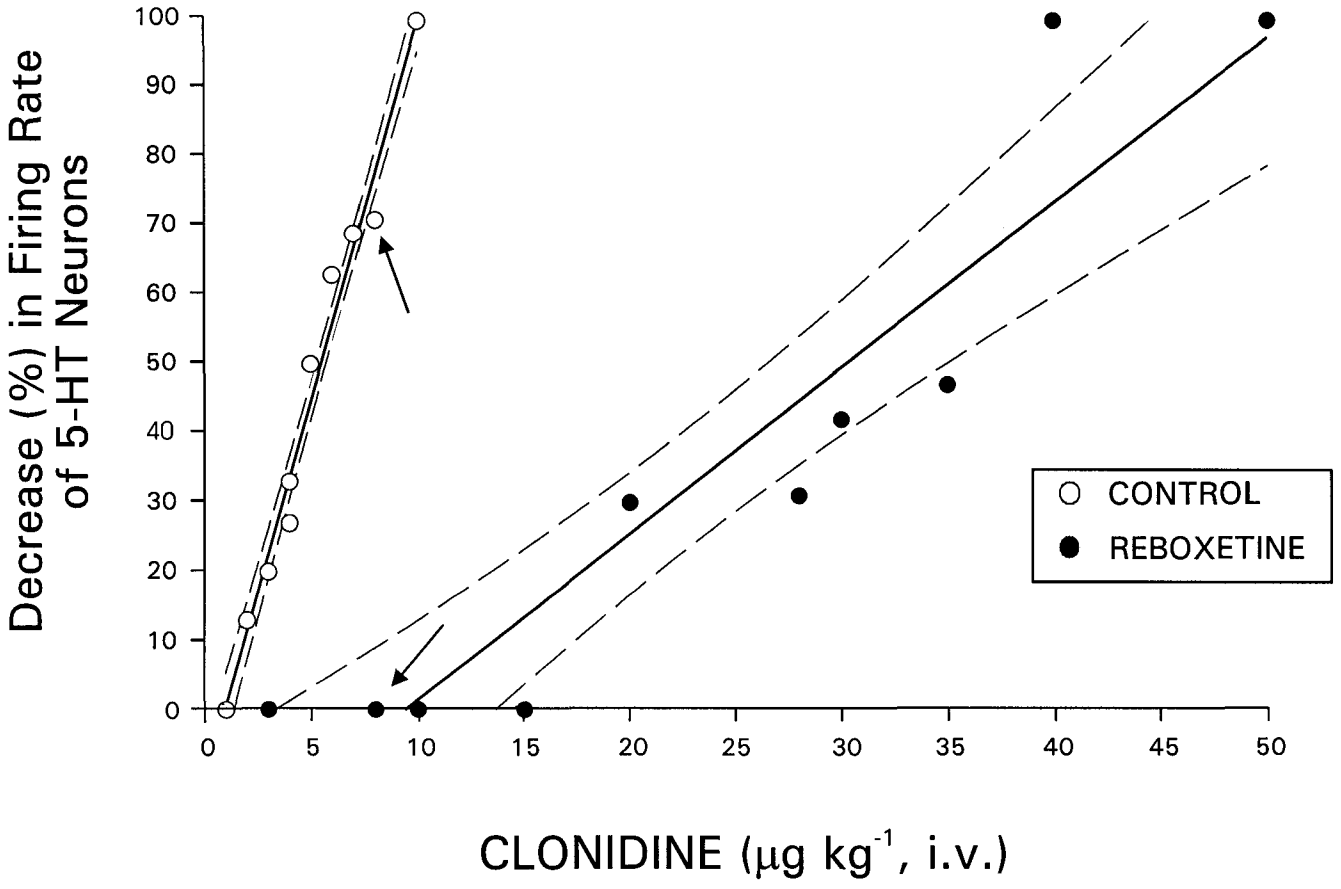

A
CONTROL
50
25
0
Spikes / 10 sec.
CLONIDINE
(8 μg kg-1)
IDAZOXAN
(1 mg kg-1)
B
REBOXETINE X 21 Days
CLONIDINE
(8 μg kg-1)
50
25
0
Spikes / 10 sec.
CLONIDINE
(8 μg kg-1)
CLONIDINE
(12 μg kg-1)
LSD
(10 μg kg-1)
WAY 100,635
(100 μg kg-1)
1 min

Figure XIII.


Decrease (%) in Firing Rate of 5-HT Neurons
100
90
80
70
60
50
40
30
20
10
0
0
5
10
15
20
25
30
35
40
45
50
CONTROL
REBOXETINE
CLONIDINE (μg kg-1, i.v.)

Chapter VIII.

PREFATORY REMARKS

Reboxetine is a selective NA reuptake inhibitor antidepressant devoid of tricyclic structure. It displayed similar effects on monoamine neuron activity during an acute and sustained administration as that of the TCA desipramine (Chapter VII). However, in rats treated with reboxetine for 21-days, NA neuron firing activity decreased further in comparison to that of the desipramine group. Given that 5-HT and NA systems send reciprocal projections to each other in the brainstem, we envisaged that this further attenuation on NA activity might be due to compromised 5-HT reuptake function. We decided to assess if reboxetine is capable of altering 5-HT reuptake function during an acute and sustained administration with this agent in the hippocampus. Furthermore, given that an enhanced 5-HT_{1A} and adrenergic receptor sensitivity in many forebrain structures has been observed with TCAs, and this effect being linked to its moiety and beneficial effects of this antidepressant class, we assessed whether reboxetine would induce the same phenomenon. Lastly, because all antidepressants directed at the NA system is capable of desensitizing α_2-heteroceptors controlling 5-HT release, we decided to assess if reboxetine also follows this trend. Thus, the impact of a selective NA agent antidepressant devoid of TCA moiety on the 5-HT system was for the first time put to the test.

This article entitled "Effects of the selective norepinephrine reuptake inhibitor reboxetine on norepinephrine and serotonin transmission in the rat hippocampus" by myself and Pierre Blier is currently "In Press" in Neuropsychopharmacology.

EFFECTS OF THE SELECTIVE NOREPINEPHRINE REUPTAKE INHIBITOR REBOXETINE ON NOREPINEPHRINE AND SEROTONIN TRANSMISSION IN THE RAT HIPPOCAMPUS

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Short Running Title: Reboxetine on NE and 5-HT transmission

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Date: April 20, 2001

ABSTRACT

Given that norepinephrine (NE) and serotonin (5-HT) neurons are implicated in the mechanisms of action of antidepressant drugs and both project to the hippocampus, the impact of acute and long-term administration of the selective NE reuptake inhibitor reboxetine was assessed on CA_3 pyramidal neuron firing in this postsynaptic structure. Cumulative injections of reboxetine (1-4 mg/kg, i.v.) dose-dependently increased the recovery time of the firing of these neurons following iontophoretic applications of NE, but not 5-HT. In rats treated with reboxetine for 2.5 mg/kg/day for 21 days, a robust increase in the recovery time following NE applications was observed, and a small but significant prolongation occurred following 5-HT applications. In controls and reboxetine-treated rats, 1 and 5 Hz stimulations of the afferent 5-HT bundle to the hippocampus, which allows determination of terminal 5-HT_{1B} autoreceptor sensitivity, produced similar frequency-dependent decreases in pyramidal neuron firing in both groups. However, after low and high doses of clonidine (10 and 400 μg/kg, i.v.), which assesses α_2-adrenergic auto- and heteroreceptor sensitivity, respectively, only the effect of the high dose of clonidine was attenuated. Interestingly, administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 induced a 140% increase in basal pyramidal neuron firing in reboxetine as compared to saline treated rats. This increase in tonic activation of postsynaptic 5-HT_{1A} receptors might be attributable in part to a desensitization of

α_2-adrenergic heteroreceptors, presumably resulting from sustained NE reuptake inhibition. These results indicate that even a selective NE reuptake inhibitor can modulate 5-HT transmission.

KEYWORDS: antidepresssants, α_2-adrenoceptors, clonidine, 5-HT_{1A} receptors, 5-HT_{1B} receptors, locus coeruleus.

INTRODUCTION

The major classes of antidepressant drugs, including the tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRIs), modify serotonin (5-HT) and/or norepinephrine (NE) neurotransmission through which they likely exert their therapeutic effects in anxiety and affective disorders (see Blier and de Montigny et al., 1999). It has been postulated that antidepressant drugs selective for either the 5-HT or NE system act via independent mechanisms. Furthermore, these "selective" drugs display side effect profiles indicative of their neurotransmitter specificity. This, however, does not preclude that antidepressant agents specific for one monoaminergic transporter, or receptor subtype, may exert their therapeutic action through interactions between the 5-HT and NE systems. For example, SSRIs induce a gradual decrease in the spontaneous firing activity of NE neurons after long-term administration (Szabo et al., 1999, 2000), via a complex neuronal circuitry (Szabo and Blier, 2000a), which may contribute to their beneficial and/or side effects depending on the symptomatic profile of the patients (Blier, 2000). On the other hand, the selective NE reuptake inhibitor desipramine increases the synaptic availability of NE but also alters 5-HT parameters after long-term administration, such as enhancing extracellular 5-HT concentrations and the responsiveness of 5-HT receptors in postsynaptic structures (de Montigny and Aghajanian, 1978; Wang and Aghajanian, 1980; Menkes et al., 1981; Yoshioka et al., 1995). This enhanced synaptic availability

of NE may be due to a decreased sensitivity of α_2-adrenergic heteroceptors located on 5-HT terminals that normally induce a negative feedback regulation on 5-HT release (Mongeau et al., 1993; Yoshioka et al., 1995). Interestingly, all TCA drugs independent of their capacity to inhibit the reuptake of 5-HT and/or NE, progressively enhance the responsiveness of postsynaptic 5-HT_{1A} receptors with a time-course congruent to the delayed onset of action of these drugs in major depression (de Montigny and Aghajanian, 1978; Heninger et al., 1984; Chaput et al., 1991). Due to the lack of effective antidepressant drugs selective for the NE transporter not belonging to the TCA family, it has been difficult to assess whether the effects of desipramine on 5-HT transmission is attributable to its TCA moiety (because at least one of these drugs does not block NE reuptake) or to NE reuptake blockade per se.

Reboxetine is not a TCA and it is a selective NE reuptake inhibitor. It is currently the only antidepressant agent of its kind in clinical use in Europe. Given that NE and 5-HT monoaminergic brainstem nuclei project to the hippocampus, the impact of acute and long-term administration of reboxetine was assessed on CA_3 pyramidal neuron firing in this brain region generally thought to be implicated in at least some aspects of depression. It is not currently known whether atrophy in the hippocampus atrophy represent a depressive state or trait (Sheline et al., 1999; Bremner et al., 2000), however antidepressant treatments have been shown to induce adaptative changes in this structure (see Malberg et al., 2000 for review). The effect of reboxetine on NE transmission in this manuscript was not directly assessed as Sacchetti et al., 1999 already concluded that acute and

sustained treatment with reboxetine lead to similar increases in extracellular levels of NE without producing any adaptive changes in α_2-autoreceptor sensitivity in the hippocampus. However, given the importance of receptors on 5-HT terminals in the hippocampus, which become altered after long-term antidepressant treatment, α_2-adrenergic heteroceptor and 5-HT_{1B} autoreceptor function was assessed using electrical stimulation of the afferent 5-HT bundle to this postsynaptic structure.

MATERIALS AND METHODS

Animals and Treatments

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 to 325 g and were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water ad libitum, at a room temperature of 21 ± 2°C). Rats were anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instuments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, a catheter was inserted in a lateral tail vein for systemic i.v. injection of drugs. All experiments were performed in compliance with NIH guidelines and the Canadian Council on Animal Care.

In sustained treatment regimens, rats were anaesthetized with halothane containing a 2 to 1 O_2/N_2O mixture for subcutaneous implantation of osmotic Alzet 2ML4 minipumps (ALZA, Palo Alto, CA). The rats were tested with the minipumps in place in order to mimic the clinical condition whereby patients present an antidepressant response while taking their medication. Rats were treated with reboxetine (2.5 mg kg^{-1} day^{-1}) or the saline vehicle for 21 days delivered by osmotic minipumps. This dose was chosen because it produced a similar degree of attenuation of LC neuronal firing after a two-day treatment as

those obtained with regimens of desipramine and MAOIs examined in long-term studies (Szabo et al., 2000; Blier and de Montigny, 1985).

Recording from Dorsal Hippocampus CA_3 Pyramidal Neurons

Extracellular unitary recordings and microiontophoresis of drugs onto pyramidal neurons in the CA_3 region of the dorsal hippocampus were conducted with five-barrelled micropipettes, pulled conventionally with the tips broken to a diameter of 9 to12 μm under microscopic control. The central barrel, used for recording, was filled with a 2 M NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulfate (5 mM in 200mM NaCl, pH 4), NE bitartrate (20 mM in 200mM NaCl, pH 4), quisqualate (1.5 mM in 200mM NaCl, pH 8), and a 2 M NaCl solution used for automatic current balancing. All drug solutions were injected as cations and retained with a -10 nA current between ejections. Pyramidal neurons were identified by their large amplitude (0.5 mV to 1.2 mV) and long-duration (0.8 msec to 1.2 msec) simple spike alternating with complex spike discharges (Kandel and Spencer, 1961). These characteristics readily allow the on-line differentiation of pyramidal neurons from interneurons. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anaesthesia, small ejection currents of quisqualate (0 to 5 nA) were used to activate them within their physiological firing rate (8 – 15 Hz; Ranck, 1975). Furthermore, the level of cellular activation of the pyramidal neurons does not alter the estimates of neuronal responsiveness (Brunel and de Montigny, 1987). To evaluate the effectiveness of reboxetine on the blockade of

NE and 5-HT transporter reuptake, the recovery of the firing activity of pyramidal neurons following the microiontophoretic application of NE and 5-HT was assessed using the recovery time 50 (RT_{50} value). The RT_{50} value is defined as the time in seconds required by the neurons to recover 50% of the initial firing frequency from termination of microiontophoretic application (de Montigny et al., 1980). The RT_{50} value has also been shown to be a reproducible measure and a reliable index of the *in vivo* activity of the 5-HT and NE reuptake process which is independent from postsynaptic neuronal responsiveness (de Montigny et al., 1980; Piñeyro et al., 1994). The neuronal responsiveness to microiontophoretic applications of NE and 5-HT was assessed and expressed as the number of spikes suppressed. This approach avoids an interference of the recovery of firing which is largely dependent on the activity of the reuptake transporters (Chaput et al., 1986). The sensitivity of neurons to NE or 5-HT was evaluated by counting the number of spikes suppressed during drug ejections.

Stimulation of the 5-HT pathway

To activate the 5-HT projections originating from the dorsal and median raphe to the dorsal hippocampus (Hensler et al., 1994), a bipolar electrode (NE-100; David Kopf, Tujunga, CA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda, and 8.3 mm below the cortical surface. A stimulator (S8800; Grass Instrument, Quincy, Mass; USA) delivered 200 square pulses of 0.5 ms at a frequency of 1 or 5 Hz at an intensity of 300 μA. The duration of suppression of pyramidal neurons firing

activity produced by stimulation was measured on-line using an oscilloscope with memory (1201B; Hewlett Packard; Palo Alto, CA). The effect of the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT into the synaptic cleft (Blier and de Montigny et al., 1983, 1985; Chaput et al., 1986). In order to determine the function of the terminal 5-HT autoreceptors, two series of stimulations (1 and 5 Hz) were carried out, while recording the same neurons. Since it has been previously demonstrated that the activation of the terminal 5-HT autoreceptors decrease the release of 5-HT, thus increasing the frequency of stimulation from 1 to 5 Hz results in a greater activation of terminal 5-HT autoreceptors (Blier et al., 1989; Göthert et al., 1980). Also, the effect of 1 Hz stimulations was determined while recording from the same neurons before and after the successive intravenous injection of a low dose (10 μg/kg) and high dose (400 μg/kg) of clonidine. The effects of the electrical stimulation on CA_3 pyramidal neuron firing rate following low and high doses of clonidine allows for the assessment of the sensitivity of α_2-adrenergic auto- and heteroreceptors, respectively (Mongeau et al., 1994a). This is supported by previous experiments showing that in rats pretreated with the NE neurotoxin 6-hydroxydopamine, the inhibitory effects of the high dose of clonidine was abolished, but the enhancing action of the low dose did not change (Mongeau et al., 1993). Furthermore, prolonged treatment with the monoamine oxidase inhibitor befloxatone selectively attenuates the effect of the high dose of clonidine in intact rats but not in NE-lesion rats (Mongeau et al., 1994a).

Tonic Activation of 5-HT_{1A} Receptors on Dorsal Hippocampus CA_3 Pyramidal Neurons

The selective 5-HT_{1A} receptor antagonist WAY 100,635 (Fletcher et al., 1996) was used to assess the degree of 5-HT_{1A} receptor-mediated inhibition of CA_3 pyramidal neurons induced by the sustained administration of reboxetine for 21 days. The degree to which the antagonists could disinhibit the firing of hippocampal neurons has been determined to be a measure of the tonic activation of postsynaptic 5-HT_{1A} receptors (Haddjeri et al., 1998). In reboxetine-treated rats, if an increase in extracellular levels of 5-HT in the raphe region were present, WAY 100,635 would restore 5-HT neuron firing activity. However, this is probably not the case as it was previously documented that the firing activity of dorsal raphe 5-HT neurons, and the responsiveness of 5-HT_{1A} autoreceptors controlling these neurons, are not altered after a prolonged reboxetine administration (Szabo and Blier, 2000b). Nevertheless, because WAY 100,635 was given systemically, it would simultaneously be blocking the effects of 5-HT on postsynaptic neurons, thereby canceling out the effect of WAY 100,635 on the somatodendritic autoreceptors. Indeed, if the action of the antagonist at the somatodendritic 5-HT_{1A} autoreceptors were influencing the activity of hippocampus neurons, it would serve to further inhibit their firing rate due to an increased release of 5-HT into the target area. Therefore, it was assumed that any increases in firing activity observed during the administration of WAY 100,635 would be a reflection of the action of the antagonist at postsynaptic 5-HT_{1A} receptors. Thus, given that WAY 100,635 antagonizes the action of

exogenous 5-HT at postsynaptic 5-HT_{1A} receptors, CA_3 pyramidal neuron activity would be a direct measure of the tonic level of activation of these receptors by extracellular 5-HT. Prior to the intravenous administration of four successive 25 μg/kg doses of WAY 100,635, the firing activity of the quisqualate-activated CA_3 pyramidal neurons was decreased to about 5 Hz in order to more readily allow the detection of enhancements in firing following administration of the antagonist in control and treated rats. After a steady baseline firing activity was established, an injection of saline always preceded the WAY 100,635 injections.

Drugs

The following drugs were used: reboxetine (Pharmacia and UpJohn, Kalamazoo, MI, U.S.A.); clonidine and WAY 100,635 (RBI, Natick, MA, U.S.A.); 5-HT creatinine sulfate, NE bitartrate, and quisqualate were purchased from Sigma Chemical (St Louis, MO, USA). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water and injected in a volume of less than 0.2 ml.

Statistical analysis

Results were expressed as means ± SEM. The *n* refers to the number of neurons tested in the figures, however, in the results section *n* corresponds to the number of rats tested. The statistical significant difference between the means of iontophoretic application of NE and 5-HT on CA_3 pyramidal neurons

firing activity to intravenous reboxetine injections and long-term reboxetine administration on RT_{50} values as well as the number of spikes suppressed was assessed with two-way analysis of variance (ANOVA). The difference between the effects of 1 and 5 Hz stimulation frequencies of the 5-HT pathway on the duration of suppression of firing of CA_3 pyramidal neurons was assessed with the paired Student's *t*-test. Possible differences in the magnitude of the effects of 1 Hz electrical stimulation frequencies of the 5-HT pathway to intravenous clonidine injections in control and 21-day reboxetine treated rats were assessed via one-way ANOVA. Lastly, the effects of cumulative injections of reboxetine in control rats, and incremental doses of WAY 100,635 injections on the firing activity of CA_3 pyramidal neurons in control and 21-day reboxetine treated rats were assessed using the one-way repeated measures ANOVA. Post-hoc pairwise multiple comparison procedures were performed with the Tukey test and the Student-Newman-Keuls method for one-way and two-way ANOVAs, respectively.

RESULTS

Effect of acute reboxetine injections on the response of CA_3 pyramidal neurons to microiontophoretic applications of NE and 5-HT

The average number of spikes suppressed from baseline firing activity of hippocampus neurons during microiontophoretic applications of NE and 5-HT provides a reliable index of the sensitivity of postsynaptic α_2-adrenergic and 5-HT_{1A} receptors, respectively (Curet and de Montigny, 1988a; Rueter et al., 1998). These values generated from microiontophoretic applications of NE and 5-HT on the firing activity of CA_3 pyramidal neurons was significant ($F_{2,122}$ = 15.4 and $F_{2,120}$ =11.3, respectively; $P < 0.001$ for both monoamines) and current-dependent ($P < 0.05$ for both monoamines), meaning that increasing ejection currents enhanced the number of spikes suppressed following NE and 5-HT ejections from the micropipette, examples of which are provided in figure 1. The recovery time necessary for pyramidal neurons to regain 50% of their firing rate (RT_{50} value) after microiontophoretic ejections of NE and 5-HT provides an index of the function of the reuptake transporters for these monoamines (de Montigny et al., 1980; Piñeyro et al., 1994). The RT_{50} values generated from microiontophoretic applications of NE and 5-HT on the firing activity of CA_3 pyramidal neurons were significantly prolonged ($F_{2,122}$ = 35.0 and $F_{2,120}$ = 27.1, respectively; $P < 0.001$ for both monoamines) and also current-dependent (P <0.05 for both monoamines), examples of which is also provided in figure 1.

Cumulative doses of 1, 2, and 4 mg/kg of reboxetine were i.v. injected in succession and reduced the firing activity of CA_3 pyramidal neurons to 15%, 13%, and 23%, respectively, but did not significantly differ from baseline or each other ($F_{3,12}$ = 1.6, P = 0.248; n = 4 rats), similar to desipramine (Curet et al., 1992). Reboxetine injections did not significantly influence the number of spikes suppressed by microiontophoretic applications of NE ($F_{3,121}$ = 0.1, P = 1.0) and 5-HT ($F_{3,119}$ = 0.3, P = 0.8). There was no statistically significant interaction between the different doses of reboxetine injected and current of NE and 5-HT ejected on the number of spikes suppressed ($F_{6,118}$ = 0.1, P = 0.990 and $F_{6,116}$ = 0.4, P = 0.87, respectively). In contrast, 1, 2, and 4 mg/kg injections of reboxetine prolonged the RT_{50} values across all of the currents employed on CA_3 pyramidal neurons firing for NE current ejections as compared to controls ($F_{3,122}$ = 58.4, P < 0.001; figure 2), but not to that of 5-HT ($F_{3,119}$ = 1.7, P = 0.176; figure **2**). The effect of reboxetine on this parameter for NE was dose-dependent and reached a plateau at the cumulative dose of 2 mg/kg (P < 0.05; figure 3). Importantly, the lack of effect observed for 5-HT across reboxetine injections was unrelated to the current used ($F_{6,116}$ = 0.2, P = 0.97).

Effect of 21-day reboxetine administration on the response of CA_3 pyramidal neurons to microiontophoretic applications of NE and 5-HT

In reboxetine treated rats, a significant number of spikes were suppressed for microiontophoretic application of NE and 5-HT ($F_{2,98}$ = 22.8 and $F_{2,109}$ = 9.1, respectively; P < 0.001 for both monoamines) on CA_3 pyramidal neurons. The

effect of NE and 5-HT ejections on the number of spikes suppressed was current-dependent ($P < 0.05$ for both monoamines), however, it did not differ among controls and treated rats ($F_{1,99} = 3.2$, $P = 0.08$ and $F_{1,110} = 0.4$, $P = 0.55$, respectively; figure 4). This lack of difference observed in reboxetine-treated rats as compared to controls on the number of spikes suppressed from NE and 5-HT current ejections were not due to the effects of reboxetine treatment ($F_{2,98} = 0.5$, $P = 0.95$ and $F_{2,109} = 0.9$, $P = 0.41$, respectively). Similarly, the RT_{50} value for NE and 5-HT applications on CA_3 neurons in reboxetine-treated rats also increased in a current-dependent manner ($P < 0.05$ for both monoamines; figure 5A and 5B, respectively). In contrast to the number of spikes suppressed obtained for 5-HT ejections, where no difference was detected regardless of drug treatment, an enhancement resulted in the RT_{50} values to microiontophoretic applications of NE and 5-HT in reboxetine treated rats on the firing activity of CA_3 neurons as compared to controls ($F_{2,152} = 79.7$ and $F_{2,158} = 58.7$, respectively, $P = 0.001$ for both monoamines). Furthermore, this difference was also present in the subset of neurons for which all currents were used: the RT_{50} values for NE and 5-HT were almost exactly the same and still significantly different from the control values ($F_{2,152} = 35.8$ and $F_{2,92} = 19.4$, respectively, $P < 0.001$). This prolongation of the effect of 5-HT ejections, as indicated by an enhanced RT_{50} value on CA_3 neuron firing, was much less robust than that observed for NE (figure 5A and 5B), but was nevertheless statistically significant ($F_{1,159} = 46.8$, $P < 0.001$). In addition, this effect of NE or 5-HT on the RT_{50} value did not depend on the current used ($F_{2,152} = 2.6$, $P = 0.079$ and $F_{2,159} = 0.5$, $P = 0.59$, respectively).

Effect of long-term reboxetine treatment on the effectiveness of electrical stimulation of the afferent 5-HT fibers to the hippocampus

The net effect of long-term reboxetine treatment on 5-HT transmission was determined by stimulating the ascending 5-HT pathway at a frequency (1 Hz) similar to the spontaneous firing rate of 5-HT neurons (Vandermaelen and Aghajanian, 1983). A brief suppression of firing of CA_3 neurons results from electrical stimulation of the 5-HT pathway due to the release of 5-HT mediated through postsynaptic 5-HT_{1A} receptors (Chaput et al., 1986; Chaput and de Montigny et al.,1988). The effectiveness of the electrical stimulation of the ascending 5-HT fibers at the level of the ventro-medial tegmentum on the firing activity of the postsynaptic hippocampal CA_3 pyramidal neurons at a frequency of 1 Hz did not differ ($P = 0.74$) in control and reboxetine treated rats (figure 6). To assess the function of the terminal 5-HT_{1B} autoreceptors which controls the amount of 5-HT released for each electrical impulse reaching 5-HT terminals, the ascending 5-HT pathway was subsequently stimulated at a frequency of 5 Hz while recording the firing activity of the same hippocampus CA_3 pyramidal neurons. In controls and reboxetine-treated rats, increasing the frequency of stimulation from 1 to 5 Hz induced the same 26% reduction of the duration of suppression of firing ($t_{17} = 11.87$ and $t_9 = 13.95$, respectively, $P < 0.001$ for both groups; figure 6). Thus, at a frequency of 5 Hz, the effectiveness of the electrical stimulations in reboxetine-treated rats was not different from that of the controls ($P = 0.42$; figure 6).

Effects of long-term treatment with reboxetine on the sensitivity of α_2-adrenergic auto- and heteroreceptors

Previous studies have shown that antidepressant treatments which increase the concentration of NE in the synaptic cleft desensitize the terminal α_2-adrenergic heteroreceptors located on 5-HT fibers in the dorsal hippocampus after long-term administration (Mongeau et al., 1994a,b; Yoshioka et al., 1995), however, leaving α_2-adrenergic autoreceptors normosensitive (Szabo and Blier, 2000b; Sacchetti et al., 1999; Mateo et al., 1998; Mongeau et al.,1994a; Moret and Briley, 1994). In control (n = 9 rats) and 21-day reboxetine (2.5 mg/kg/day; n = 7) treated rats, 1 Hz electrical stimulations of the ascending 5-HT pathway on the firing activity of dorsal hippocampus CA_3 pyramidal neurons produced similar suppressions of firing which did not statistically differ when compared to each other (P = 0.27; figure 7). In addition, a low dose of clonidine (10 μg/kg), which assesses the sensitivity of terminal α_2-adrenergic autoreceptors, was able to significantly increase the effectiveness of a 1 Hz stimulation to a similar degree in control (34%) and reboxetine treated (30%) rats (P < 0.001 for both; figure 7). In contrast, a high dose of clonidine (400 μg/kg), which assesses the sensitivity of terminal α_2-adrenergic heteroceptors, significantly reduced the effectiveness of 1 Hz electrical stimulations as compared to the stimulations without clonidine injection in controls (25 %), but not in 21-day reboxetine treated rats (P < 0.001 and P = 0.79, respectively; figure 7).

Effect of long-term treatment with reboxetine on the tonic acivation of 5-HT_{1A} receptors using WAY 100635

Due to a recent finding that all major classes of antidepressants induce a tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus (Haddjeri et al., 1998), the possibility that reboxetine could also influence this parameter was examined. Dorsal hippocampus CA_3 pyramidal neurons were activated by applying a small ejection current of quisqualate in controls and long-term reboxetine treated rats (n = 5 rats for both groups), an example of each is provided in figure 8. In control rats receiving saline for 21-days, the i.v. administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 (four successive doses of 25 μg/kg) did not modify the firing activity of dorsal hippocampus CA_3 pyramidal neurons (P = 0.08; figure 8A). In contrast to rats receiving reboxetine for 21 days, i.v. administration of the second dose and subsequent doses of WAY 100,635 was able to induce a marked enhancement of the firing activity of CA_3 pyramidal neurons (P < 0.001 for all doses except the first; figure 8B and 9). The effect of a final injection of WAY 100,635 (cumulative dose of 100 μg/kg) increased the firing activity of CA_3 pyramidal neurons by 140% above the basal firing rates (figure 9).

DISCUSSION

The electrophysiological results presented herein indicate that acute injections of reboxetine prolonged the duration of action of NE in the hippocampus via blockade of the NE transporter, as measured by a greater RT_{50} value for NE applications onto CA_3 pyramidal neurons (figure 1 and 2). In contrast, reboxetine injections did not alter this parameter for 5-HT applications on the same neurons, demonstrating that acute injections of reboxetine does not impact upon the function of the 5-HT transporter in this postsynaptic structure when the effect of NE is prolonged more than two-fold (figures 1 and 3). This specific effect of reboxetine on the RT_{50} values to application of NE and 5-HT on CA_3 pyramidal neuron firing is similar to results obtained with the TCA desipramine (Lacroix et al., 1991). These findings are also consistent with the results of microdialysis studies showing that acute reboxetine administration (15 mg/kg, i.p.) produces an increased level of NE in the dialysates in the frontal cortex and dorsal hippocampus without altering that of 5-HT in the striatum (Sacchetti et al., 1999). When considered together with biochemical binding data on the transporter (Wong et al., 1999), such results confirm that acutely administered reboxetine selectively blocks the NE reuptake transporter *in vivo* without altering the function of the 5-HT reuptake transporter.

The potency of selective NE reuptake inhibitors or dual NE/5-HT reuptake inhibitors to suppress the firing activity of LC NE neurons by 50% (ED_{50} value) correlate well with the dose required to produce a maximal prolongation of the

RT_{50} value for NE application on CA_3 pyramidal neuron firing. When comparing such ED_{50} and RT_{50} values for reboxetine (Szabo and Blier, 2000b) to those previously obtained with desipramine (Béïque et al., 1998; Lacroix et al., 1991), a discrepancy between the potency of these two selective NE reuptake inhibitors is apparent in the hippocampus as compared to the LC. The ED_{50} value to suppress the firing activity of LC NE neurons for desipramine (0.24 ± 0.01 mg/kg; Béïque et al., 1998) is half that reported for reboxetine (0.48 ± 0.01 mg/kg; Szabo and Blier, 2000b), whereas the effect of reboxetine to prolong the RT_{50} value to NE applications on CA_3 pyramidal neuron firing was three times greater than that of DMI: it reached a plateau at a cumulative dose of only 2 mg/kg (figure 2) while that of desipramine produced a maximal effect after 6 mg/kg (Lacroix et al., 1991). In addition, reboxetine and duloxetine (a dual NE/5-HT reuptake inhibitor) possess nearly identical ED_{50} values on LC firing suppression, but reboxetine is five times more potent in prolonging the RT_{50} value to applications of NE on CA_3 pyramidal neurons as compared to duloxetine (figure 2; Kasamo et al., 1996). These results indicate that reboxetine appears to be more potent on the NE reuptake process in the hippocampus than in the LC. The exact basis for this difference remains to be elucidated.

The sensitivity of postsynaptic α_2-adrenergic and 5-HT_{1A} receptors was not altered in the hippocampus after long-term reboxetine administration, as indicated by the number of spikes suppressed from microiontophoretic applications of NE and 5-HT on CA_3 pyramidal neuron firing being similar (figure 4). In contrast, it had been reported that 5-HT_{1A} receptors become

supersensitive in postsynaptic structures to prolonged TCA treatment (de Montigny and Aghajanian, 1978; Menkes et al., 1980; Wang and Aghajanian, 1980). Also, TCA drugs had been shown to alter adrenoceptor function, particularly to sensitize α_1-adrenoceptor in the facial motor nucleus and the lateral geniculate body and α_2-adrenoceptors in the amygdala after long-term treatment (Wang and Aghajanian, 1980; Menkes et al., 1981; Menkes et al., 1983; Freedman and Aghajanian, 1985). Prior to the advent of reboxetine, the lack of selective NE reuptake blocking drugs used clinically without a TCA moiety made it impossible to rule out structural versus NE components of such drugs in delineating the clinical impact of these properties. The present results on reboxetine are thus similar to those reported by Lacroix et al., 1991, indicating that postsynaptic α_2-adrenoceptors remained normosensitive in the hippocampus after long-term desipramine (10 mg/kg/day) treatment, but stand in opposition to an increased 5-HT_{1A} receptors sensitivity (de Montigny and Aghajanian, 1978). In contrast, both reboxetine and desipramine have been shown to reduce the sensitivity of 5-HT_{1A} receptors that control the firing activity of rat LC neurons after long-term treatment (Szabo and Blier, 2000b). Yet, reboxetine but not desipramine attenuates the 5-HT_{2A}-receptor mediated inhibition of LC neurons firing (Szabo and Blier, 2000b). These observations therefore clearly highlight that "similar" receptor subtypes located in various brain areas adapt differently to sustained treatment with antidepressants. Moreover, these differences among these two NE reuptake inhibitors may be due to the presence or absence of a tricyclic strucuture.

Consistent with the results obtained with acute reboxetine injections, rats treated with reboxetine for 21-days also presented an increase in the RT_{50} value to NE application (figure 5A). However, in contrast to results obtained with 5-HT in the acute reboxetine experiments, long-term reboxetine treatment also increased the RT_{50} value for 5-HT (figure 5B). This slight increase in the RT_{50} value observed with 5-HT after prolonged administration does not detract from reboxetine being an effective NE reuptake blocker after acute and long-term administration, but it provides evidence that this agent may produce an alteration in 5-HT transporter activity (figure 5B). In this regard, studies have shown that NE and 5-HT transporters are both regulated by protein kinase C (Miller and Hoffman, 1994; Qian et al., 1997; Apparsundaram et al., 1998). Consequently, this small inhibitory action of reboxetine on 5-HT reuptake actually may result more from intracellular events than from a direct physical inhibitory interaction of reboxetine with the 5-HT reuptake binding sites. This alteration in 5-HT reuptake function may be germane to the observation of a small but significant NE reuptake inhibition produced after a sustained SSRI treatment with paroxetine (Owens et al., 2000). The functional significance of these phenomena, given their small magnitude, are not known and must be put into perspective when considering the impact of these selective agents on antidepressant drug efficacy.

The sensitivity of somatodendritic α_2-adrenergic autoreceptors which control the firing activity of LC NE neurons do not desensitize after a 21-day reboxetine administration in control rats, therefore explaining their sustained attenuation of firing activity (Szabo and Blier, 2000b). Similarly, the terminal α_2-

adrenergic autoreceptors mediating the release of NE in the hippocampus, remained normosensitive in 21-day reboxetine treated rats (figure 7). These results are fully consistent with the reports showing that the effect of clonidine on NE dialysates is unaltered in 14-day reboxetine treated rats in the hippocampus, but attenuated in the frontal cortex (Invernizzi et al., 2000, 2001). However, drugs which increase the synaptic availability of NE desensitize α_2-adrenergic heteroceptors located on 5-HT terminals in the hippocampus and may account for an increase in synaptic 5-HT availability in the same structure (Mongeau et al., 1994a; Yoshioka et al., 1995) and other postsynaptic structures (Blier and Bouchard, 1994). It was thus decided to assess whether reboxetine desensitized the terminal α_2-adrenergic heteroceptors located on 5-HT terminals, as previously reported with desipramine (Mongeau et al., 1993; Yoshioka et al., 1995). Reboxetine did desensitize terminal α_2-adrenergic heteroceptors as determined by the attenuated response to the high dose of clonidine (400 μg/kg) on hippocampal neurons (figure 7B). This effect is likely mediated by an increased availability of NE because the MAOI befloxatone could no longer desensitize this heteroceptor in NE lesioned rats (Mongeau et al., 1994a).

The amount of 5-HT being released per impulse reaching 5-HT terminals was not altered in 21-day reboxetine as compared to saline treated rats: there was no change in the efficacy of 5-HT fiber stimulation on hippocampus neuron firing as demonstrated in figure 6. This paradigm assesses terminal 5-HT_{1B} autoreceptors sensitivity located on 5-HT neurons controlling 5-HT release. A change in sensitivity of this receptor subtype is usually indicative of

antidepressants that potently block the reuptake of 5-HT (Mongeau et al., 1994a), such as citalopram (Chaput et al., 1991), fluvoxamine (Dong et al., 1991), fluoxetine (Blier et al., 1988), paroxetine (Chaput et al, 1991), and venlafaxine (Béïque et al., 2000). Clearly then, reboxetine does not belong to that category of antidepressant agents.

It is interesting that a drug like reboxetine which increases the synaptic availability of NE can also increase that of 5-HT in the hippocampus. As compared to controls, the 140% increase in CA_3 pyramidal neuron firing in response to i.v. administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 (figure 8B and 9), is well within the range of that produced with other antidepressant treatments (Béïque et al., 2000, Rueter et al., 1998, Haddjeri et al., 1998), with lithium addition (Haddjeri et al., 2000) and even to a greater extent with the combination of mirtazapine and paroxetine than either drug alone (Besson et al., 2000). It is interesting to note that the latter strategies are endowed with a greater antidepressant efficacy (Rouillon and Gorwood, 1998; Debonnel et al., 2000). These observations therefore provide considerable face validity for the use of the hippocampus as a brain structure relevant to antidepressant drug action. However, since the effectiveness of the stimulation of the afferent 5-HT pathway to the hippocampus is unaltered in 21-day reboxetine treated rats, a small inhibition of 5-HT reuptake, combined with decreased α_2-adrenergic heteroceptors sensitivity on 5-HT terminals (figure 7B), may then be postulated to account for the increased tonic activation of 5-HT_{1A} receptors in the hippocampus of these animals.

In conclusion, it has been shown that NE availability in the hippocampus is enhanced by reboxetine treatment via blockade of the NE transporter (Sacchetti et al., 1999). Since both the acute and long-term administration of reboxetine produced the same degree of enhancement of NE in the extracellular milieu in the same experiments in the hippocampus, the relationship between the delayed clinical response to reboxetine with respect to hippocampal function is not clear. However, the hippocampus is endowed with excitatory β-adrenoceptors, inhibitory α_1-adrenergic and α_2-adrenergic receptors (Curet and de Montigny, 1988a,b; Lacroix et al., 1991). Thus, it appears that a delayed desensitization of β-adrenoceptors, which is common to most antidepressant treatments, including desipramine, also occurs with reboxetine (Riva et al., 1989; Lacroix et al., 1991). This would lead to an overall increased inhibitory action of the treatment on the firing of pyramidal neurons mediated by a decreased function of excitatory β-adrenoceptors and an increased activation of normosensitive α-adrenergic and 5-HT_{1A} receptors. The increase in transmission at 5-HT_{1A} receptors in the hippocampus, also common to all antidepressant treatments, occurs for reboxetine, although not via their sensitization as is the case for TCA drugs, but rather via an increase in 5-HT availability. The latter effect of sustained reboxetine administration on the 5-HT system with respect to the antidepressant response is currently not known. A dietary tryptophan depletion in patients responding to reboxetine treatment for major depression could shed light on this issue.

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LEGENDS TO FIGURES

Figure 1. Integrated firing rate histograms illustrating the response of a CA_3 pyramidal neuron to microiontophoretic application of NE and 5-HT in a control rat before and after a reboxetine injection. The three circles at the bottom of the upper histogram indicate that it continues below. This neuron was activated with a quisqualate ejection current of –3 nA. Note that when compared to microiontophoretic drug application before reboxetine injection, the durations of CA_3 pyramidal neuron suppression with applications of NE are markedly increased and those for 5-HT are unchanged.

Figure 2. Histograms representing the recovery times (RT_{50}) of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of NE with varying currents in control rats receiving cumulative i.v. injections of reboxetine. The number of neurons tested is given at the bottom of each column. * $P < 0.001$ when comparing microiontophoretic NE applications using two-way ANOVA with Student-Newman-Keuls method.

Figure 3. Histograms representing recovery time (RT_{50}) of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of 5-HT with varying currents in control rats receiving cumulative injections of reboxetine. The number of neurons tested is given at the bottom of each column. Note that reboxetine

administration did not alter the current-dependent increases in the RT_{50} value as opposed to that for NE illustrated in figure 2.

Figure 4. Histograms representing the number of spikes suppressed of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of NE (A) and 5-HT (B) with varying currents in control and 21-day reboxetine (2.5 mg/kg/day) treated rats. The number of neurons tested is given at the bottom of each column. All of the currents differed from each other on the number of spikes suppressed ($P < 0.05$), however, did not differ when compared across treatment groups using the two-way ANOVA with Student-Newman-Keuls method.

Figure 5. Histograms representing recovery times (RT_{50}) of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of NE (A) and 5-HT (B) with varying currents in control and 21-day reboxetine (2.5 mg/kg/day) treated rats. The number of neurons tested is given at the bottom of each column. * $P <$ 0.001 using two-way ANOVA with Student-Newman-Keuls method.

Figure 6. Effects of 1 and 5 Hz electrical stimulations of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons in 9 control and 8 reboxetine (2.5 mg/kg/day X 21 days) treated rats. * P < 0.001 using the paired Student's *t*-test.

Figure 7. Histograms representing the effect of systemic injections of clonidine on the efficacy of the electrical stimulations of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons in control and reboxetine treated rats. * Corresponds to an enhancement and † to a decrease as compared to controls at a $P < 0.001$ significance level using a one-way ANOVA with Tukey test.

Figure 8. Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron, showing its responsiveness to microiontophoretic application of 5-HT before and after the intravenous injection of WAY 100,635 (25 μg/kg X 4) in a control (A) and 21-day reboxetine (2.5 mg/kg/day) treated rat (B). *Horizontal bars* indicate the duration of the applications (current given in nA). Note the altered effectiveness of 5-HT to suppress firing activity after administration of WAY 100,635 (100 μg/kg) in both histograms. Although the RT_{50} values to NE after WAY 100,635 in 21-day reboxetine treated rats are reduced, two other rats had slightly prolonged RT_{50} values.

Figure 9. Effects of the intravenous injection of WAY 100,635 (25 μg/kg X 4) on the percent increase in the firing activity of dorsal hippocampus CA_3 pyramidal neurons in controls and 21-day reboxetine treated rats. *$P < 0.001$ when compared to controls and each other using one-way repeated measures ANOVA and Tukey test.

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Table 1. Spikes suppressed and RT_{50} values following microiontophoretic application of 5-HT and NE

	Spikes Suppressed		RT_{50} Value (seconds)	
	NE	5-HT	NE	5-HT
Control	493 ± 23	545 ± 26	89 ± 08	59 ± 03
Acute Reboxetine Injection				
1 mg/kg	488 ± 23	543 ± 27	132 ± 08 *	57 ± 04
2 mg/kg	508 ± 34	504 ± 35	248 ± 11 *	68 ± 05
4 mg/kg	506 ± 42	522 ± 50	239 ± 14 *	66 ± 07
Sustained Reboxetine Administration				
2.5 mg/kg/day X 21 days	443 ± 21	522 ± 22	106 ± 03 *	73 ± 03 *

Data is reported as least square means for data obtained form current ejections.
*P<0.05 as compared to controls, using Two-Way ANOVA followed by multiple pairwise comparison procedures (Student-Newman-Keul

Figure I.

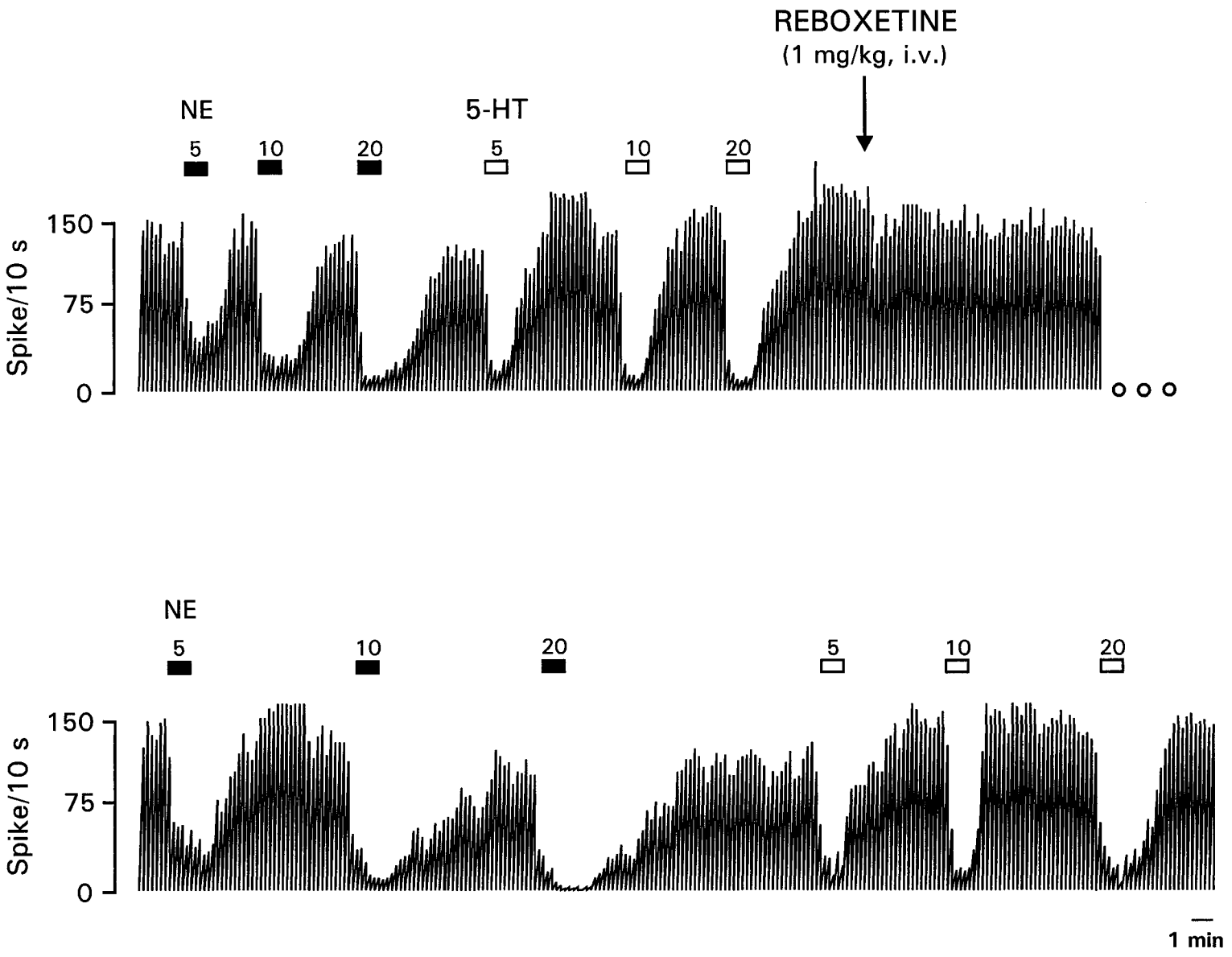

REBOXETINE
(1 mg/kg, i.v.)
NE
5-HT
5
10
20
5
10
20
150
75
0
Spike/10 s
NE
5
10
20
5
10
20
150
75
0
Spike/10 s
1 min

Figure II.

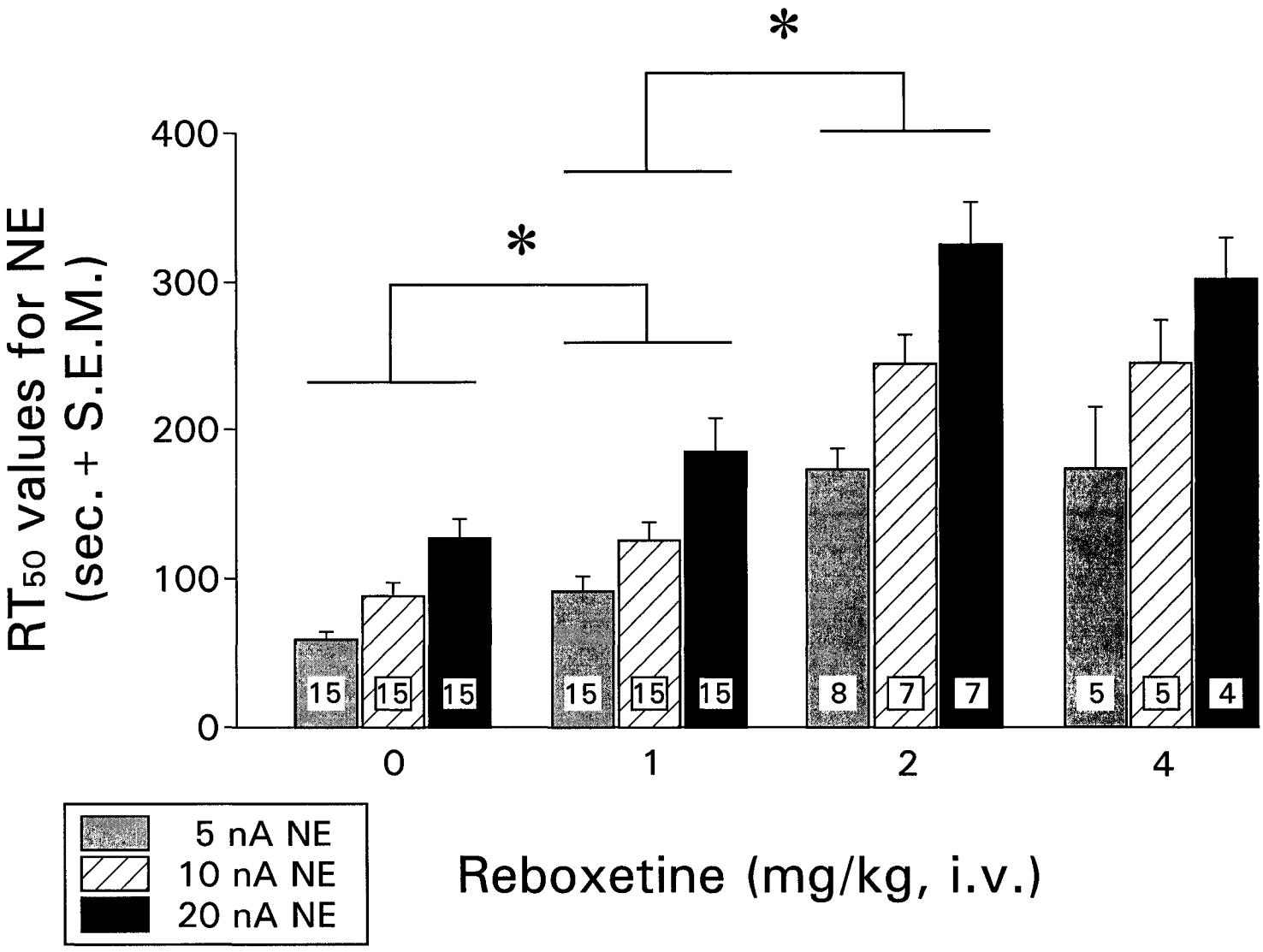

*
*
400
300
200
100
0
RT50 values for NE
(sec. + S.E.M.)
15
15
15
15
15
15
8
7
7
5
5
4
0
1
2
4
5 nA NE
10 nA NE
20 nA NE
Reboxetine (mg/kg, i.v.)

Figure III.

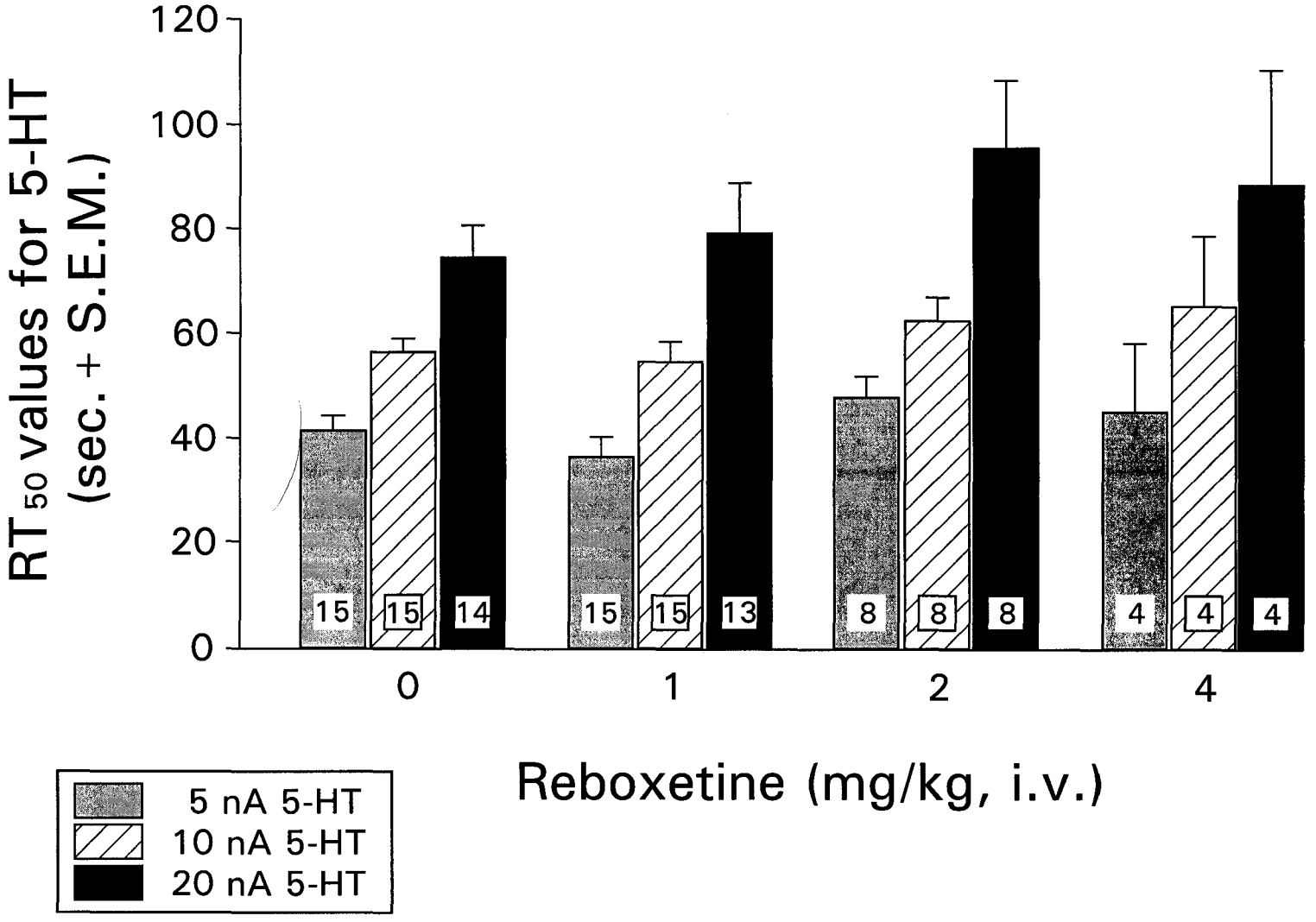

RT50 values for 5-HT
(sec. + S.E.M.)
120
100
80
60
40
20
0
15
15
14
15
15
13
8
8
8
4
4
4
0
1
2
4
Reboxetine (mg/kg, i.v.)
5 nA 5-HT
10 nA 5-HT
20 nA 5-HT

Figure IV.

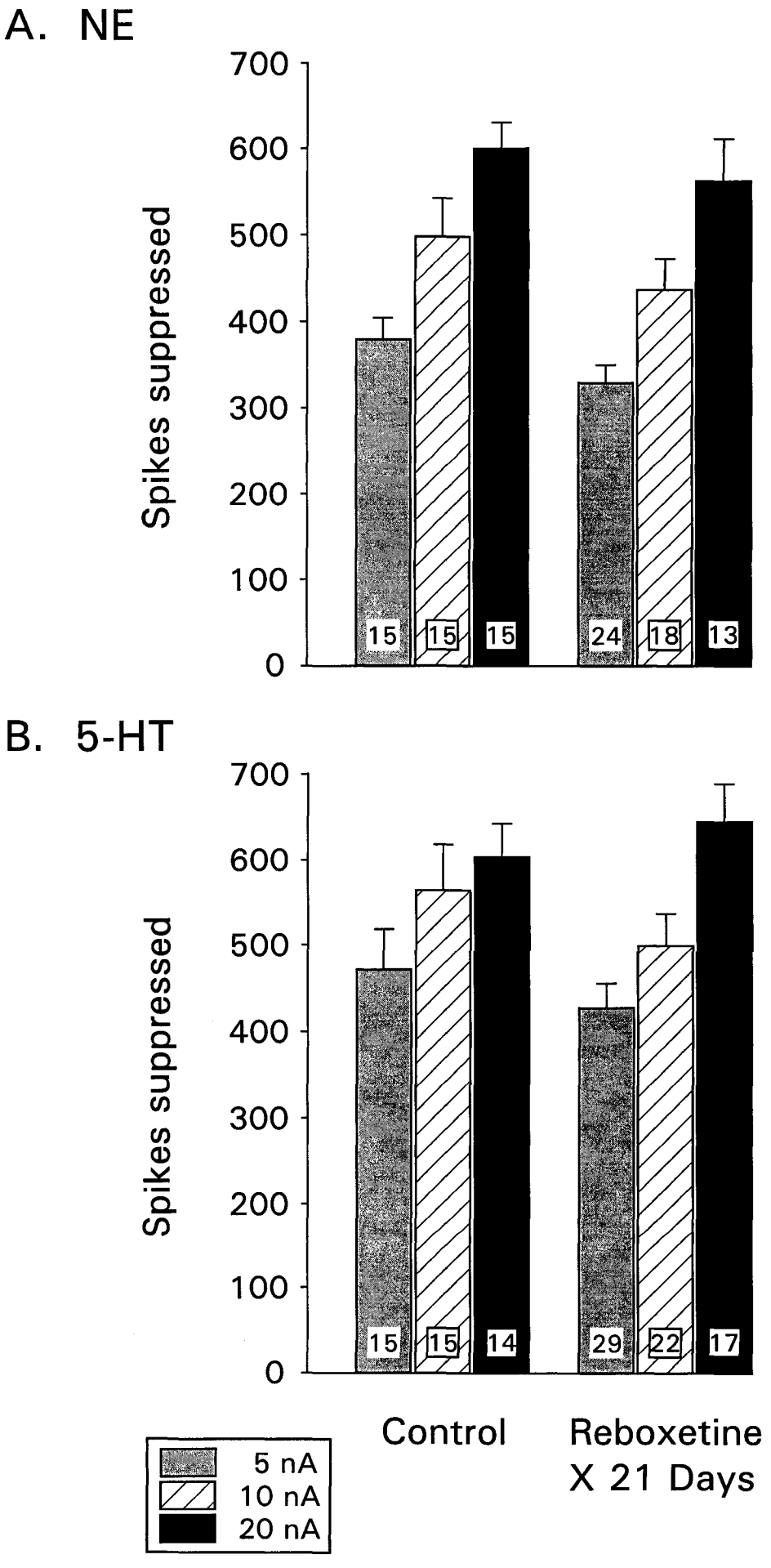

A. NE
Spikes suppressed
700
600
500
400
300
200
100
0
15
15
15
24
18
13
B. 5-HT
Spikes suppressed
700
600
500
400
300
200
100
0
15
15
14
29
22
17
Control
Reboxetine
X 21 Days
5 nA
10 nA
20 nA

Figure V.

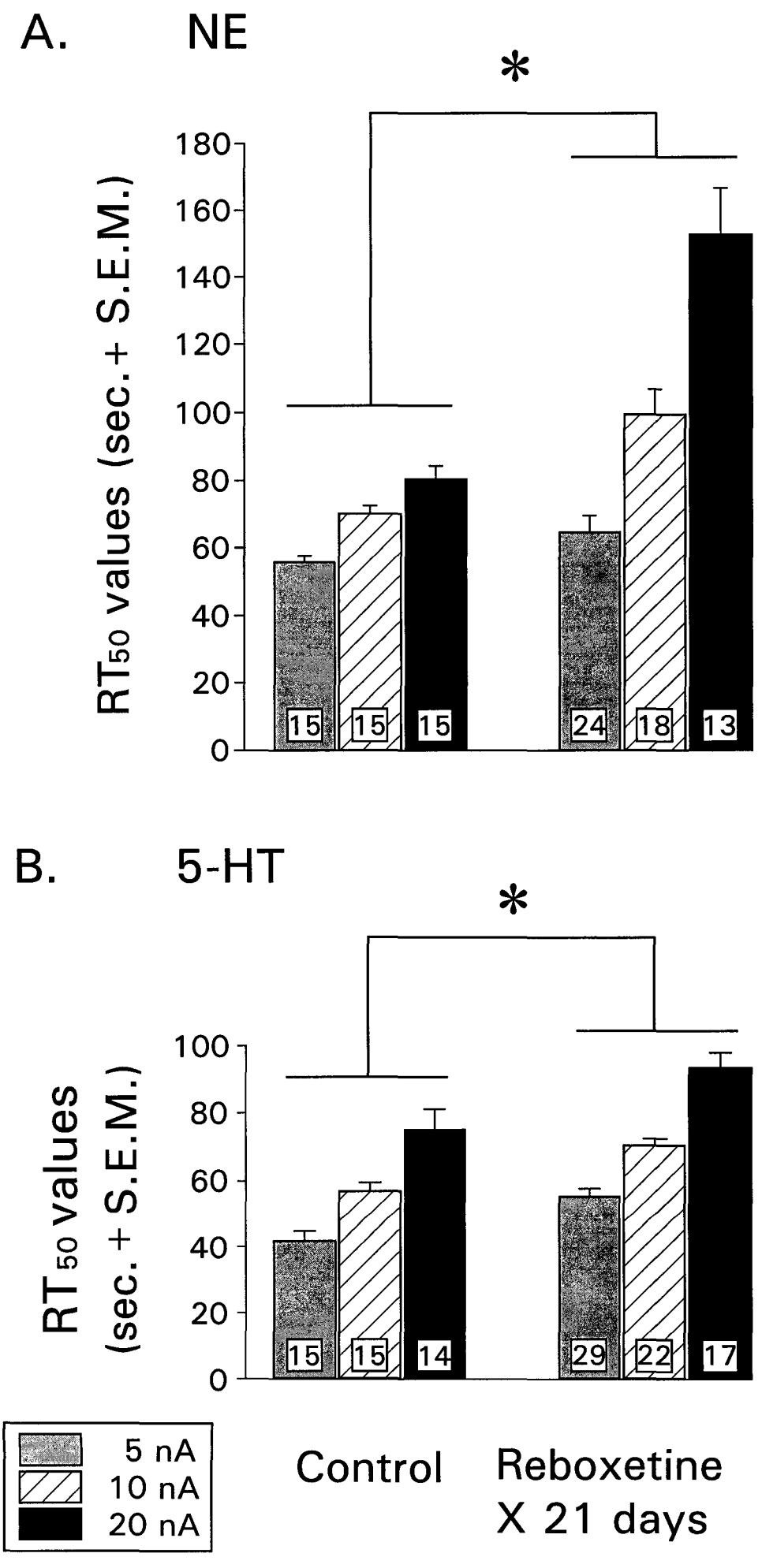

A.
NE
*
180
160
140
120
100
80
60
40
20
0
RT50 values (sec.+ S.E.M.)
15
15
15
24
18
13
B.
5-HT
*
100
80
60
40
20
0
RT 50 values
(sec.+ S.E.M.)
15
15
14
29
22
17
5 nA
10 nA
20 nA
Control
Reboxetine
X 21 days

Figure VI.

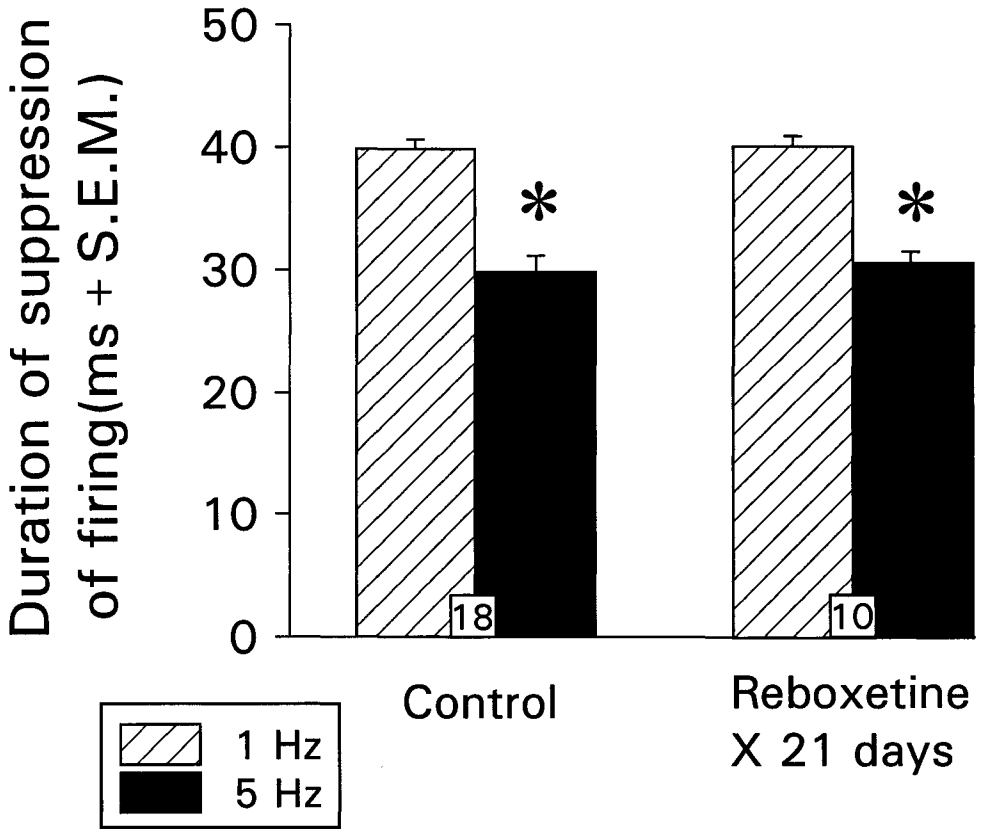
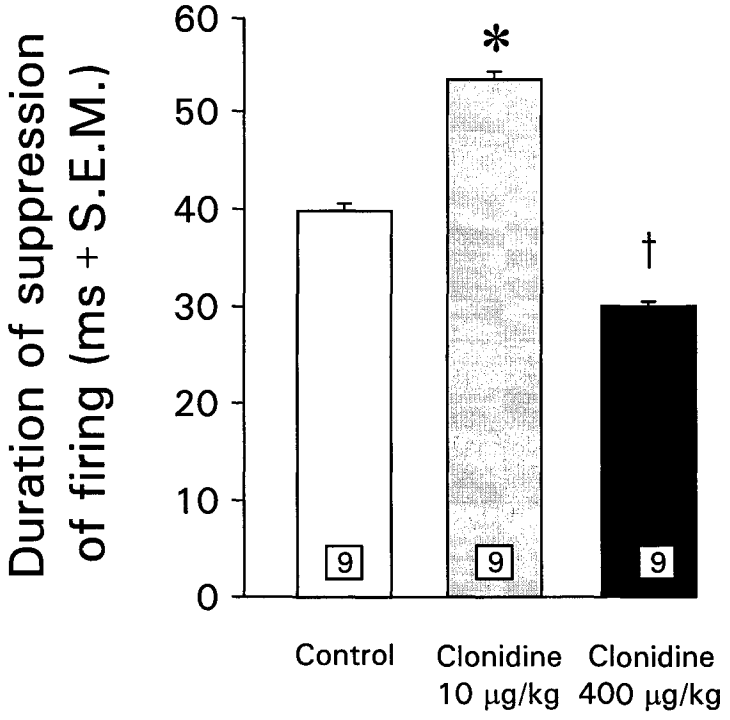

Duration of suppression of firing(ms + S.E.M.)
50
40
30
20
10
0
*
*
18
10
Control
Reboxetine X 21 days
1 Hz
5 Hz

Figure VII.

A. Control



B. Reboxetine X 21 Days

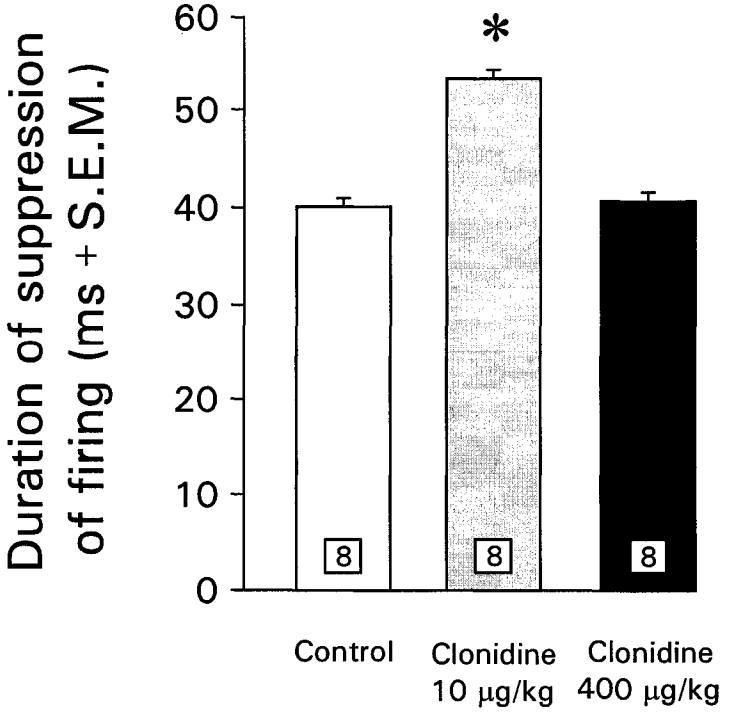
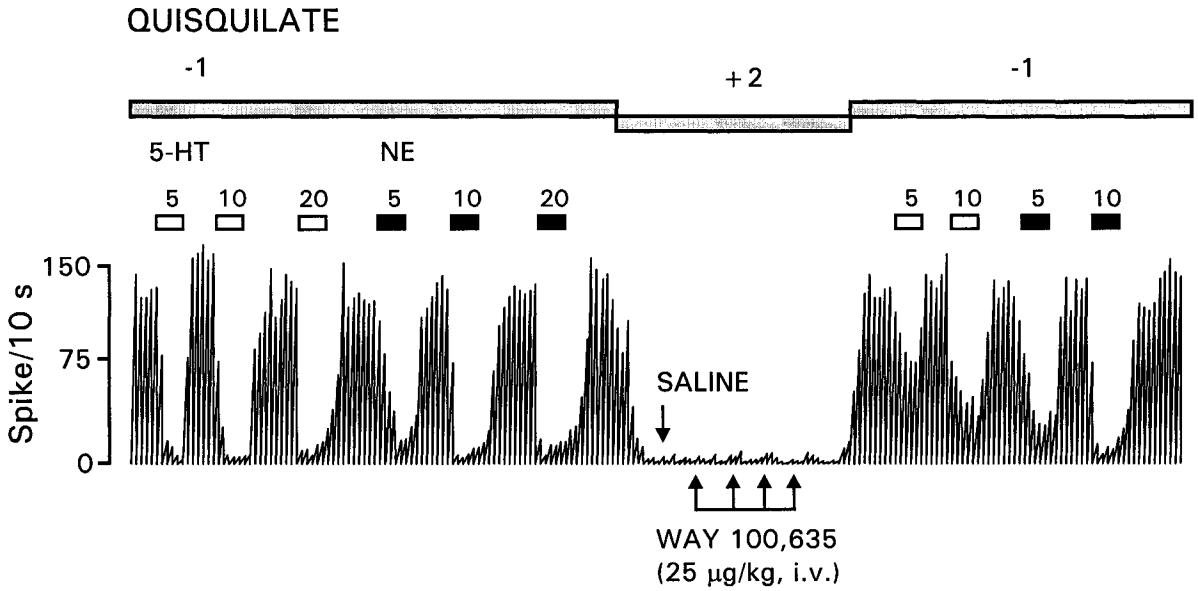


Figure VIII.

A. Control



B. Reboxetine X 21 days

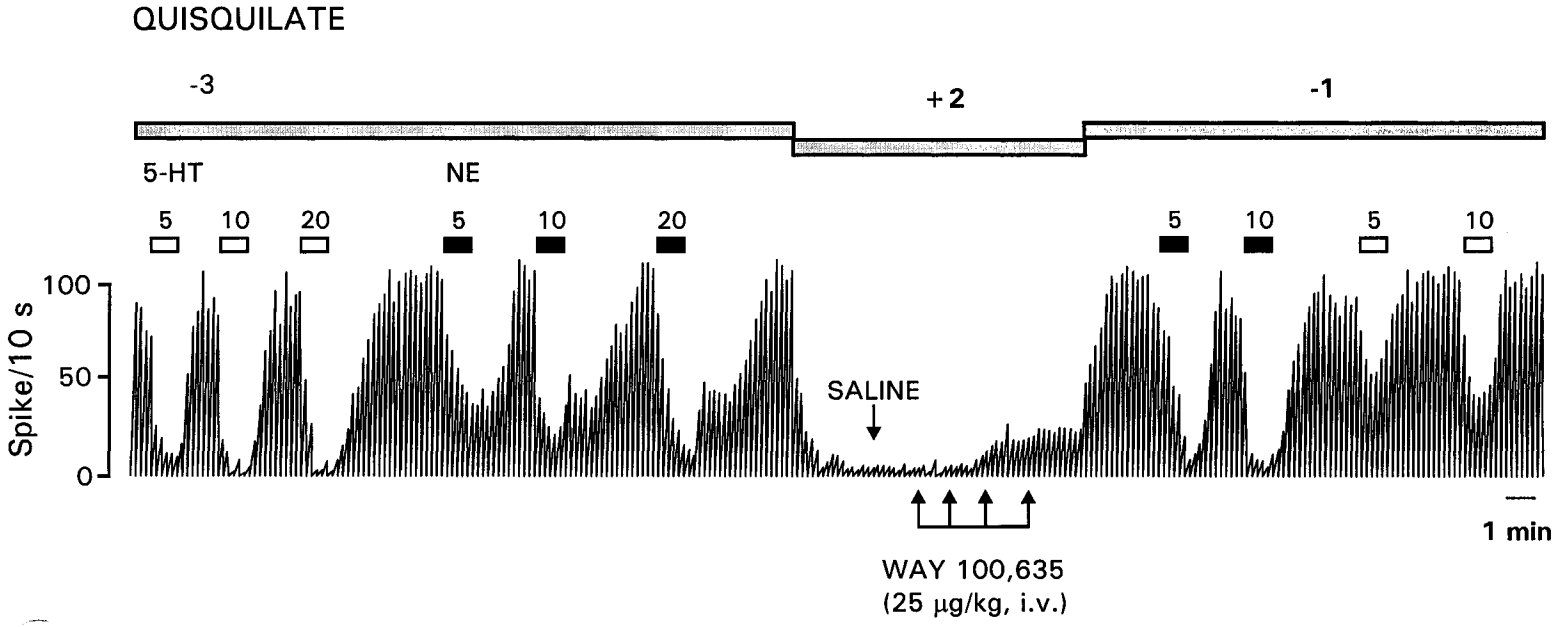
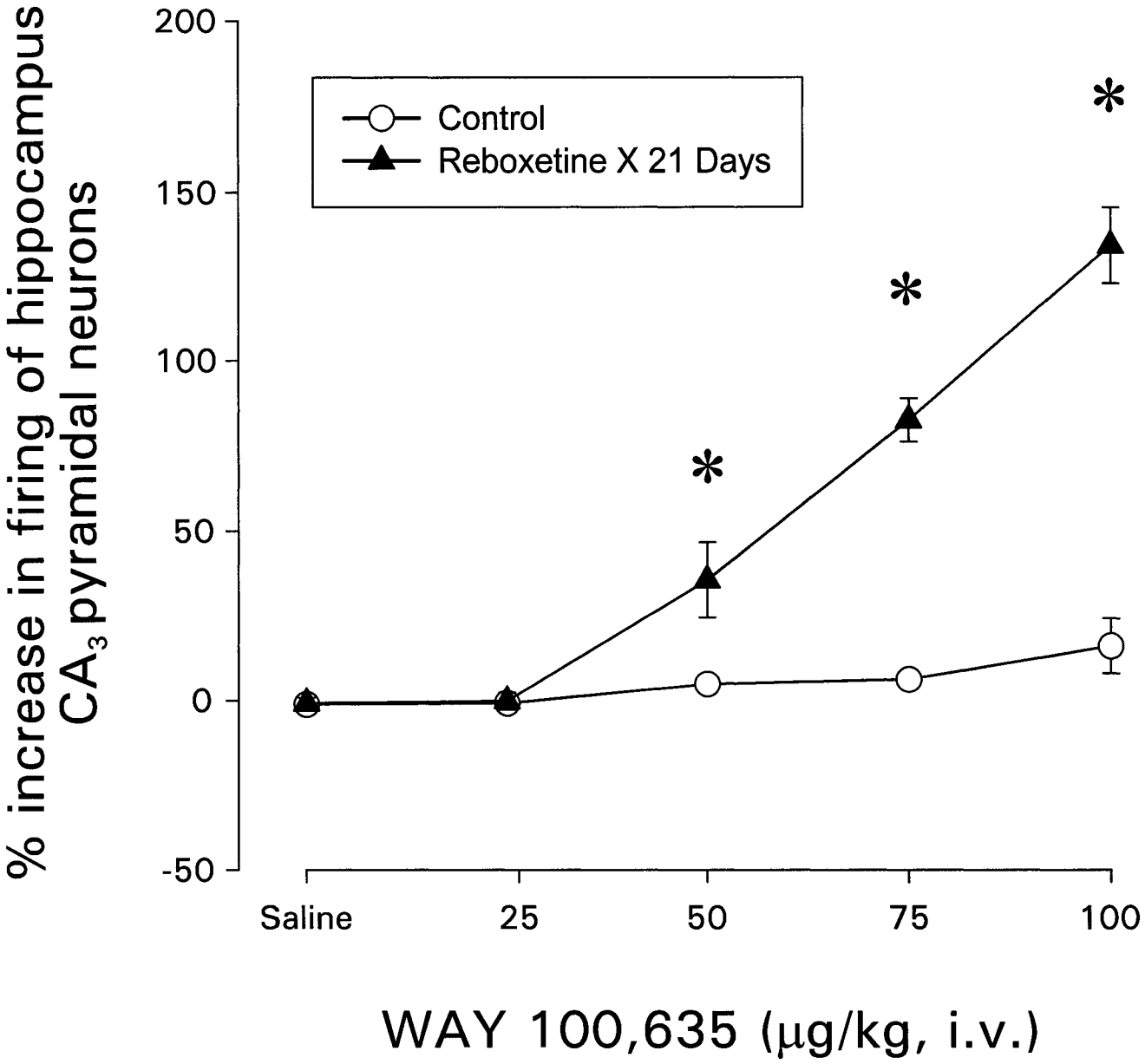


Figure IX.


% increase in firing of hippocampus CA3 pyramidal neurons
200
150
100
50
0
-50
Control
Reboxetine X 21 Days
*
*
*
Saline
25
50
75
100
WAY 100,635 (µg/kg, i.v.)

CHAPTER IX.

GENERAL DISCUSSION:

The experiments presented in this document were undertaken to further characterize interactions between two brainstem neuronal systems involved in the regulation of anxiety and affective disorders. In support of a monoamine dysfunction hypothesis related to these disorders, the results in this document contributes further to this knowledge and provides the manner by which the 5-HT and NA systems modulate each other. Novel insight into the mechanism of action of antidepressant agents is also conveyed. It is concluded from these studies that the 5-HT system, and antidepressants targeting this monoamine, regulate other neurotransmitters following a sustained administration to impart a more direct effect on LC NA firing. Antidepressant induced adaptations and cross-talk between these two neurotransmitters at the cell body and terminal levels account for results obtained with selective agents on one monoaminergic system being able to alter the other. The inter-modulation between NA and 5-HT, as well as their receptor adaptations, is likely dictating the therapeutic effectiveness of antidepressants during a sustained administration.

The first study (Chapter II) unveiled an important finding, indicative of being published as a special report, and served as the impetus for subsequent experiments presented in this document. It was reported in chapter II that the firing activity of LC NA neurons is attenuated after 21-days, but not during a 2-day SSRI administration. Taken with evidence from microiontophoretic studies demonstrating that application of 5-HT onto LC NA neurons (Gorea et al., 1991), and systemic injections of SSRIs (Béïque et al., 1999) fail to alter the firing activity of these neurons, it appears that the attenuation on this nucleus may be due to an alteration on the 5-HT system. A study measuring the regional cerebral blood flow (rCBF) with glucose in long-term paroxetine treated rats (an index which correlates with neuronal activity) indicates that the LC was nearly the only brain area attenuated by this parameter (Freo et al., 2000). It is interesting to envisage that if depressed or anxious patients have an altered/attenuated 5-

HT tone on LC NA neurons, alarming external or internal stimuli would trigger dysregulation of this nucleus and permit NA perturbations to occur at times of stress (Szabo and Blier, 2001, located in the appendix; Goddard and Charney, 1997). This attenuation on LC activity during a long-term SSRI administration is congruent with the delayed onset of action of these agents in anxiety and affective disorders. In support of this contention, administration of the α_2-adrenoceptor antagonist yohimbine enhances the activity of LC NA neurons in the rat and can also produce anxiety in healthy volunteers and trigger panic attacks in patients with panic disorder (Charney et al., 1984, Charney and Henninger, 1986). With this premise in mind, elucidation of the function of possible 5-HT receptor subtypes that mediate the attenuation of LC NA firing during a long-term SSRI treatment was deemed important. More relating to the effect of antidepressants with respect to anxiety will be mentioned in the concluding paragraphs of this discussion.

Augmented 5-HT release in the LC is likely contributing to the attenuation on NA neuron firing observed during a long-term SSRI treatment. Although, 5-HT neurons in the MR and pericoeruleus area may be contributing, 5-HT neurons in the DR appear to be of prime importance in mediating this effect (see section 4.2.). It has been documented by Chaput et al., (1986) and replicated by others that SSRIs attenuate the firing activity of 5-HT neurons upon an acute administration (see section 5.5). Somatodendritic 5-HT_{1A} and terminal 5-$HT_{1B/D}$ autoreceptors desensitize to restore and increase firing activity and release capacity of 5-HT neurons during a long-term SSRI treatment, respectively. The results with SSRIs on LC firing presented in chapter II and III may now be used to also explain the peculiar results on LC activity obtained with amiflamine nearly 15 years ago (Blier and de Montigny, 1986). This MAOI-A preferentially inactivates 5-HT and emulates the SSRI induced adaptations on 5-HT neurons during a long-term administration (see sections 5.3 and 5.5). The only difference is that amiflamine augments the availability of 5-HT due to catabolic inhibition verses transporter blockade. It is now not surprising that amiflamine alters LC activity and in a manner *identical* to results obtained with citalopram during a prolonged administration. The attenuation on LC activity during a long-term

SSRI and amiflamine treatment results from increased availability of 5-HT that leads to desensitization of autoreceptors controlling 5-HT activity to increase transmission in this nucleus.

Recent results demonstrate that the spontaneous firing activity of 5-HT neurons in the DR of rats treated with citalopram and paroxetine did not differ from each other at various time points during a sustained administration (figure 1). This was important to assess as these agents were used in the studies presented in chapters II and III and rendered the conclusion that attenuated LC activity is attributed to alterations of DR neurons and 5-HT release. The firing activity of 5-HT neurons in rats treated with paroxetine when recorded at day 14 did not appear to fully recover back to basal firing rates. This effect, however, did not reach statistical significance and possibly reflects sample size. Nonetheless, this may reflect less of an excitatory NA tone to the DR. Indeed, a greater reduction on the spontaneous firing activity of NA neurons in rats treated with paroxetine versus citalopram was obtained (Chapter I and II). This is attributed to paroxetine possessing a moderate affinity for ACh receptors (Chapter II). Thus, in the presence of 1) 5-HT transporter blockade, 2) normalized 5-HT firing rate, and 3) augmented 5-HT release capabilities; a net enhancement of 5-HT in the LC occurs during a long-term SSRI treatment (see section 5; table 4 in the introduction) to attenuate NA neuron firing activity. Experiments assessing 5-HT availability in the LC during systemic and sustained SSRI treatments are needed.

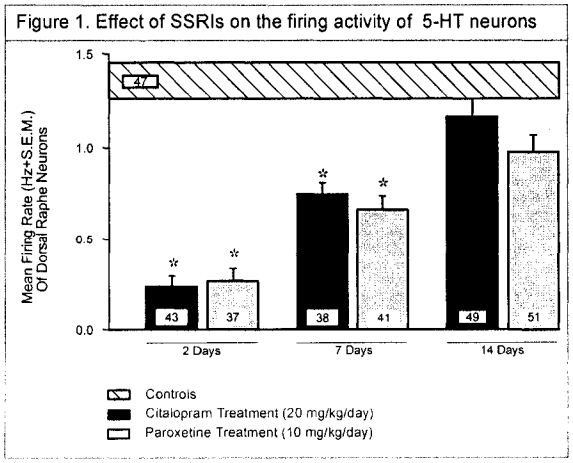


Figure 1: Effect of SSRIs on the spontaneous firing activity of DR 5-HT neurons. The hatched area represents the range (SEM X 2) of the mean firing activity of neurons recorded in control rats. *P<0.05 (Dunn's Method) when compared to the control. value. The number of neurons recorded is given in boxes. Three rats were tested for each treatment group.

The lack of effect on NA neuron firing activity during an acute and subacute SSRI administration is probably due to a balance between 5-HT reuptake blockade and the lack of autoreceptor desensitization controlling the firing activity of 5-HT neurons. This would result in the amount of 5-HT to the LC to be unaltered and represents a spectacular homeostatic mechanism of the brain. Furthermore, this is consistent with this is the lack of altered NA levels in the hippocampus and frontal cortex of rats receiving an i.p. injection of fluoxetine and citalopram, respectively (Page and Abercrombie, 1999; Hatanaka et al., 2000). A balance between 5-HT activity and availability may also explain why Mateo et al., (2000) observed an attenuation on NA neuron firing upon a direct application of an SSRI in the LC whereas Grant and Weiss (2001) replicated our results demonstrating decreased NA activity during a long-term SSRI treatment only (chapter II and III).

Given that a long-term treatment with desipramine, but not with the TCA imipramine nor the SSRI fluoxetine desensitizes 5-HT_3 receptors modulating [^{3}H] NA release from rat hippocampal slices (Mongeau et al., 1994) may be interesting with respect to inter-modulation of the 5-HT and NA systems. It would appear that these 5-HT_3 receptors are important in modulating NA release as SSRIs would presumably overactivate them and increase NA release. This may be an important homeostatic mechanism of the NA system with respect to 5-HT where upon a long-term SSRI treatment NA activity is decreased. On the other hand, since desipramine augments NA concentrations, desensitization of 5-HT_3 receptors may be a mechanism whereby through the influence of 5-HT is able to counteract these effects. Indeed, these same phenomena may also be occurring at the level of the brainstem. Alterations on 5-HT and NA neuron firing and release during SSRI treatments are illustrated in figure 2. The firing activity of neurons in the DR and LC (figure 2A), as well as the release of neurotransmitters in these nuclei (figure 2B), are concordant with the overall effects that NA and 5-HT imparts on each other. The reciprocal projections and inter-modulations between these two nuclei at the level of the brainstem are envisaged to be of major significance in the treatment of anxiety and affective disorders.

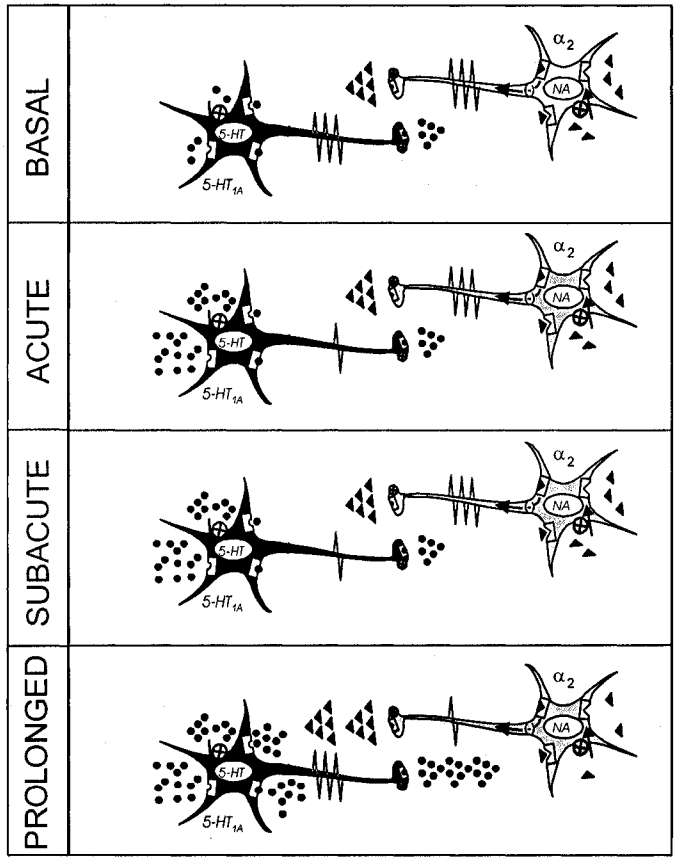
Figure 2. Relationship Between the Effect of SSRI Treatments on 5-HT and NA Neurons

A.

	5-HT	NA
	Firing Activity	
Acute (I.V.)	↓	Ø
Short-Term (2 Days)	↓	Ø
Long-Term (21 Days)	Ø	↓

Figure 1. (A) Impact on the firing activity of 5-HT and NA neurons after various treatments. (B) Schematic representation of the effect of various treatments on NA and 5-HT release. Note: as opposed to (A), figure (B) represents the amount of neurotransmitter in the projection area but have yet to be directly confirmed. Increased 5-HT in the raphe during acute and subacute treatments is due to transporter reuptake blockade. The desensitization of 5-HT autoreceptors during a prolonged treatment lead to increased 5-HT transmission. The increase in NA transmission with a slight decrease of NA in the LC during a prolonged treatment represents enhanced action at excitatory 5-HT_3 receptors (possibly on NA terminals) and attenuated NA activity, respectively. This diagram represents a hypothetical framework for future experiments. Triangles represent NA and circles represent 5-HT. The green and blue cogwheels indicate transporter reuptake processes for 5-HT and NA, respectively. The boxes on the neurons correspond to receptors.

B.



Experiments carried out in Chapter III confirmed previous reports that activation of 5-HT_{1A} receptors with 8-OH-DPAT augments LC NA neuron activity

while agonism of 5-HT_2 receptors with DOI attenuates firing (Chiang and Aston-Jones 1993; Piercey et al., 1994). This enhancement on LC NA neuron firing produced by the 5-HT_{1A} receptor agonist 8-OH-DPAT is abolished in rats undergoing a long-term treatment with SSRIs (chapter III). 5-HT_{1A} receptors appear to induce a tonic regulation on LC activity as antagonism by WAY 100,635 completely suppressed NA neuron firing (chapter IV; figure 1). This desensitization of 5-HT_{1A} receptors may be responsible for the observed attenuation on LC activity observed during a long-term SSRI treatment. These results were also documented with the NA reuptake inhibitor desipramine (chapters III and VII).

The inhibitory effects of a systemic administration of DOI was only slightly blunted in long-term SSRI treated rats and not altered in the desipramine treatment group. Given these results, it was hypothesized that a prolonged SSRI administration leads to enhance 5-HT in the LC and induce a greater activation of slightly desensitized 5-HT_2 receptors. This overactivation occurs in the presence of an abolished 5-HT_{1A} receptor response to yield attenuated NA neuron firing and may be integral to these phenomena. On the other hand, the rapid and robust decrease on LC activity observed in the presence of the NA reuptake inhibitor/TCA desipramine is primarily due to α_2-adrenoceptor overactivation (Chapter III). This is exemplified further by the alteration on NA neuron activity being reversed upon a subsequent injection of the α_2-adrenoceptor antagonist idazoxan (chapter VII, figure 1), and can be blocked by a pretreatment with this agent (Lacroix et al., 1991; Mateo et al., 1998). It is concluded that all antidepressants that inhibit the reuptake of 5-HT or NA attenuate NA neuron firing and desensitize 5-HT_{1A} receptors controlling LC activity. Indeed, this alteration on 5-HT_{1A} receptors occurs with a time course that parallels the beneficial effects of these agents in anxiety and affective disorders.

Enhanced activation of 5-HT_{2A} receptors in the presence of an abolished 5-HT_{1A} receptor response on LC activity occurs during prolonged 5-HT transporter reuptake blockade (chapter IV). These adaptations are regarded as being pivotal in mediating the attenuation on NA neuron firing during a long-term

treatment with SSRIs (chapter III). The 5-HT_{2A} receptor subtype was initially targeted in mediating the attenuation on LC activity during a long-term SSRI administration as: 1) systemic administration of DOI induces a decrease in NA neuron activity and has been demonstrated to be a preferential 5-HT_{2A} receptor agonist *in vivo* (Alukah et al., 1988), 2) YM992 and flibanserin are endowed with 5-HT_{2A} receptor antagonistic properties and blocks the effects of DOI on the firing activity of LC NA neurons upon pre-injection (see chapter VI and unpublished observations, respectively), and 3) 5-HT_{2A} but not 5-HT_{2C} receptors have been localized in the LC. Results in chapter IV demonstrate that a systemic administration of MDL 100,907 did not alter NA neuron activity in rats treated with saline, however, was able to augment LC activity by 28% in rats undergoing a 21-day SSRI treatment. This puts into evidence that 5-HT_{2A} receptors are activated to a greater extent in long-term SSRI treated rats. Augmented NA neuron firing activity following an injection of MDL 100,907 in SSRI treated rats resulted in a non-significant difference as compared to the firing activity of NA neurons recorded in control rats (Chapter IV). Thus, a tonic activation of 5-HT_{2A} receptors controlling LC activity envelops during a long-term SSRI treatment and accounts for the attenuation presented on NA neurons with this antidepressant class.

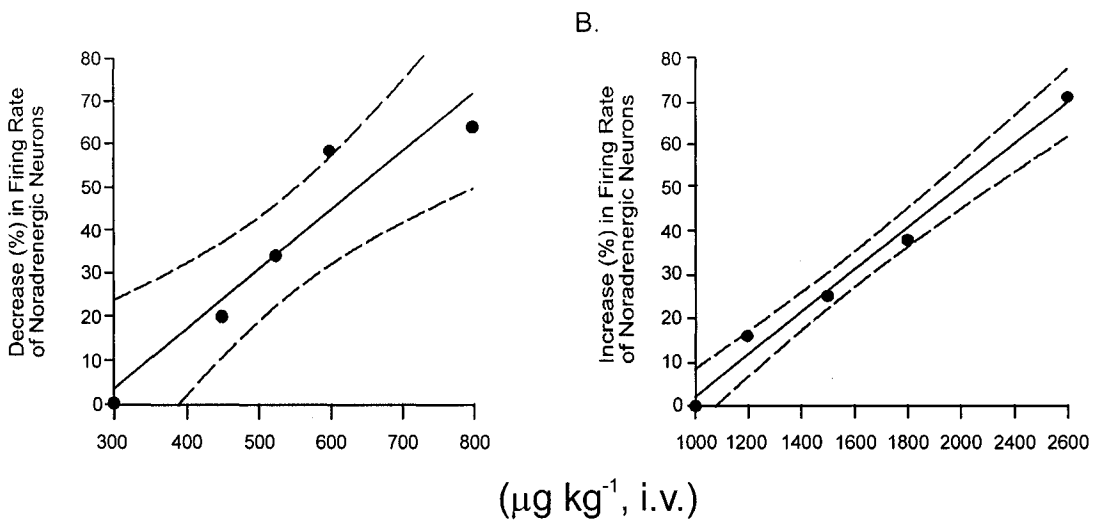
In contrast to the SSRIs, other classes of antidepressants are able to induce their inhibitory effects on LC firing via an overactivation of α_2-adrenoceptors (see section 5). Alterations on α_2-adrenoceptors contributing to attenuate NA activity during a prolonged SSRI treatment was assessed in spite of the evidence already obtained with MDL 100,907. Interesting, paroxetine has been demonstrated to alter the reuptake of NA transporters (Owens et al., 1997; 2000; Gilmore et al., 2001). It is anticipated that if a NA reuptake blockade phenomenon occurs with paroxetine, overactivation of α_2-adrenoceptors would result and attenuate NA neuron firing. Indeed, this is the mechanism whereby reboxetine and desipramine induce their robust effects on LC firing (Chapter III and VII). It was concluded from the results in chapter IV that an alteration in NA transporter function during a long-term treatment with paroxetine is not

responsible for attenuating NA activity. This is reflective of the injection of the selective α_2-adrenocepetor antagonist idazoxan producing the same relative degree of enhancement (~53 %) in long-term SSRI treated rats as compared to rats treated with saline. Given that the spontaneous firing activity of LC neurons in long-term SSRI treated rats are attenuated may precisely account for NA neurons not being able to reach the same absolute value as reported in controls following a systemic administration of idazoxan (chapter IV, figure 8). Thus, a mechanism other than altered activation of α_2-adrenoceptors are involved in the action of SSRIs on LC activity and represents a true 5-HT effect being mediated by overactivation of 5-HT_{2A} receptors (chapter IV). This is elegantly highlighted by the example in chapter IV, figure 7B illustrating that a systemic injection of MDL 100,907 is capable of producing a further increase on NA neuron firing subsequent to an idazoxan injection in SSRI treated rats.

Desensitized 5-HT_{2A} receptors being responsible for mediating the attenuation on LC activity during a long-term SSRI treatment may be disturbing at first glance. In fact, subsensitive 5-HT_{2A} receptors likely envelops as a result of the enhanced 5-HT transmission during a long-term SSRI treatment (section 5.5). A slightly desensitized 5-HT_{2A} receptors response may account for the lack of complete attenuation on NA neuron firing to prolonged SSRI treatment. Indeed, an abolished 5-HT_{1A} receptor response to 8-OH-DPAT during a prolonged SSRI treatment should yield the same effect as if these receptors were antagonized by WAY 100,635 and shut down LC activity. Alterations in, or activations of, 5-HT_{1B} and 5-HT_{2C} receptors controlling LC firing activity during prolonged SSRI treatments cannot be ruled out and may also be contributing to the effects on LC activity. To this end, 5-HT_{1B} receptors have been demonstrated *in vitro* to control glutamate and GABA release in this nucleus (Bobker and Williams, 1989). This receptor is also envisaged to be in high proportions in the LC (Weissman and Nanopoulos, 1985). 5-HT_{2C} receptors when antagonized by ritanserin augment LC activity (Vandermaelen et al., 1982). Recent results indicate that activation of 5-HT_{1B} receptors with RU24969 dose-dependently attenuates LC activity (figure 3A). As the dose of RU24969 is

increased, NA neuron activity becomes augmented and likely reflects the recruitment of 5-HT_{1A} receptors at these doses (figure 3B). Indeed, the inhibitory and excitatory effects of RU24969 is effectively reversed by the 5-HT_{1B} receptor antagonist GR12957 and the 5-HT_{1A} receptor antagonist WAY 100,635, respectively (unpublished observation). The low dose of RU24969 that corresponds to the activation of 5-HT_{1B} receptors on LC firing should be assessed during a long-term antidepressant treatment.

Figure 3. Effect of the 5-$HT_{1B/A}$ receptor agonist RU24969 on LC NA neuron firing



It would appear plausible that activation of 5-HT_{1A} receptors controlling DR 5-HT neuron activity upon a systemic administration of 8-OH-DPAT could reduce 5-HT neuron driven release in the LC and augment NA activity (section 5.1.). However, 5-HT_{1A} receptors have also been localized in the LC and most likely exert an effect on NA neurons. These 5-HT_{1A} receptors in the LC are not on 5-HT elements as a 5,7-DHT treatment did not significantly alter the [^{3}H]8-OH-DPAT binding profile in this nucleus (Weissman-Nanopoulos et al., 1985). As illustrated in table 2., of the introduction, this 5-HT neurotoxin destroys DR versus MR neurons. Given the impact that 5-HT from the DR exerts on NA neuron firing (see section 4), coupled with inhibitory effects 5-HT_{1A} receptors induce on 5-HT activity, elucidation of whether ligands directed at this receptor alter LC activity

being dependent on 5-HT neurons was mandatory. The effects of 8-OH-DPAT, as that previously shown for WAY 100,635 is dependent on intact 5-HT neurons as a 5,7-DHT treatment abolished these effects (Haddjeri et al., 1997 and chapter IV). However, these receptors probably do not correspond to 5-HT_{1A} receptors controlling 5-HT neurons as: 1) systemic injection of the selective 5-HT_{1A} receptor antagonist WAY 100,635 does not reliably alter 5-HT firing activity, 2) a dose greater than the ED_{100} of 8-OH-DPAT on 5-HT neuron firing is required to produce an observable effect on the LC, and 3) long-term treatment with desipramine and reboxetine fails to alter 5-HT activity and 5-HT_{1A} autoreceptor sensitivity, but desensitize 5-HT_{1A} receptors controlling LC NA neurons (chapter VII). It is concluded that the effects of 5-HT_{1A} receptor ligands on LC NA neuron firing are not solely due to somatodendritic 5-HT_{1A} autoreceptors or 5-HT_{1A} receptor effects on medial prefrontal cortex neurons projecting to the DR, but are dependent on intact 5-HT neurons (chapter IV). On the other hand, alterations in the sensitivity of 5-HT_1 autoreceptors as a result of a prolonged SSRI treatment would still be necessary to produce a net increase of 5-HT in the LC and mediate the inhibitory effects on firing observed in this nucleus. This temporal-dependent increase of 5-HT in the LC (figure 2) may desensitize 5-HT_{1A} receptors located in the vicinity of the LC to impart the attenuation on NA neuron firing with this antidepressant class, presumably through a 5-HT_{2A} receptor mechanism (chapter IV).

It appeared peculiar as to the mechanism involved by which 5-HT_{1A} receptors that control LC activity would become desensitized during prolonged treatments with antidepressants that inhibit the reuptake of NA (chapter III and IV). A possible explanation may be related to these antidepressants being able to augment the synaptic availability of NA and desensitize inhibitory α_2-heteroceptors located on 5-HT terminals. Indeed, this phenomenon occurs with antidepressant agents that augment NA concentrations in the hippocampus (Mongeau et al., 1994; chapter VIII). A desensitization of α_2-heteroceptors on 5-HT terminals in the LC during a long-term treatment with NA reuptake inhibitors would enhance 5-HT in this nucleus and may lead to the abolished 5-HT_{1A}

receptor effects on NA neuron firing observed in chapter III and VII. With this reasoning in mind, the once peculiar results of (-) mirtazepine attenuating NA neuron firing may now be explained. (-) mirtazapine preferentially activates α_2-adrenergic heteroceptors in the hippocampus to enhance 5-HT release in this structure, but does not activate α_2-autoreceptors on NA neurons (Haddjeri et al., 1998). This agent may also be preferentially antagonizing α_2-adrenergic heteroceptors on 5-HT terminals in the LC to mediate the observed attenuation on NA neuron firing as opposed to the augmentation with the racemic mixture (Haddjeri et al., 1998). Indeed, only the effect of (-) mirtazapine on LC activity is dependent on an intact 5-HT system (Haddjeri et al., 1998). A microdyalisis study in the LC monitoring 5-HT levels in rats receiving a systemic injection of (-) mirtazapine would help to validate this claim.

Adaptations occurring on 5-HT neurons, with the aid of desensitized 5-HT_{1A} receptors postsynaptic to 5-HT and NA neurons, attenuates LC activity through activation of 5-HT_{2A} receptors during a long-term SSRI treatment. Given this, it had to be evaluated whether the effects of 5-HT_2 receptor ligands on LC activity are mediated through alterations in the firing activity of DR 5-HT neurons that project to the LC. At face value this would not appear so given that a systemic injection of DOI attenuates 5-HT activity and release in projection areas (Done and Sharp, 1992). An attenuated 5-HT tone in the LC would thus be excitatory and not inhibitory on NA neuron activity. Indeed, the effect of a systemic administration of DOI on NA neuron firing is not dependent on intact DR 5-HT neurons as this response is largely unaffected in 5,7-DHT treated rats (chapter IV). Thus, 5-HT_{1A} and 5-HT_{2A} receptors controlling NA neuron firing are postsynaptic to 5-HT neurons and possibly located in the vicinity of the LC. The impact of the former receptor on this nucleus is entirely dependent on 5-HT neurons.

The proposed relationship between the 5-HT_{1A} and 5-HT_{2A} receptors in yielding effects on LC NA activity had to be experimentally defined. It was demonstrated previously that a systemic administration of spiperone abolished the inhibitory effects of a subsequent injection of WAY 100,635 on NA neuron

firing (Haddjeri et al., 1997). Regretfully, it cannot be definitively concluded whether the effect of spiperone was due to 5-HT_{2A} receptor antagonism or attributed to its DA and 5-HT_{1A} receptor affinity. Figure 1., in Chapter IV indicates that the inhibitory effect of the selective 5-HT_{1A} receptor antagonist WAY 100,635 on LC NA neuron firing is completely reversed by a subsequent administration of the selective 5-HT_{2A} receptor antagonist MDL 100,907. This solidifies that 5-HT_{2A} receptors are mediating the effects of 5-HT_{1A} receptor ligands on NA neuron firing. From the experiments accumulated in this document the hypothesis that 5-HT_{1A} receptor ligands mediate effects on NA activity via 1) intact 5-HT neuron terminals to augment 2) 5-HT release in the LC and 3) regulate the activation of 5-HT_{2A} receptors were able to be made.

Since the proposed sequence of events of 5-HT_{1A} receptor effects on LC NA neuron activity being mediated through alterations of 5-HT_{2A} receptor activations, the location of these receptors postsynaptic to 5-HT and NA neurons was further scrutinized. From piecing together previously published literature regarding 5-HT_{1A} and 5-HT_2 receptor agonistic effects on LC firing in combination with the data presented in this document, a speculative neuronal circuitry to account for the 5-HT ligand mediated effects on NA neuron activity was able to be put forward (chapter IV). More importantly for my interests, the cascade of events accounting for the mechanisms by which SSRIs mediate effects on LC firing could now be explained with a circuitry. The results in this document bridge the existing literature regarding the location of 5-HT_{1A} receptors in the LC and strongly supports it as being on glutamatergic neurons projecting to this nucleus (see section 3.1.1; chapter IV). It was later realized that a circuitry similar to that formulated in chapter IV., has already been proposed by Singewald and Phillippu, (1998) through the utilization of push-pull superfusion techniques in the LC (figure 4).

Figure 4. Effects of 5-HT on LC NA neurons relating to Stress

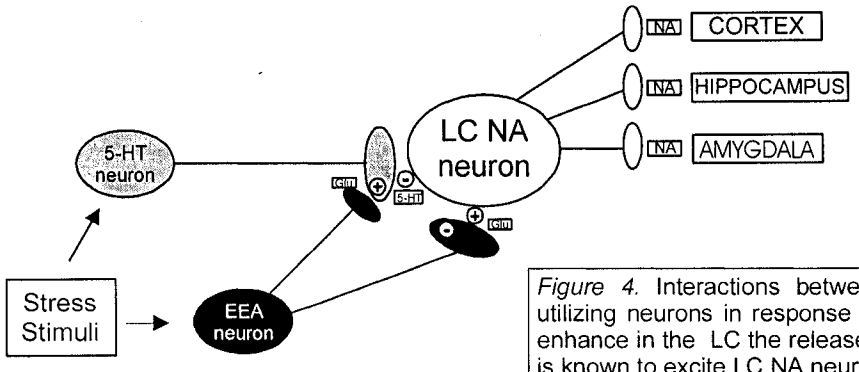


Figure 4. Interactions between EAA- and 5-HT utilizing neurons in response to stress. Stressors enhance in the LC the release of glutamate, which is known to excite LC NA neurons. Increased 5-HT release in response to stress is largely mediated indirectly by EEA via stimulation of presynaptic NMDA receptors, located on 5-HT neurons. 5-HT inhibits stress induced glutamate release and in this way may prevent a deleterious overstimulation of LC neurons during stress.

(reproduced from Singewald and Phillippu, 1998)

The EAA and glutamatergic neurons detailed in figure 4., and chapter IV, respectively, may arise from the nucleus paragigantocellularis (PGi) in the rostral ventrolateral medulla. Evidence supporting this comes from: 1) the PGi sends a potent excitatory input to the LC and mediates its action on the firing activity of NA neurons primarily via non-NMDA receptors (Ennis and Aston-Jones 1989), 2) 5-HT_{1A} receptors have been localized in the PGi (Helke et al., 1997) and functionally demonstrated to be on glutamate axon terminals projecting to the LC inhibiting release (Bobker and Williams, 1989; Singewald et al., 1998; Kaehler et al., 1999). Given that the effects of 5-HT_{1A} receptor ligands on NA neuron firing is dependent on intact 5-HT neurons (chapter IV), 3) anatomical evidence in the LC shows that 5-HT neuron terminals in this nucleus are endowed with kainate receptors and could accept the EAA input (Van Bockstaele, 2000). It is proposed that inhibitory 5-HT_{1A} receptors located on glutamate neurons projecting to the LC would synapse on 5-HT terminals to mediate the 5-HT_{1A} receptor effects on LC NA neuron firing (chapter IV; figure 9).

Activation of inhibitory 5-HT_{1A} receptors on glutamate terminals would attenuate the amount of EAAs to 5-HT neuron terminals endowed with kainate receptors (and possibly other EAA receptors) to decrease 5-HT release in the LC (chapter IV, figure 9). As 5-HT is inhibitory to LC activity, an attenuation in the

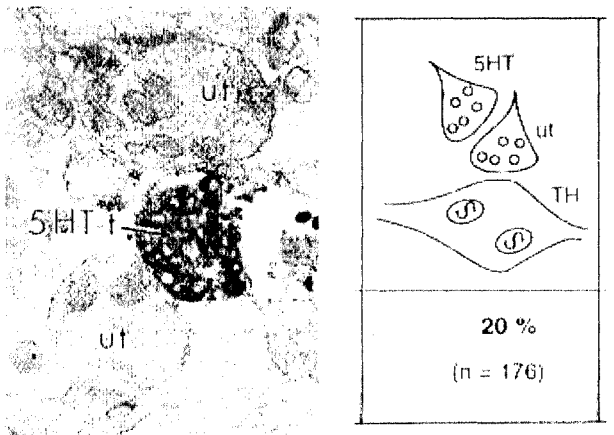
amount of this neurotransmitter in the LC would release the tonic inhibitory tone on NA neurons and augment firing as observed with 8-OH-DPAT (Chapter III). Again, it should be re-emphasized that the formulation of this speculative neuronal circuitry is reflective of data presented in this document. It is also consistent with previous results obtained from our laboratory and others, not to mention being able to explain phenomena once looked at as peculiar. Nevertheless, it is open to further scrutiny and is currently the focus of ongoing experimentation.

The possible effecter systems on NA neurons responsible for mediating the alteration of LC activity due to 5-HT in this nucleus were next pondered. In chapter IV, it was concluded that the inhibitory effect of DOI on NA activity is not dependent on 5-HT neurons. Rather, there is convincing evidence supporting that the 5-HT_{2A} receptor effects of DOI on LC firing activity is mediated through local alterations of GABA in this nucleus as: 1) the inhibitory effects of a systemic injection of DOI is blocked by an iontophoretic application of the selective $GABA_A$ receptor antagonist bicuculline on NA neurons (Chiang and Aston-Jones, 1993), 2) the nucleus PrH sends a potent inhibitory GABAergic input to the LC (Ennis and Aston-Jones, 1989), 3) destruction of this nucleus abolishes the inhibitory effects of systemic DOI injection (Gorea et al., 1991) and, 4) application of DOI directly in the PrH does not alter LC activity (Gorea et al., 1991). Furthermore, 5-HT_{2A} receptors have been documented in the LC of rats and humans, while NA neurons fail to present a hybridization signal for these receptors (Pompietto et al., 1994; Lopez-Gimenez et al., 1999). Thus, evidence of an excitatory 5-HT_{2A} receptor subtype being localized on GABAergic terminals in the LC is likely. It is also fitting that 5-HT_{1A} receptor effects on NA neuron firing are mediated by an alteration of local 5-HT to 5-HT_{2A} receptors present on GABA terminals in this nucleus given the proposed circuitry (chapter IV; figure 1).

Albeit, difficult to assess receptor location with systemic administration of agents used in our experimental paradigms, *in vivo* experiments are the optimal design for this research to be linked to the clinical effects of antidepressants in patients. Despite these caveats, anatomical evidence obtained with electron

microscopy reveals a multi-synaptic influence on 5-HT terminals being identical to the schematic representation of the proposed neuronal circuitry in chapter IV (Van Bockstaele, 2000; Figure 5A). The unlabelled terminals that surround those of 5-HT in the LC as represented in figure 5A, may very well contain glutamate and GABA. Evidence for the synaptic connections between 5-HT terminals is now anatomically provided. Furthermore, most of the 5-HT terminals in the LC impinge on an unlabeled neuron terminal (possibly GABA) that then synapses on a tyrosine hydroxylase element in the LC (figure 5B).

Figure 5. Location of 5-HT neuron terminals in the LC



(reproduced from Van Bockstaele, 2000)

Figure 5. (A) 5-HT-labeled axon terminal is surrounded by two unlabelled terminals (*ut*) in the LC. (B) Most of the 5-HT terminals encountered formed a synapse on an *ut* terminal which then made contact with a tyrosine hydroxylase labeled neuron.

Experimental effort directed at strengthening the proposed sequence of events responsible for the 5-HT mediated effects on LC activity was further warranted despite the compelling results presented in chapter IV. In chapter V, iontophoretic application of antagonists in the LC directed at key receptors represented in the proposed circuitry was carried out. First, the effects of glutamate and kainate on NA neuron firing applied by iontophoresis being effectively blocked by the co-application of the EAA antagonist kynurenate was ensured. Second, results of Ennis and Aston-Jones (1989) demonstrating that iontophoretic application of GABA is attenuated in the presence of the selective

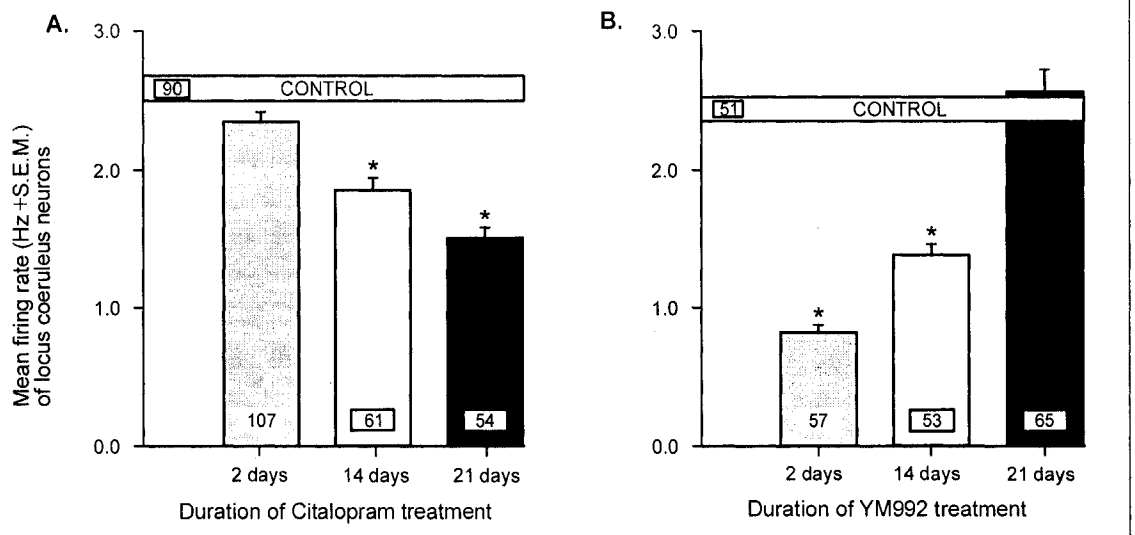
$GABA_A$ receptor antagonist bicuculline were replicated. It was additionally desired to compare effects of 5-HT_{1A} receptor ligands obtained with multi-barreled pipettes to the alterations presented on LC NA firing activity obtained previously with single barreled electrodes (Chapter III), basal firing activity of these NA neurons becoming not being significantly altered by agents in the pipette is imperative. The results of these experiments would not only disclose mechanisms relating to the lack of effect reported with 5-HT_{1A} receptor ligands on NA neuron firing that occurs during prolonged antidepressant treatments, but add insight in whether this may represent a confound of attenuated NA activity. Regretfully, it has been reported that bicuculline and kynurenate by just being present in the pipette augment and inhibits the firing activity of LC NA neurons due to a small leak, respectively. Attempts to circumvent the leakage effect of these agents on NA activity was made by ensuring that both antagonists were in the microelectrode at all times. Indeed, this resulted in a non-significant difference in LC activity as compared to values obtained with single barreled pippets (Chapter V; table 1).

More importantly for the validation of the proposed circuitry, kynurenate applied by iontophoresis abolished the augmentation and inhibition produced by systemic administration of 8-OH-DPAT and WAY 100,635 on LC NA activity (chapter V). These results are consistent with the proposed neuronal circuitry in that alterations in EAAs to 5-HT terminals in the LC are necessary to mediate the effects of 5-HT_{1A} receptor ligands on NA neurons. Furthermore, the effects of the 5-HT_{1A} receptor ligands are proposed to ultimately mediate their impact through 5-HT_{2A} receptors and modulate the $GABA_A$ receptor tone directly on NA neurons. With this reasoning it is logical that iontophoretic application of the selective $GABA_A$ receptor antagonist bicuculline effectively attenuates the inhibitory response of systemically administered WAY 100,635 on LC NA neurons (chapter V, figure I). The effect of WAY 100,635 on LC firing was not completely suppressed by iontophoretic bicuculline (chapter V). Importantly, this does not reflect a lack of effective $GABA_A$ receptor blockade with bicuculline but likely

represents a $GABA_B$ receptor component that envelops as a result (Shefner and Osmanovic, 1991).

Great efforts have been taken to experimentally confirm and provide a circuitry detailing the mechanism by which 5-HT ligands and a long-term SSRI treatment attenuates LC activity. This latter effect is due to increased activation of local 5-HT_{2A} receptors in the LC via a multisynaptic circuitry. The complexity of this circuitry may account for why the inhibitory influence on 5-HT of LC NA activity documented more than 20 years ago have until now been accounted for. Given the proposed circuitry and SSRI induced effects imparted on LC activity, it was then deemed interesting to conduct experiments by which 5-HT transporters and 5-HT_{2A} receptors are both initially blocked. These experiments were appropriately carried out with YM992 (chapter VI). A robust attenuation on LC NA firing activity occurs during a 2-day administration with YM992. However, an effect opposite to this was expected given the proposed circuitry. Just as surprising were the results demonstrating that LC NA neuron activity recovered back to basal levels during a sustained 21-day administration of YM992. Thus, the sequence of events on NA neuron firing with YM992 during a sustained treatment is inverted as compared to citalopram. This is represented by the results illustrated in figure 6., being mirror images of each other.

Figure 6. Effect of Citalopram and YM992 on LC NA neuron firing during a sustained administration



(reproduced from chapters III and VI, with permission)

As a 2-day treatment with YM992 displayed an identical decrease on LC activity to that of reports with NA reuptake inhibitors (chapter III and VII). Overactivation of α_2-adrenoceptors in mediating this effect was then postulated. Indeed, microdialysis studies have indicated that systemic injection of YM992 is capable of enhancing NA concentrations in postsynaptic structures to a much greater extent than that of SSRIs, and even SSRIs plus 5-HT_{2A} receptor antagonism together (Hatanaka et al., 2000). YM992 may thus possess a special characteristic that would enable a robust effect to be imparted on NA release. Its chemical configuration, however, does not resemble that of catecholamine releasers (i.e., amphetamines). To identify mechanisms related to the recovery of LC NA neuron firing during a prolonged administration, alterations in α_2-adrenoceptor sensitivity as mediating these effects was next targeted. Indicated by the dose-response curve for clonidine being displaced to the right (chapter VI), a long-term treatment with YM992 desensitized α_2-adrenocepetors and may be responsible for recovery on LC NA neurons. This phenomenon has not been observed in rats receiving a long-term treatment with desipramine or reboxetine (chapter III and VII). Further experiments are needed with a combination of various SSRIs and 5-HT_2 receptor antagonists to assess

whether these alterations on NA neuron firing are in fact due to a distinct property of YM992 or is attributed to these 5-HT components being blocked.

Extensive evidence in this document has put forth a great deal of results demonstrating the relationship between the effects of 5-HTT blockade on the NA system. Attention was then shifted towards the reverse relationship and elucidation of the impact of NAT reuptake blockade on the 5-HT system. The advent of reboxetine, an antidepressant that possesses NAT reuptake affinity without TCA moiety, may provide insight into the reports of a greater efficacy obtained with TCAs in the treatment of depression (table 1., in the introduction). Reboxetine did not produce an alteration on 5-HT activity during a 2- and 21-day sustained administration (chapter VII). This is similar to results previously obtained with desipramine (Lacroix et al., 1991). As mentioned earlier, reboxetine, as with all antidepressants tested thus far, desensitize 5-HT_{1A} receptors controlling LC activity. Unlike desipramine, reboxetine produced a minor shift to the right in the dose-response curve for DOI. Interestingly, this shift was similar to that observed with SSRIs (Chapter III). As with the SSRIs, reboxetine may also be augmenting 5-HT in the LC after a long-term administration but presumably via different mechanisms.

A discrepancy between the dose of reboxetine and desipramine acutely administered to obtain the ED_{100} on the suppression of NA neuron firing as compared with the dose necessary to produce roughly a 70% sustained attenuation on LC activity exists (chapter VII). Furthermore, when the dose of reboxetine required to obtain a 70% inhibition on NA neuron firing in the 2-day treatment group was used to treat rats for 21-days, a greater attenuation on LC NA firing was observed (chapter VII). This is in contrast to results obtained in rats treated with desipramine (chapter VII). As 5-HT is inhibitory on NA neuron firing, a small alteration in the 5-HT reuptake function in this nucleus with reboxetine may be responsible for the further attenuation of NA activity occurring during a long-term treatment (chapter VII). A microdialysis study monitoring 5-HT levels in the LC during a long-term reboxetine administration would aid to further this prospectus.

Under acute and chronic treatment conditions, reboxetine yielded results in accord with being a potent NA reuptake blocker (chapter VIII). Furthermore, the sensitivity of α_2-adrenoceptors controlling CA_3 pyramidal neuron firing activity remained normosensitive during a long-term administration with reboxetine. These results are similar to that demonstrated with desipramine (Lacroix et al., 1991). The potency of selective NA reuptake inhibitors or dual NA/5-HT reuptake inhibitors to suppress the firing activity of LC NA neurons (ED_{100} value) correlate well with the dose required to induce a maximal prolongation of the RT_{50} value to NA application on CA_3 pyramidal neuron firing. Comparison of the ED_{100} and RT_{50} values for reboxetine (chapter VII) to those previously obtained with desipramine (figure 7; Béïque et al., 1998; Lacroix et al., 1991) yields a discrepancy in the potency of these two selective NA reuptake inhibitors in the hippocampus versus the LC. The ED_{100} value required to suppress the firing activity of LC NA neurons for desipramine (Béïque et al., 1998) is half that reported for reboxetine (chapter VII), whereas the effect of reboxetine to prolong the RT_{50} value to NA applications on CA_3 pyramidal neuron firing is three times greater than that of desipramine: it reached a plateau at a cumulative dose of only 2 mg/kg (chapter IV and figure 7) while that of desipramine produced a maximal effect after 6 mg/kg (see figure 7; Lacroix et al., 1991). In addition, reboxetine and duloxetine (a dual 5-HT/NA reuptake inhibitor) possess nearly identical ED_{100} values on the suppression of LC NA neuron firing but reboxetine is five times more potent to prolong the RT_{50} value to applications of NA on CA_3 pyramidal neurons in comparison to duloxetine (chapter VIII, figure 2; Kasamo et al., 1996). These results indicate that reboxetine is more potent on the NA reuptake process in the hippocampus than in the LC. The exact basis for this difference remains to be elucidated. Of possible interest, the dose required to produce a maximal RT_{50} value on CA_3 pyramidal neurons of the antidepressants in figure 7., when multiplied to the power of three gives the approximate clinically recommended dose used in treating patients with major depression. This could represent a useful index and a model in gauging the clinically effective dose of future NA reuptake agents from rat studies. More research with other selective

NA reuptake antidepressant agents with a potential clinical application is needed to assess whether this relationship holds true.

Figure 7. Differential Effectiveness of NA Reuptake Inhibitors in the Brain

	Locus Coeruleus	Dorsal Hippocampus	
	ED_{100} (mg/kg)	Maximal RT_{50} (mg/kg)	Potency Ratio
DESIPRAMINE	0.6	6	10.0
DULOXETINE	1.0	8	8.0
REBOXETINE	1.25	2	1.6

Similar to that of reports with desipramine, reboxetine did not alter 5-HT reuptake inhibition properties in the hippocampus upon acute administration (chapter VIII). A sustained 21-day treatment with reboxetine did in fact decrease 5-HT reuptake function in the hippocampus, but to a much lesser degree than NA (chapter VIII). Attenuated 5-HT reuptake function in the hippocampus did not occur after a sustained desipramine treatment (Lacroix et al., 1991). In contrast to that of TCAs, the sensitivity of 5-HT_{1A} receptors in the hippocampus remained normal and not sensitized following a prolonged reboxetine treatment (chapter VIII). This is important as TCAs are able to alter the sensitivity of 5-HT_{1A} receptors in many postsynaptic brain structures. This sensitization effect of TCAs has been postulated to be due to chemical structure independent of NA reuptake blockade and is also thought of as beneficial to the antidepressant response. The results in chapter VIII., provide evidence to confirm this assertion as a NA reuptake inhibitor antidepressant without TCA moiety, as with all major classes of antidepressants fails to produce this sensitization phenomenon (see section 5.4).

Agents that augment NA concentrations in the hippocampus is able to desensitize α_2-adrenergic heteroceptors controlling 5-HT release in this structure

(Mongeau et al., 1994). In addition to enhancing NA availability, reboxetine produced a tonic activation of 5-HT_{1A} receptors in the hippocampus during a long-term administration similar to that obtained with SSRIs (Haddjeri et al., 1998). Desensitization of α_2-heteroceptors combined with the inhibition of 5-HT reuptake function may be the mechanism by which a tonic activation of 5-HT_{1A} receptors in the hippocampus occurs with reboxetine (chapter VIII). Of interest, lithium addition to various antidepressants is capable of enhancing further this tonic activation on CA_3 pyramidal neurons (see Haddjeri et al., 1998, located in the appendix). A robust impact of 5-HT on postsynaptic structures after a long-term treatment may be of added benefit to treatment with the "selective" NA reuptake blocker reboxetine as compared to other antidepressants (Massana et al., 1999). Indeed, lithium augmentation has been documented to be effective in treatment resistant major depressive patients (de Montigny,1994) and dual reuptake inhibitors enhancing 5-HT and NA are also more efficacious (Shaeffer et al., 1998; Silverstone and Ravindran; 1999).

Systemic administration of reboxetine attenuated CA_3 pyramidal neuron firing activity (chapter VIII). This was not evidenced in the study by Lacroix et al., (1991) utilizing desipramine. This discrepancy, as the one presented on the firing activity of LC NA neurons between these agents (see section 5.4.), may also be a reflection of methodology. A steady state concentration with desipramine in the study by Lacroix et al., (1991) may have not been achieved due to i.p. injections. Indeed, the Italian group has concluded that their variable microdialysis effects in α_2-adrenoceptor sensitivity in the prefrontal cortex between desipramine and reboxetine was due to the former being administered i.p. (Invernizzi et al., 2001). Fluctuations in plasma levels due to rapid clearance are likely preventing adaptive changes from occurring. The attenuation on CA_3 pyramidal neuron activity from NAT reuptake inhibitors during a long-term treatment may be beneficial in the antidepressant response. A downregulation of α_1- and β-adrenoceptors (which would offset each other) with an increase in α_2-adrenoceptor activation would mediate the decrease presented on CA_3 neuron activity (see figure 9., in the introduction). As with the LC, it is not implied here

that the absolute attenuation on CA_3 activity is important. Rather mechanisms beyond the receptor level may be contributing to treatment efficacy (see figure 4., in the introduction). Indeed, activation of 5-HT_{1A} and adrenergic receptors are linked to the trophic effects of antidepressants on the hippocampus (Duman et al., 2000). The effects of trophic fractors in mediating the antidepressant response has received much attention as of late. It is important to keep in mind that depressive patients are able to relapse when depleted of the monoamine their antidepressant biochemically targeted once in remission (Delgado et al., 1990; Delgado, 2000). This illustrates that monoamine levels are crucial to sustain affect even in the presence of morphometric growth and volume regain in the hippocampus following prolonged antidepressant treatments.

When the results in this document are extrapolated to the clinical symptomology and treatment of anxiety and affective disorders, some interesting hypotheses are raised and others critically evaluated. A hypothesis by Grant and Weiss (2001) puts forth that the absolute decrease imparted on LC activity during a prolonged antidepressant treatment may be important as major depressive and panic disorder patients are proposed to have heightened LC activity (Gold and Chrousos, 1999). The latter would be consistent with a shared feature nosological model of anxiety and affective disorders presented in figure 2., of the introduction. The LC may in fact be the contributing factor to a common underlying pathophysiology in anxiety and depression, but other neurotransmitter systems (5-HT, EAA, GABA, etc.), as well as modulators (peptides and hormones) regulating brainstem and forebrain function are likely involved (Harro and Oreland, 1996). Unfortunately, Grant and Weiss (2001) overlooked results with mirtazapine and mianserin illustrating that firing activity of NA neurons is not attenuated, but augmented and unaltered after a long-term administration, respectively (see commentary in Biological Psychiatry located in the appendix). This goes against their claim of all antidepressants being able to attenuate LC firing activity during a long-term antidepressant treatment. A more plausible hypothesis is that antidepressants irrespective of their absolute impact on LC activity may guard against perturbations on this nucleus and the development of

anxiety symptoms. An elegant study by Copland et al., (1997) provides clinical data strengthening this hypothesis. It was demonstrated that panic disorder patients challenged with clonidine possess "elevated NA volatility" being measured by plasma MHPG levels. Eleven of these patients were then rechallenged after 12 weeks of fluoxetine treatment and a significant reduction in MHPG volatility during clonidine administration enveloped. Of interest, the patients whom received fluoxetine and had the greatest between-visit reductions of basal MHPG levels were more likely to show clinical improvement (Copland et al., 1997). This is also consistent with the attenuating effects of fluvoxamine on yohimbine-induced anxiety in panic disorder patients (Goddard et al., 1993).

In conclusion, all antidepressant agents alter the activity of NA and/or 5-HT neurons. The crosstalk and inter-modulation between these neurotransmitters at the cell body and terminal regions is important in mediating antidepressant action. Given that anxiety and affective disorders are due, in part, to alterations in 5-HT and/or NA, the results in this document brings to life the functional relationship between these disorders. Further investigation on the reciprocal interactions between NA and 5-HT is warranted. This may ultimately result in the discovery of more efficacious treatment strategies at a time when anxiety and affective disorders are projected to be of great burden to our society.

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Appendix

REVIEW

RESPONSE OF THE NOREPINEPHRINE SYSTEM TO ANTIDEPRESSANT DRUGS

by

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Teaching Objectives:

1) The impact of environmental factors on the noradrenergic system

2) The importance of the noradrenegic system in the symptomotology of some neuropsychiatric disorders

3) The role of noradrenaline in pharmacotherapy of neuropsychiatric disorders

CME questions:

Type A

1) The firing activity of noradrenergic neurons in the locus coeruleus is inhibited:

   A. By significant blood pressure decreases
   B. During opiate withdrawal
   C. During REM sleep
   D. By noxious stimuli

2) The following lead(s) to increases in locus coeruleus noradrenergic neuronal firing:

   A. Long-term administration of a SSRI
   B. The acute administration of a benzodiazepine
   C. Injection of the α_2-adrenergic antagonist yohimbine
   D. Subacute bupropion administration

Type K

1) An enhancement of noradrenergic transmission is believed to be important in the therapeutic action of antidepressants in the following disorders:

   A. Post Traumatic Stress Disorder
   B. Panic Disorder
   C. Depression
   D. Obsessive Compulsive Disorder

2) The following antidepressant drugs rapidly attenuate noradrenergic neuronal firing:

   A. Monoamine oxidase inhibitors of the A subtype
   B. Secondary amine tricyclic antidepressants
   C. Reboxetine
   D. Mirtazapine

ABSTRACT:

Environmental stimuli and drugs affect the norepinephrine (NE) system and may be linked to the manifestation and treatment of anxiety and affective disorders. The activity of locus coeruleus (LC) NE neurons in the brainstem can alter the function of forebrain structures associated with several psychiatric disorders. In particular, NE neurons send and receive projections from sensory afferents, limbic, and cortical areas implicated in the higher order brain malfunctions and symptomatology of anxiety and affective disorders. In turn, anxiolytic and antidepressant drugs are able to offset perturbations of NE activity and forebrain structures with a time course congruent with their therapeutic action. All antidepressant drugs, even the agents selective for other biogenic amines or peptides also act on the NE system. In the present review, the role of antidepressant agents on NE neurons are summarized and extrapolated to treatment of neuropsychiatric disorders with emphasis placed on their mechanism of action.

INTRODUCTION:

Norepinephrine Systems in Anxiety and Affective Disorders

The norepinephrine (NE) system has been implicated in anxiety and affective disorders, but there is a lack of consensus about whether enhanced NE levels are beneficial or maladaptive. Patients with affective disorders often present increased plasma NE and 3-methoxy 4-hydrophenolglycol (MHPG) levels, the main metabolite of NE, suggesting a hyper-adrenergic state [1]. However, most antidepressant classes augment NE concentrations and this may appear counterproductive to treatment of these disorders. On the other hand, a reduction in cerebral spinal fluid (CSF) and urinary MHPG levels are reported in major depressives and suggests a decreased NE availability [2]. In contrast, anxiety disorder patients, except for those with obsessive compulsive disorder, have the exact opposite characteristics as compared to major depressives in that they possess reduced plasma NE concentrations and slightly enhanced MHPG urinary levels [3]. Interestingly, depressed patients with panic attacks also have elevated levels of urinary MHPG [4]. In the presence of increased NE availability, anxiety disorder patients have a decreased GABA level and this may represent an important link between benzodiazepines being able to facilitate $GABA_A$ transmission and the capacity of these drugs to attenuate NE levels and firing [5,6]. A hyper-adrenergic state has also been reported in mania and opiate withdrawal [7,8], conditions in which antidepressants are not effective. In fact, antidepressants may even be harmful in some bipolar patients by inducing rapid cycling between states of depression and mania that correlate with urinary NE levels [7]. However, agents like lithium and the α_2-adrenoceptor agonist clonidine that are used in the management of these conditions also alter NE function [9,8]. Thus, the NE system is consistently altered in one fashion or another in many psychiatric disorders, however only a fraction of MHPG in these peripheral specimens is

derived from the brain and this information should thus be interpreted with caution. As well, one has to consider that the CSF MHPG may be derived more from descending pathways to the spinal cord than from ascending NE projections to the forebrain which may not have the same physiological properties.

A more direct index of activity of the NE system is the firing rate of locus coeruleus (LC) NE neurons in the brainstem that are responsible for approximately 90% of the NE innervation of the forebrain. The firing rate of NE neurons is largely proportional to the amount of NE released. Electrical stimulation of the LC produces fear behavior in monkeys [10], and exposure of freely moving cats to dangerous or threatening situations results in increased LC NE firing [11]. Activation of NE neurons via blockade of the cell body and nerve terminal α_2-adrenoceptors with yohimbine can trigger panic attacks in patients with panic disorders and even produce anxiety in healthy volunteers [12]. On the other hand, bilateral lesions of the LC are associated with a decrease in fear behavior [10], and a decrease of NE neuronal firing in this nucleus with clonidine may attenuate panic, post traumatic stress disorder (PTSD) and opiate withdrawal symptoms [12,13]. In addition to the psychological effects attributed to anxiety, the somatic symptoms associated with these disorders correlate with altered LC firing. For instance, in fearful situations LC firing in rats is enhanced [10]. Also, in blood volume depletion, or blood pressure decrease, the firing activity of NE neurons is attenuated [14]. Furthermore this may be important when put into the context of the LC system in affect may be involved in the context of medical causes and impact (i.e., post-stroke depression [15] and coronary artery disease [16]). Thus, LC NE neuronal activity appears critical in the manifestation of some affective and anxiety disorders and their treatment.

The LC receives afferent information from sensory systems responsible for monitoring internal and external environments. Internal monitoring occurs primarily via glutamatergic and GABA influences on NE neurons originating in the rostral medulla [17]. Interestingly, LC firing activity is suppressed during REM sleep [18]. External information is relayed through glutamatergic afferents from orbital, prefrontal, insular, and infralimbic cortical regions [12]. LC NE neurons may affect anxiety-related processing by receiving these sensory cues and then subsequently sending pertinent information to multiple brain areas, such as amygdala, hippocampus, hypothalamus, cortex and spinal cord [12]. PTSD and major depression patients present impaired functioning of the hippocampus (i.e., explicit memory; for review see [19]) and even atrophy of this structure [20, 21]. Antidepressants have been shown to reverse the attenuated cerebral blood flow in the frontal cortex and also to induce neurogenesis in the hippocampus, possibly via a neurotrophic factor dependent mechanism [22,23]. Indeed, the NE system densely innervates these structures and antidepressant-induced alterations occur with a time course of about two weeks which is congruent with their therapeutic effects. Given that PTSD patients often experience flashbacks, and that patients with anxiety disorders may have phobias linked to traumatic events [24], these structures do seem relevant to the clinical condition. Extensive research on anxiety and affective disorders has focused on the frontal cortex, hippocampus and the 5-HT system, all of which are regulated by the NE system. The importance of some of these alterations of the NE system in relation to the drug-induced modifications is discussed below.

Antidepressants and the Norepinephrine System:

Monoamine oxidase inhibitors (MAOIs) on the NE system

The MAOIs were the first effective drugs used in the treatment of major depression and subsequently found to have therapeutic actions in some anxiety disorders as well. MAO exists in two forms, A and the B, in the mammalian brain. *In vivo*, NE and 5-HT are deaminated primarily by the A form, whereas the B form of MAO preferentially deaminates dopamine. The effectiveness of clorgyline and moclobemide, two selective MAO-A inhibitors, in major depression gives strength to the idea that enhanced synaptic availability of NE and/or 5-HT in mediating their therapeutic effects. Furthermore, deprenyl when used at a dose selective for MAO-B is not effective in depression [25].

Repeated administration of clorgyline or phenelzine, the latter drug being a non-selective MAOI, produces an early and sustained decrease in the firing activity of rat LC NE neurons [26]. This sustained attenuation of NE neuron activity is due to the overactivation of inhibitory α_2-autoreceptors by increased NE synaptic concentrations in the LC [26]. These drugs also produce an initial decrease in the firing activity of dorsal raphe 5-HT neurons, but in contrast to NE neurons, this is followed by a progressive and complete recovery of their activity [26]. As expected, deprenyl does not alter the activity of NE or 5-HT neurons [26].

MAOI-induced NE alterations have been reported in the hippocampus and other forebrain structures. A reduced cyclic AMP response to NE occurs in the limbic forebrain after long-term treatment with MAOIs [27]. Most classes of antidepressant agents also downregulate β-adrenoceptors, but this effect may not be germane to their efficacy as antagonists of these receptors are not antidepressant. Rather, β-adrenoceptor blockers are mildly effective by acting peripherally to attenuate the somatic manifestations of anxiety. The fact that LC neuron activity

is similarly decreased during 2 and 21-day MAOI treatments seems to shed doubt that modifications of NE neurotransmission, *per se*, account for the delayed antidepressant effect of MAOIs. However, long-term but not subacute administration of the MAOI tranylcypromine enhances the synaptic availability of NE in the rat frontal cortex [28]. Although the exact basis for this delayed enhancement is not known, it is fully consistent with the delayed onset of antidepressant action of this non-selective MAO A/B inhibitor.

Effect of selective NE reuptake inhibitors on NE activity: TCA and reboxetine

TCAs are effective in the treatment of some anxiety disorders and depression. Desipramine and other TCAs with a secondary amine in their side chain are more potent NE reuptake inhibitors than their tertiary aminated analogues that are more potent inhibitors of 5-HT reuptake. Most TCAs block several receptor subtypes, leading to significant side effects, but alterations of the sensitivity of NE and 5-HT receptors are postulated to be mediating, in part, some of the beneficial effects of these agents [29, 30]. Because some of these side-effects are mostly due to the tricyclic structure of these agents, reboxetine was developed as a non-TCA drug to selectively inhibit the NE transporter. It is currently the only antidepressant agent of its kind in clinical use in Europe and is effective in the treatment of major depression and anxiety disorders [31].

Desipramine and reboxetine suppress NE neuron firing activity following acute systemic injection (figure 1.)[29, 30]. A prolonged treatment with desipramine or reboxetine results in a robust and sustained attenuation on NE neuron firing, emulating the effects observed with MAOI-A inhibitors [26]. As with MAOI-A antidepressants, the attenuation of NE neuron firing activity by desipramine and reboxetine is due to an overactivation of α_2-adrenergic

autoreceptors. Following their long-term administration, these autoreceptors do not densensitize, thus explaining the lack of recovery of firing rate of NE neurons (figure 2.)[26]. Reboxetine and desipramine lead to similar increases in extracellular levels of NE after acute or sustained regimens without consistently producing any adaptive changes in terminal α_2-adrenergic autoreceptor sensitivity in the hippocampus, but desensitizes those in the cortex [32,33]. These agents have however been shown to enhance the synaptic availability of 5-HT in the hippocampus [34, 35]. This is due in part to a decreased sensitivity of α_2-adrenergic heteroceptors located on 5-HT terminals, which normally induce a negative feedback regulation on 5-HT release, a phenomenon common to all drugs that increase the synaptic availability of NE [35]. Also, the responsiveness of 5-HT receptors in forebrain areas is increased in TCA treated rats relating to its chemical structure whereas 5-HT reuptake function in the hippocampus is marginally but significantly attenuated in reboxetine-treated rats [34, 35]. The latter effect of sustained reboxetine administration on the 5-HT system with respect to the antidepressant response is currently not known.

Mirtazapine and the NE system: relevance of α_2-adrenoceptors

Given the importance of α_2-adrenoceptors in mediating the effects on NE firing activity and release, it is not surprising that drugs that block α_2-adrenoceptors are antidepressants. Mianserin and mirtazapine are two antidepressants that possess α_2-adrenoceptor antagonistic properties, but other characteristics as well. Blockade of 5-HT_2 receptors may be important as they have been shown to be upregulated in affective disorder patients [36]. Yohimbine and idazoxan are α_2-adrenoceptors antagonists that augment LC NE firing [12]. Whereas the former agent is able to induce panic [12], the latter may not possess antidepressant efficacy possibly due

SSRIs [51]. Taken together, these observations indicate that 5-HT can modulate sensory and internal inputs to the LC being mediated via glutamate, 5-HT and GABA transmission. The importance of the interactions between these neurotransmitters is thus once again underscored by these observations.

antidepressants, or in remission off drugs, can induce relapse [39]. Given that these NE and 5-HT receptors not only control their respective network, but the others as well through reciprocal interactions, the NE system just cannot be studied on its own. Therefore, the interplay between the NE and the 5-HT systems, as well as other hormonal, neurotransmitter, and neuropeptide modulators are important as well as in the understanding of the physiological/clinical roles of the NE system.

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FIGURES:

Legends to figure 1:

Figure 1. Integrated firing rate histogram of a locus coeruleus norepinephrine (NE) neuron showing that the non-tricyclic antidepressant and selective NE reuptake inhibitor reboxetine produces a decrease in the firing activity. Subsequent injection of the tricyclic antidepressant and selective NE reuptake inhibitor desipramine also suppressed the firing activity of this NE neuron, while the α_2-adrenoceptor antagonist idazoxan restored the firing activity.

Legends to figure 2:

Figure 2. Schematic representation of the effects of sustained administration of a norepinephrine (NE) reuptake inhibitor administration on locus coeruleus neurons. The black circles represent NE molecules, yellow rectangles α_2-adrenoceptors, the white rectangles α_1-adrenoceptors, and blue rectangles = β-adrenoceptors. Note that following a prolonged treatment with a NE reuptake blocker, β-adrenoceptors are downregulated as depicted by their disappearance from the postsynaptic element. The α-adrenoceptors would thus become overactivated.. The number of red arrows in the postsynaptic element would be proportional to NE transmission.

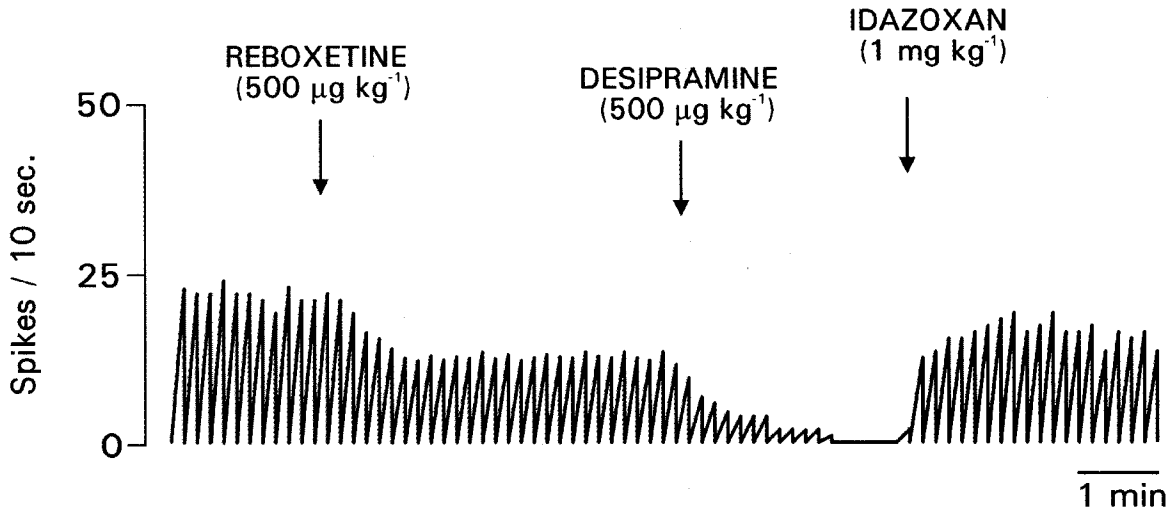
Legends to figure 3:

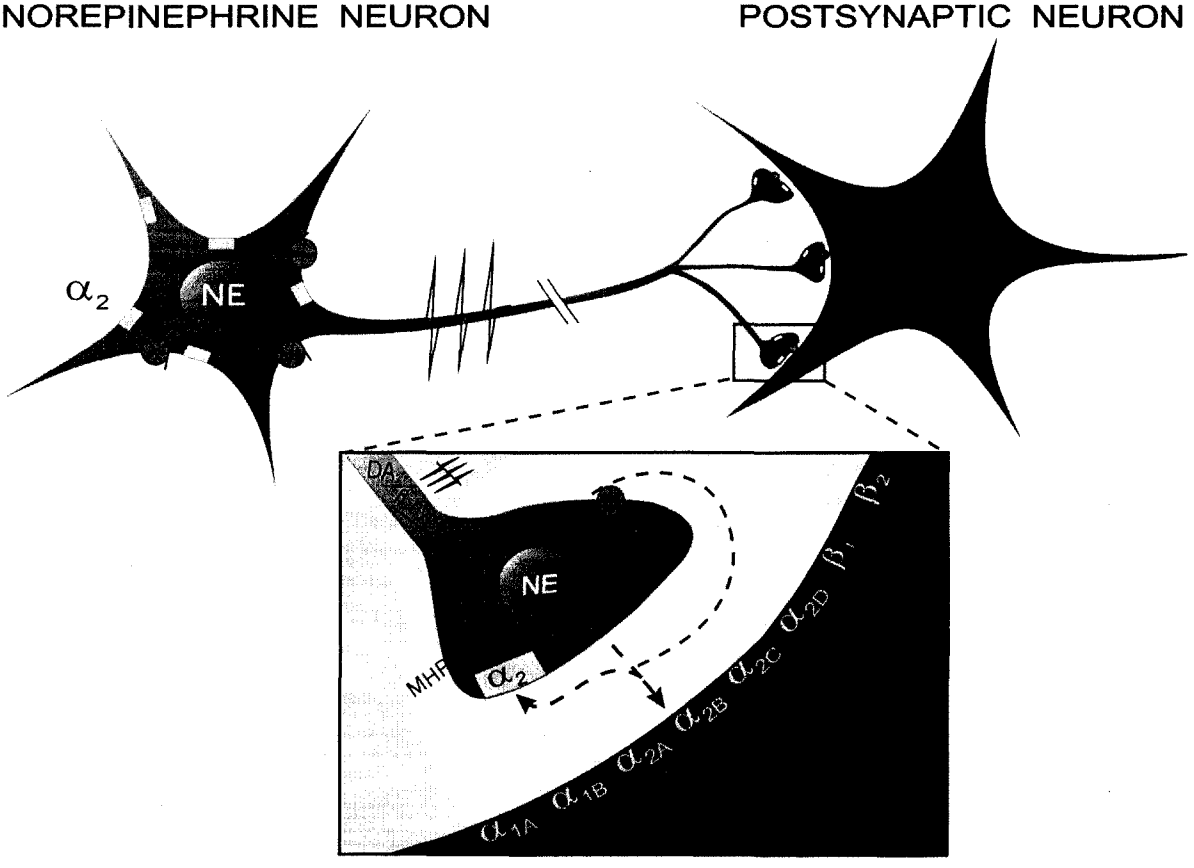
Figure 3. Schematic representation of a NE neuron projecting to a postsynaptic structure. The numerous subtypes of adrenergic receptors are presented and the green cogwheels depict the NE reuptake transporter. DA stands for dopamine, MAO for monoamine oxidase inhibitor, and MHPG for 3-methoxy 4-hydrophenolglycol. The (-) sign indicates an inhibitory action of the terminal α_2-adrenergic autoreceptor on NE release.

TABLES:

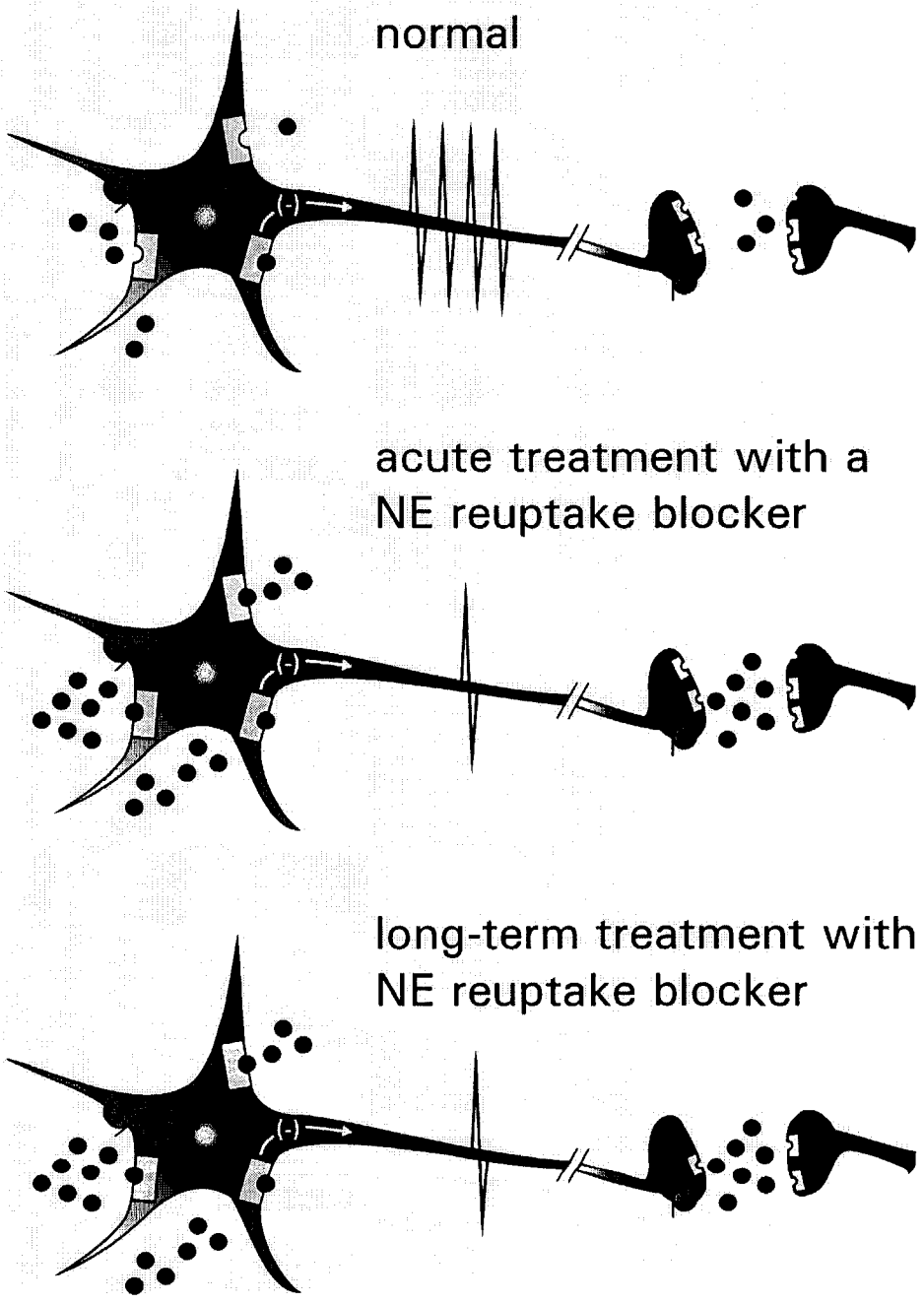
Legends to table 1:

Table 1. ↓ = decrease; ↑ = increase; Ø = no change; N.D. = not determined; MAOI = monoamine oxidase inhibitor; TCA = tricyclic antidepressant; NE = norepinephrine; 5-HT = serotonin; SSRI = selective serotonin reuptake inhibitor.


REBOXETINE
(500 µg kg-1)
DESIPRAMINE
(500 µg kg-1)
IDAZOXAN
(1 mg kg-1)
50
25
0
Spikes / 10 sec.
1 min


NOREPINEPHRINE NEURON
POSTSYNAPTIC NEURON
α_2
NE
NE
MHP
α_2
α_{1A} α_{1B} α_{2A} α_{2B} α_{2C} α_{2D} β_1 β_2

NE neurotransmission and NE reuptake inhibition



Antidepressant Effect on the Firing Activity of Locus Coeruleus Norepinephrine Neurons in Rats

Antidepressant Class	Drug	Acute	Long-Term
MAOI	phenelzine	↓	↓
TCA	clorgyline	↓	↓
	desipramine	↓	↓
	imipramine	↓	↓
NE reuptake inhibitor	reboxetine	↓	↓
α_2-adrenergic antagonist	mirtazapine	↑	↑
Dual NE/5-HT reuptake inhibitors	venlafaxine	↓	↓
	milnacipran	↓	↓
	duloxetine	↓	↓
SSRI	paroxetine	Ø	↓
	citalopram	Ø	↓
NE releaser	bupropion	↓	N.D.

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Enhancement of Serotoninergic Function — A Sometimes Insufficient Cause of Antidepressant Action

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INTRODUCTION

In the last three decades considerable evidence, both pre-clinical and clinical, has accumulated to make the central serotonin (5-HT) system the cornerstone of the antidepressant response, although most tricyclic antidepressant drugs (TCAs) block the reuptake of 5-HT and norepinephrine (NE), whereas monoamine oxidase inhibitors (MAOIs) increase brain levels of 5-HT and NE. The advent of selective 5-HT reuptake inhibitors (SSRIs) in the modern armamentarium to treat major depression solidified the notion that an enhancement of 5-HT neurotransmission was essential to obtain an attenuation of the depressive syndrome. At about the same time in the Department of Psychiatry at Yale University, data were being generated showing unequivocally that a dietary depletion of the amino acid precursor of 5-HT, tryptophan, could produce within five hours a relapse of depression in patients who had recently responded to antidepressant drug belong to various classes (Delgado *et al.*, 1990).

Selective 5-HT reuptake inhibitors as agents specific to the 5-HT neurons?

Although some SSRIs are not generally considered to be entirely selective for the 5-HT reuptake transporter, like fluoxetine, most others do not interfere at all with the NE carrier at usual clinical regimens. Upon acute systemic administration of any SSRI, brain penetration occurs within minutes which leads to a diminution of the firing rate of 5-HT neurons, which has been shown to result from activation of the cell body 5-HT_{1A} autoreceptor (de Montigny *et al.*, 1981). The reasons for such a preferential accumulation of 5-HT in the brainstem vs the forebrain is addressed in this supplement by Artigas *et al.* (2000). The 5-HT_{1A} autoreceptors desensitize over the course of a two-week treatment (Blier and de Montigny, 1983; Table 1). Since 5-HT release is impulse-flow dependent, 5-HT neurons can then release more neurotransmitter in the presence of 5-HT reuptake inhibition. Furthermore, 5-HT release occurs without the usual inhibitory action of the terminal 5-HT_{1B} autoreceptor as it also undergoes desensitization similar to its cell body counterpart (Chaput *et al.*, 1986). Since the responsiveness of most, but not all, postsynaptic neurons to 5-HT is unaltered, long-term SSRI treatment leads to enhanced 5-HT neurotransmission (Blier *et al.*, 1988). That this alteration is responsible for the antidepressant effect of SSRIs, is indicated by the reversal of the therapeutic effect of fluoxetine responders by a dietary depletion of tryptophan (Delgado *et al.*, 1999).

Since SSRIs enhance 5-HT transmission in the ascending 5-HT pathways to the forebrain, it was reasoned that it could also increase it in descending projections, such as that to the NE containing neurons of the locus coeruleus (Figure 1). It was demonstrated that 5-HT exerts a tonic inhibitory effect on the spontaneous firing rate of NE neurons because in rats treated with a selective 5-HT neurotoxine, 5,7-dihydroxytryptamine, their discharge frequency was nearly doubled (Haddjeri *et al.*, 1997). In rats that received either the SSRI paroxetine or citalopram, there was a time-dependent decrease in the firing rate of NE neurons over a 21-day period (Szabo *et al.*, 1999, 2000). These results clearly show that, due to the neuroanatomical disposition of the 5-HT projections, exquisitely selective 5-HT agents such as SSRIs have a marked effect on an important parameter controlling NE

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neuron function. On the one hand, this time-dependent decrease of NE neuronal firing could explain, at least in part, the therapeutic action of SSRIs on anxiety symptoms and perhaps even in panic disorder, a condition generally believed to be linked to a hyperadrenergic state. On the other hand, this phenomenon could possibly account for the fatigue or asthenia which occasionally persists in depressed patients otherwise improved by their SSRI regimen (Feighner and Boyer, 1991). Indeed, fatigue and sedation are common side effects of the α_2-adrenergic agonist clonidine, which suppresses NE neuron firing, when used in the treatment of hypertension. Consequently, although SSRIs are selective agents, they do produce effects that are not limited to the 5-HT system.

Mirtazapine, an α_2-adrenergic antagonist that enhances 5-HT$_1$ transmission?

Mirtazapine is an effective antidepressant which by virtue of its antagonism of α_2-adrenoceptors enhances NE neuron firing and NE release throughout the brain upon acute administration (de Boer *et al.*, 1994; Haddjeri *et al.*, 1996). However, since NE neurons send a projection to the dorsal raphe, the acute effect of this antidepressant drug was studied on the firing of 5-HT neurons. Mirtazapine also produced a dose-dependent, although transient, increase in firing rate of 5-HT neurons, presumably by enhancing the degree of activation of excitatory α_1-adrenoceptors on 5-HT neurons (Figure 1). In order to provide support for this assertion, the effect of mirtazapine on the firing of 5-HT neurons was studied in NE-lesioned rats. Mirtazapine no longer enhanced the firing rate of 5-HT neurons in the absence of NE neurons, thus clearly putting into evidence the indirect action of mirtazapine on 5-HT neurons (Haddjeri *et al.*, 1996).

Upon sustained and long-term (21 days) of mirtazapine administration, the firing rate of NE neurons was significantly increased by about 30 per cent, but that 5-HT neurons even more so, by at least 75 per cent. It can thus be concluded that long-term mirtazapine administration leads to enhanced NE and 5-HT release (Haddjeri *et al.*, 1996). In order to determine in humans which system is responsible for the antidepressant response of mirtazapine, Delgado and co-workers carried out sequential tryptophan and catecholamine depletions in depressive patients that responded to mirtazapine. Both interventions produced a relapse of depression symptomatology indicating that both systems contribute to the antidepressant effect of mirtazapine (Delgado *et al.*, 1999).

Tricyclic antidepressants, an heterogenous class of agents

TCAs have various actions on monoamine reuptake: while chlomipramine potently blocks the reuptake of both 5-HT and NE, other agents like imipramine and amitriptyline are much less potent 5-HT reuptake blockers. Desipramine is a selective NE reuptake blocker, yet trimipramine and iprindole do not inhibit monoamine reuptake transporters at all (Hyttel, 1982). Such observations prompted the search for a common mechanism of action of these drugs. It was observed that there was a progressive sensitization to 5-HT in several brain regions over a two-week treatment with all the various types of TCAs mentioned above (see Blier and de Montigny, 1994, for a review). In the hippocampus, 5-HT$_{1A}$ receptors were subsequently identified to be responsible for this increased responsiveness to 5-HT, while in the facial motor nucleus, the enhanced response to 5-HT is mediated by a 5-HT$_2$ receptor subtype. In the amygdala and the lateral geniculate body, the subtype of 5-HT receptors mediating the response to 5-HT, which is also enhanced after long-term tricyclic administration, has yet to be identified. However, in some brain regions, there was a concomitant enhanced responsiveness to both 5-HT and NE. These areas include the facial motor nucleus, the lateral geniculate body, and the amygdala, where the response to NE is mediated by excitatory α_1-adrenoceptors in the first two structures and α_2-adrenoceptors in the last one. It is therefore possible that 5-HT receptors and NE receptors in these brain structures are coupled to the same transducing mechanisms. These observations also raise the possibility that TCAs could be acting in part via an enhancement of NE neurotransmission. In the case of the selective NE reuptake blocker desipramine, Delgado *et al.* (1999) have observed that catecholamine, but not tryptophan depletion, produces a relapse of depressive symptoms in patients who had responded to this agent.

Definite evidence that an enhancement of NE transmission *per se* can produce an antidepressant response will probably have to come from the study of the effects of the effective antidepressant reboxetine on the central 5-HT and NE system. Indeed, this selective and potent NE reuptake blocker does not belong to the TCA family. However, one should

depressant drugs on monoaminergic systems therefore allows a rational approach for the treatment of resistant depressed patients.

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Effect of the selective noradrenergic reuptake inhibitor reboxetine on the firing activity of noradrenaline and serotonin neurons

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Keywords: 5-HT_{1A} receptors, α_2-adrenoceptors, antidepressant, dorsal raphe, locus coeruleus, rat

Abstract

Reboxetine is a non-tricyclic antidepressant with selective noradrenergic (NA) reuptake-blocking effects. The effects of acute and sustained administration of reboxetine, on the firing activity of locus coeruleus NA neurons and dorsal raphe 5-HT neurons, were assessed using *in vivo* extracellular unitary recording in rats anaesthetized with chloral hydrate. Reboxetine (0.1–1.25 mg/kg, i.v.) dose-dependently decreased the firing activity of NA neurons (ED_{50} = 480 ± 14 μg/kg). A 2-day treatment with reboxetine at 1.25, 2.5, 5, or 10 mg/kg per day (using osmotic minipumps implanted subcutaneously) produced significant decreases of 52%, 68%, 81%, and 83%, respectively, of NA firing activity. When the reboxetine treatment (2.5 mg/kg per day) duration was prolonged to 7 days, a 66% decrease in NA firing activity was observed which further decreased to 80% after 21 days of treatment. In contrast, 5-HT neuron firing rate remained unaltered following short- and long-term reboxetine treatments. The suppressant effect of the α_2-adrenoceptor agonist clonidine on the firing activity of NA neurons was unchanged in long-term reboxetine-treated rats, but its effect on the firing activity of 5-HT neurons was blunted. The enhancement of NA firing activity by the 5-HT_{1A} agonist 8-OH-DPAT was abolished in long-term reboxetine-treated rats, whereas, the inhibitory effect of the 5-HT_2 agonist DOI was attenuated by about three-fold. In conclusion, sustained NA reuptake blockade by reboxetine lead to profound alterations in the function of NA neurons and of 5-HT receptors modulating their firing activity.

Introduction

Alterations in central noradrenaline (NA) and serotonin (5-HT) function have been implicated in the pathophysiology of anxiety and affective disorders (Thase & Howland, 1995). Since the advent of the selective serotonin (5-HT) reuptake inhibitors (SSRIs), the NA hypotheses related to the diathesis of anxiety and affective disorders have been overshadowed by 5-HT, mainly because of the absence of non-tricyclic drugs selective for NA reuptake. Agents which block the reuptake of NA and/or 5-HT do so within hours of administration, however, a therapeutic response is not achieved in major depressive or panic disorder patients until about two to three weeks of sustained administration. It was recently reported that sustained administration of SSRIs produces a progressive decrease in the firing activity of locus coeruleus NA neurons in the rat brain, which parallels the retarded onset of action of antidepressants in major depression and panic disorder patients (Szabo *et al.*, 1999, 2000). Thus, antidepressant drugs 'selective' for one system may produce an alteration in another neuronal system involved in mediating the therapeutic and/or side-effect profiles.

The importance of the 5-HT and NA systems as well as their reciprocal interactions should be taken into account when considering antidepressant treatments, as these may ultimately dictate their effectiveness, or lack, thereof. Located on the cell bodies and terminals of NA neurons, α_2-adrenergic autoreceptors induce an inhibitory action on NA neuron firing activity and release, respectively (Svensson *et al.*, 1975; Curet & de Montigny, 1989). In the presence of a NA reuptake blocker, α_2-adrenergic autoreceptors become overactivated via increased concentrations of endogenous NA attenuating the firing activity of locus coeruleus NA neurons (Lacroix *et al.*, 1991; Kasamo *et al.*, 1996; Mongeau *et al.*, 1998; Béïque *et al.* 2000; Szabo *et al.* 2000). Dorsal raphe 5-HT neurons receive NA projections from the locus coeruleus (Loizou, 1969; Anderson *et al.*, 1977; Baraban & Aghajanian, 1980; Clement *et al.*, 1992). These NA projections modulate the activity of 5-HT neurons in the dorsal raphe nucleus via excitatory α_1-adrenoceptors (Baraban & Aghajanian, 1980). In turn, NA neurons of the locus coeruleus receive dense 5-HT projections of which 50% arises from the dorsal raphe (Kaehler *et al.*, 1999) and others most probably coming from pericoerulear 5-HT neurons (Aston-Jones *et al.*, 1991), which exert an inhibitory role.

Reboxetine is a non-tricyclic antidepressant drug that potently and selectively inhibits NA uptake (Wong *et al.* 2000). Given the major importance of such reciprocal interactions between NA and 5-HT neurons (Haddjeri *et al.*, 1997; Szabo *et al.*, 1999, 2000), acute and sustained treatment regimens of reboxetine were carried out in rats to assess whether their effect on the spontaneous firing activity of NA and 5-HT neurons differed as compared to previous studies performed with desipramine and SSRIs (Szabo *et al.* 2000). The responsiveness of NA and 5-HT neurons to the α_2-adrenoceptor agonist clonidine was also examined after long-term reboxetine administration. In addition, the effect of systemic administration of

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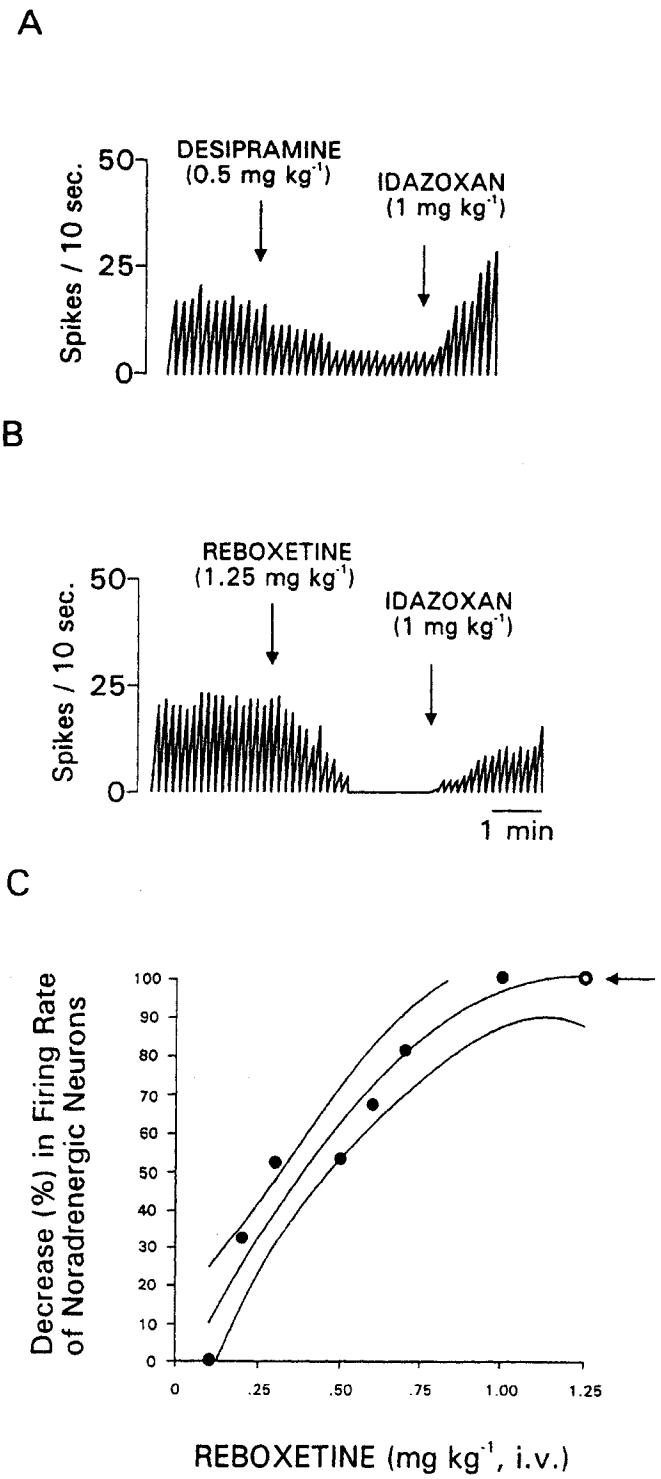
the 5-HT_{1A} and 5-HT_2 receptor agonists 8-OH-DPAT and DOI, respectively, on NA firing activity were assessed in long-term reboxetine-treated rats to determine whether sustained NA reuptake blockade could lead to changes in 5-HT modulation of NA neuronal firing activity.



Methods

Animals

The experiments were carried out in male Sprague–Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300 and 325 g. Rats were kept under standard laboratory conditions (12 h light : 12 h dark cycle, lights on 0700 h, with access to food and water *ad libitum*). Rats were anaesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments, Tujunga, CA, USA). Supplemental doses (100 mg/kg, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37 °C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc., Saint-Hyacinthe, Quebec, Canada). Prior to electrophysiological recording, a catheter was inserted into a lateral tail vein for systemic i.v. injection of drugs. All experiments were performed in compliance with NIH guidelines and the Canadian Council on Animal Care.

Sustained reboxetine treatments

Rats were anaesthetized with halothane containing a 2 : 1 O_2/N_2O mixture for subcutaneous implantation of osmotic minipumps (ALZA, Palo Alto, CA, USA). The rats were tested with the minipumps in place in order to mimic the clinical condition whereby patients present an antidepressant response while taking their medication. They were treated with varying doses of reboxetine (1.25, 2.5, 5, or 10 mg/kg per day) or the saline vehicle for either 2, 7, or 21 days delivered by osmotic minipumps. In calculating the concentration of antidepressant drug solution needed to effectively reach the desired (mg/kg per day) dose, an estimate was made of the weight of the rat at the middle of treatment time by assuming that the rat gains ≈ 50 g/week, and this value was used to prepare the solution.

Electrophysiological experiments

Extracellular unit recordings of locus coeruleus NA and dorsal raphe 5-HT neurons were conducted with single-barrelled glass micropipettes preloaded with fibreglass filaments (to facilitate filling) pulled in a conventional manner, with the tips broken back to 1–3 μm and filled with a 2 M NaCl solution. Their impedance range was between 2–4 MΩ. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for locus coeruleus neurons recordings. Locus coeruleus NA neurons were recorded with micropipettes lowered at –0.7 mm interaural and 1.1–1.4 mm lateral. Spontaneously active NA neurons of the locus coeruleus were identified using the following criteria: a regular firing rate (1–5 Hz) and a positive action potential of long duration (0.8–1.2 ms) exhibiting a characteristic burst discharge in response to a nociceptive

FIG. 1. Integrated firing rate histogram of a locus coeruleus NA neuron illustrating the effects of intravenous administration of the tricyclic antidepressant (TCA) and selective NA reuptake inhibitor desipramine producing a decrease in the firing activity, a subsequent injection the α_2-adrenoceptor antagonist idazoxan reversed the effects in a control rat (A). Integrated firing rate histogram of a locus coeruleus NA neuron showing that the non-TCA selective NA reuptake inhibitor reboxetine decreased, while idazoxan resurrected, the firing activity of the NA neuron (B). Relationship between the degree of suppression of locus coeruleus NA firing activity and doses of reboxetine administered intravenously in untreated rats. Only, the initial response of a single NA neuron to the fir dose of reboxetine in each rat was used to construct the curve. The arro pointing to the data point on the dose–response curve corresponds to the dose used in Fig. 1B. Outer lines represent the standard error of the regression line.

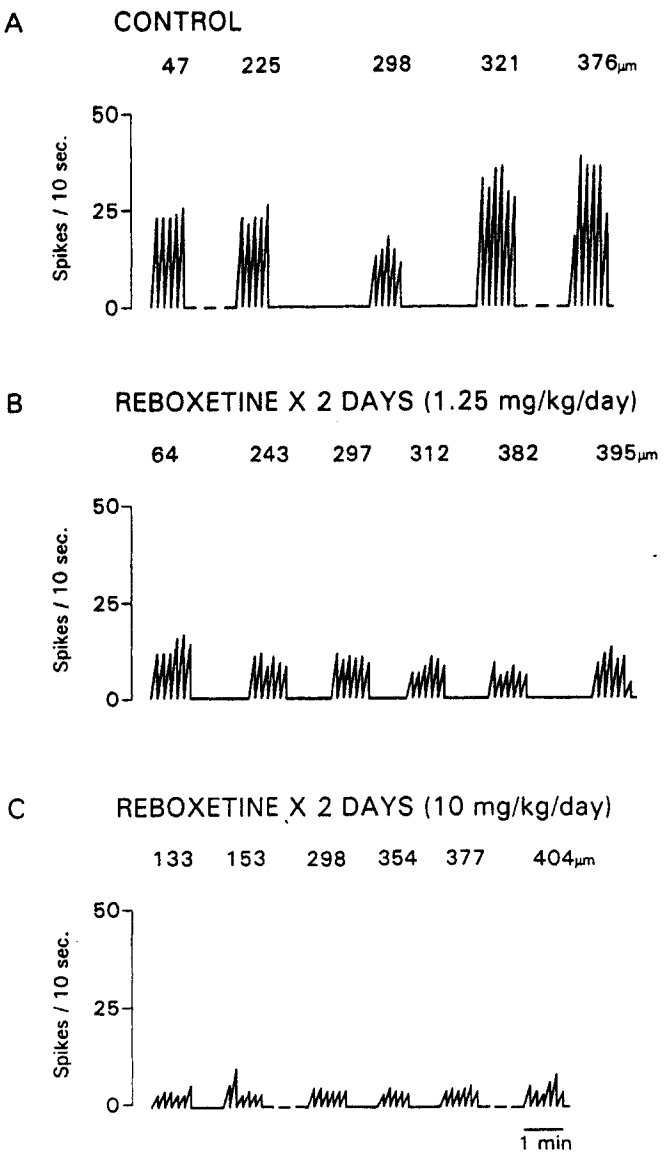


FIG. 2. Integrated firing rate histograms of locus coeruleus NA neurons, recorded in single electrode descents in the locus coeruleus showing their spontaneous firing activity in (A) control, (B) 2-day reboxetine treatment (1.25 mg/kg per day) and (C) 2-day reboxetine treatment (10 mg/kg per day). The dotted lines in between neurons indicate approximately 5-min time lapses. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

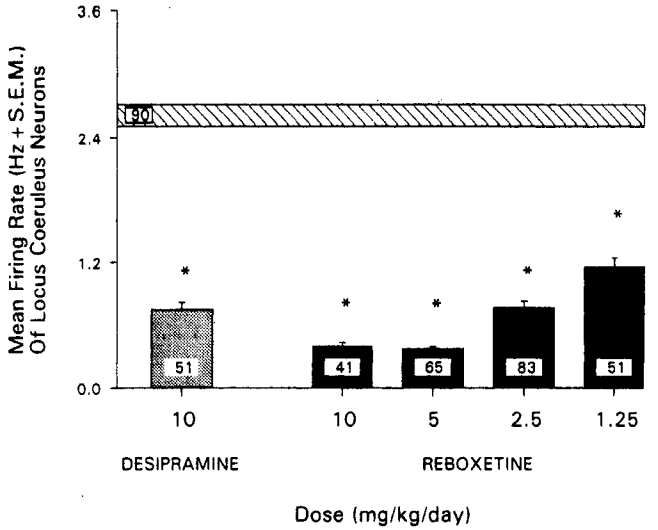


FIG. 3. Effects of 2-day reboxetine (10, 5, 2.5, and 1.25 mg/kg per day; $n = 3$ for each dose) and desipramine (10 mg/kg per day; $n = 3$) treatments on the spontaneous firing activity of locus coeruleus neurons. The striped area represents the range (SEM × 2) of the mean firing activity of neurons recorded in control rats ($n = 3$). $^*P < 0.05$ (Dunn's Method) when compared to the control value. The number of neurons recorded is displayed in each box.

pinch of the contralateral hind paw (Aghajanian *et al.*, 1977; Aghajanian & Vandermaelen, 1982). Noradrenergic neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of NA neurons in the treated animals, four to five electrode descents were carried out through this nucleus in control and reboxetine-treated rats.

For dorsal raphe recordings, the pipette was positioned 1 mm anterior to lambda on the midline and lowered into the dorsal raphe; 5-HT neurons were usually encountered at a depth of between 5.5 and 6.5 mm from the surface of the brain. 5-HT neurons were identified by their characteristic slow (0.5–2.5 Hz) and regular firing rate and long-duration (0.8–1.2 ms) positive action potential (Baraban & Aghajanian, 1980; Aghajanian & Vandermaelen, 1982). In order to assess possible changes in the firing activity of 5-HT neurons during the course of sustained reboxetine administration, four to five electrode decents were carried out in each control and treated rat: the first 1 mm anterior to lambda on the midline, the following two 200 μm anterior and posterior to the first descent, and the last two 200 μm on either side of the first track.

Dose–response curves

Dose–response curves for the alteration of NA and 5-HT neuron firing activity were obtained for systemic (i.v.) administration of reboxetine and the selective α_2-adrenoceptor agonist clonidine in untreated rats. After systemic injection of reboxetine or clonidine, the selective α_2-adrenoceptor antagonist idazoxan was administered in order to reverse the decrease in firing activity produced by the previously injected compound. In long-term (21 days) reboxetine- and vehicle-treated rats, the 5-HT_{1A} receptor agonist 8-OH-DPAT, the 5-HT_2 receptor agonist DOI and clonidine were injected while recording the firing activity of locus coeruleus NA neurons. In addition, clonidine was also systemically injected in long-term reboxetine and vehicle treated rats and the effect on 5-HT neuron firing rate were assessed. Changes in the firing activity are expressed as percentages of the baseline firing rate and were measured after systemic drug administration. Dose–response curves were constructed for 8-OH-DPAT, DOI and clonidine in control and long-term reboxetine-treated animals using only the first dose injected to each rat to generate the curves and estimates of effective doses 50 (ED_{50}).

Drugs

The following drugs were used: reboxetine (Pharmacia & UpJohn, Kalamazoo, MI, USA); desipramine HCL, [(+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane hydrochloride] (DOI), (±)-8-hydroxy-2-(di-n-propylamino)tetralin hydrobromide (8-OH-DPAT), ritanserin, idazoxan, clonidine, and WAY 100635 (RBI, Natick, MA, U.S.A.); MDL100907 (Hoechst Marion Roussel, Cincinnati, OH, U.S.A.); and LSD (lysergic acid diethylamide; Ministry of Health and Welfare,

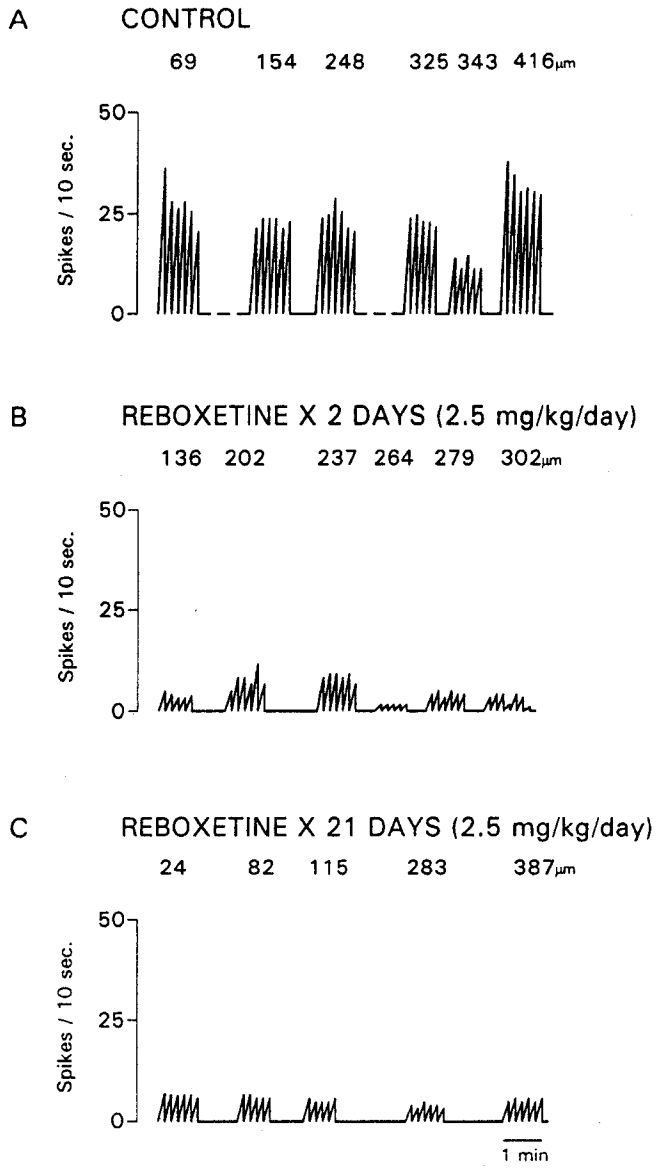


FIG. 4. Integrated firing rate histograms of NA neurons, recorded in single electrode descents in the locus coeruleus showing their spontaneous firing activity in (A) control (n = 3), (B) 2-day reboxetine treatment and (C) 21-day reboxetine treatment. The dotted lines in between neurons indicate approximately 5-min time lapses. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

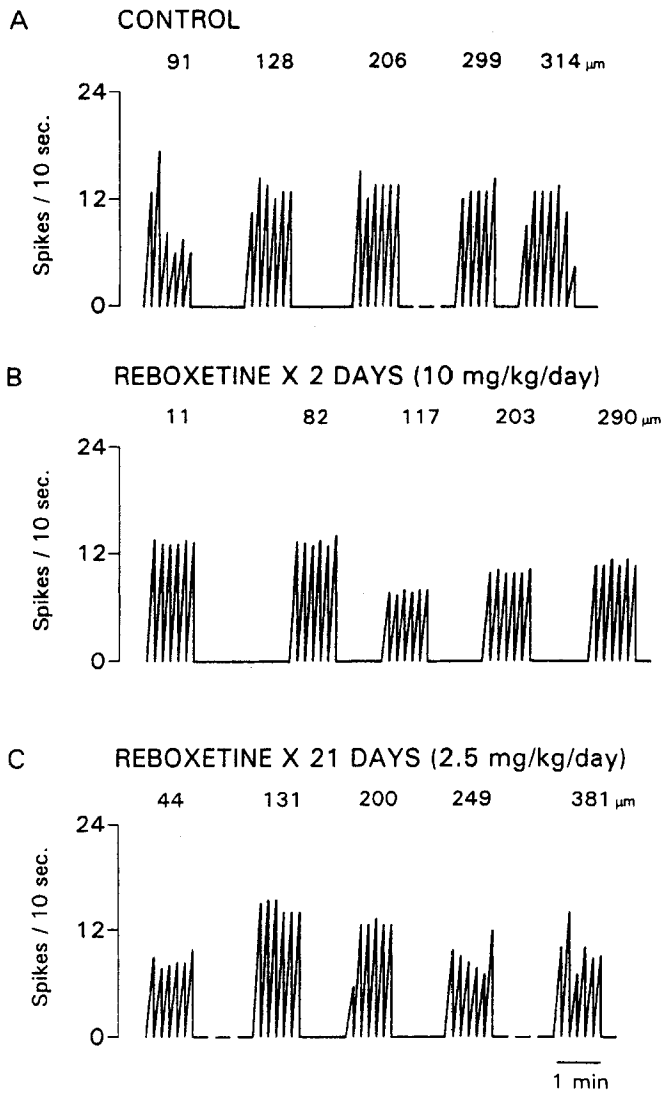


FIG. 5. Integrated firing rate histograms of 5-HT neurons, recorded in single electrode descents in the dorsal raphe showing their spontaneous firing activity in (A) control, (B) 2-day reboxetine treatment and (C) 21-day reboxetine treatment. The dotted lines in between neurons indicate approximately 5-min time lapses. The number above each neuron indicates the depth at which each neuron was recorded from the ventral border of the Sylvius aqueduct.

Ottawa, Canada). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water while ritanserin was solubilized in acetic acid and then diluted with distilled water to the appropriate concentration.

Statistical comparisons

All results were expressed as mean (± SEM) of single neuron values. Statistical comparisons of values obtained in treated and control rats were carried out using one-way analysis of variance on ranks. Dunnett's multiple comparison test was used to assess the difference between control and treated groups. Correlational coefficients (r-values) for the dose–response relationship observed in the locus coeruleus were calculated using simple linear/curvilinear regression analysis. The SEM for the ED_{50} values for the locus coeruleus were calculated by regression analysis, with the Y-value of 50 used as the regressor. Difference between the two regressions in the controls and treated rats were assessed by comparing their ED_{50} values using the confidence intervals method. The 95% confidence limit was determined from the Student's t distribution.

Results

Effect of the acute administration of reboxetine on the firing activity of NA neurons

A single i.v. dose of reboxetine was administered to each naive rat while recording a spontaneously active NA neuron (n = 6). Reboxetine (0.1–1.25 mg/kg) induced a dose-dependent decrease of the firing rate of the NA neurons (Fig. 1C), an example of which is provided in Fig. 1B. Maximal suppression of NA firing activity was

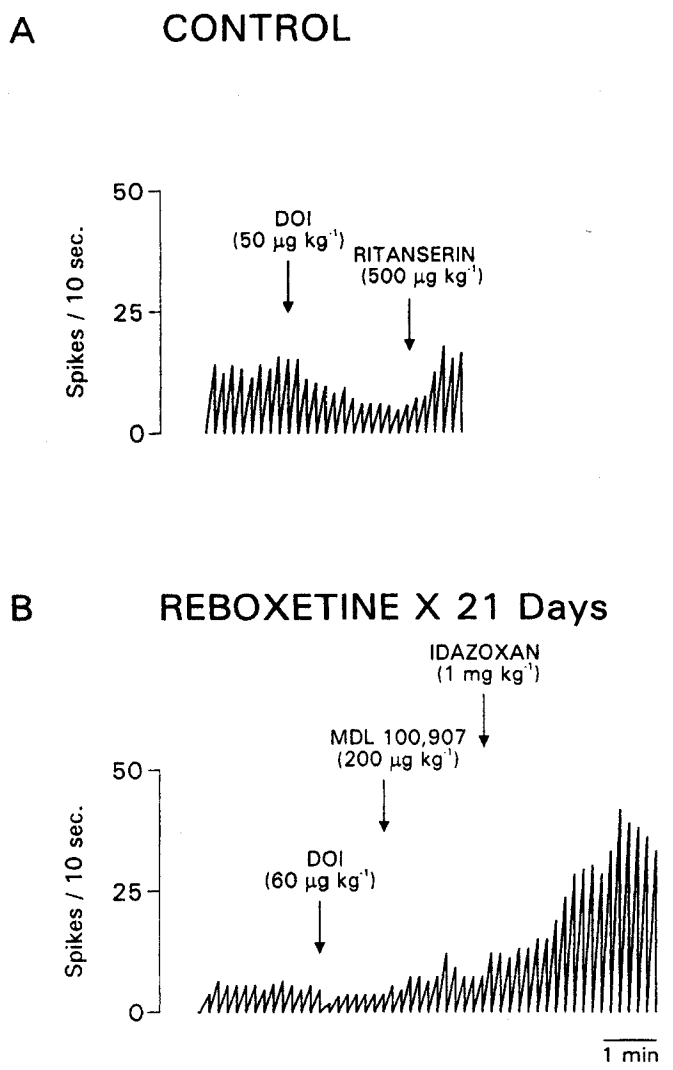


FIG. 6. (A) Integrated firing rate histograms of locus coeruleus NA neurons illustrating the effects of intravenous administration of the preferential 5-HT_{2A} agonist DOI that produced suppression of firing activity; a subsequent injection of the 5-HT_2 antagonist ritanserin reversed the effects in a control rat. (B) Integrated firing rate histograms of locus coeruleus NA neurons illustrating the effects of intravenous administration of the preferential 5-HT_{2A} agonist DOI that produced suppression of firing activity; a subsequent injection of the 5-HT_{2A} antagonist MDL100 907 reversed the effects in a reboxetine (2.5 mg/kg per day)-treated rat. The identity of the NA neuron recorded in the reboxetine-treated rat was also assessed by showing the excitatory effect of a subsequent intravenous administration of the α_2-adrenoceptor antagonist idazoxan.

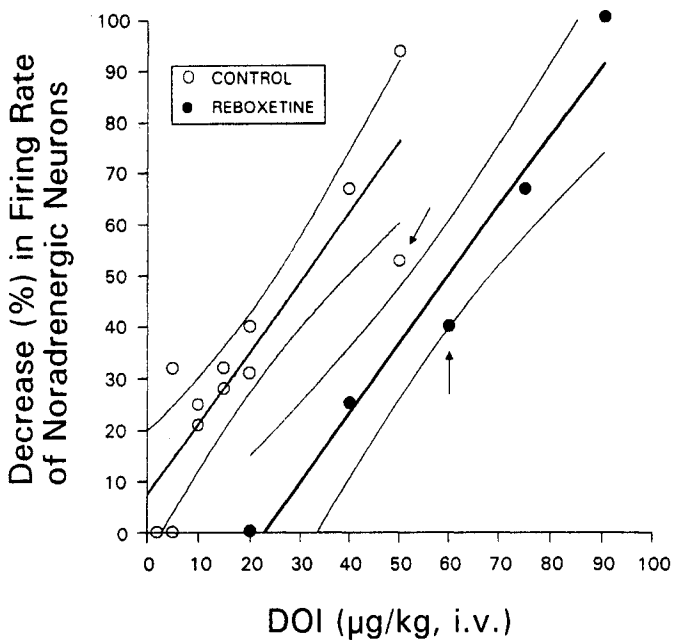


FIG. 7. Relationship between the degree of suppression of locus coeruleus NA firing activity and doses of DOI administered intravenously in controls and rats treated with reboxetine (2.5 mg/kg per day). Only, the initial response of a single NA neuron to the first dose of DOI in each rat was used to construct the curve. The arrows pointing to the data points on the dose–response curves correspond to the doses used in Fig. 6. Outer lines represent the standard error of the regression line and the straight line represents the mean (SEM × 2) of the responses obtained in controls. The shift to the right of the dose–response curve was significant ($P < 0.01$).

reached at a reboxetine dose of 1.25 mg/kg. This suppressant effect was reversed by the subsequent i.v. administration of the selective α_2-adrenoceptor antagonist idazoxan (1 mg/kg, $n = 6$). Reboxetine displayed an ED_{50} of 480 ± 14 µg/kg (Fig. 1C). Desipramine injected at 500 µg/kg decreased NA firing rate of an locus coeruleus neuron (64%) to a somewhat greater extent when compared to reboxetine injected at the same dose (52%; Fig. 1A and C).

Effect of sustained administration of reboxetine on the firing activity of locus coeruleus NA neurons

On the basis of the acute experiments with reboxetine (as above), five systematic electrode descents into the locus coeruleus nucleus were carried out in rats treated with reboxetine at various doses and time courses as well as with their respective controls. The spontaneous firing activity of NA neurons were recorded in control and reboxetine-treated rats, examples of which are provided in Fig. 2. Control groups treated with the saline vehicle under a varying time-course resulted in no significant difference in locus coeruleus spontaneous firing activity when compared to each other, these data were, therefore, merged to make up a single control group (range of firing, 0.8–3.8 Hz). A short-term treatment (2 days) with reboxetine at 1.25, 2.5, 5, or 10 mg/kg per day produced a significant decrease of 52% (range of firing, 0.5–3.8 Hz), 68% (range of firing, 0.2–2.9 Hz), 81% (range of firing, 0.2–0.8 Hz) and 83% (range of firing, 0.2–0.8 Hz), respectively, in NA firing activity as compared to controls (Fig. 3). The effect of the short-term reboxetine treatment on attenuating the firing activity of locus coeruleus NA neurons was dose-dependent and reached maximal levels at a dose of 5 mg/kg per day (Fig. 3).

Previous experiments carried out with the selective NA reuptake blocker desipramine (10 mg/kg per day) decreased the firing rate of NA neurons to the same extent after 2 and 21 days of treatment (70%; Szabo *et al.* 2000; data included in Fig. 3 for comparison). Reboxetine given at 2.5 mg/kg per day produced similar effects, at the 2-day time period, on the attenuation of locus coeruleus neurons as desipramine (10 mg/kg per day). Rats were thus treated with this dose of reboxetine for 7 and 21 days. When treatment duration with reboxetine was increased from 2 days to 7 days, a sustained decrease of 66% (range of firing, 0.3–1.6 Hz) in NA firing activity was observed which further decreased to 80% (range of firing, 0.1–0.9 Hz) after long-term treatment (21-day), examples of which are provided in Fig. 4. This greater degree of inhibition on locus coeruleus firing activity during the 21-day reboxetine treatment was

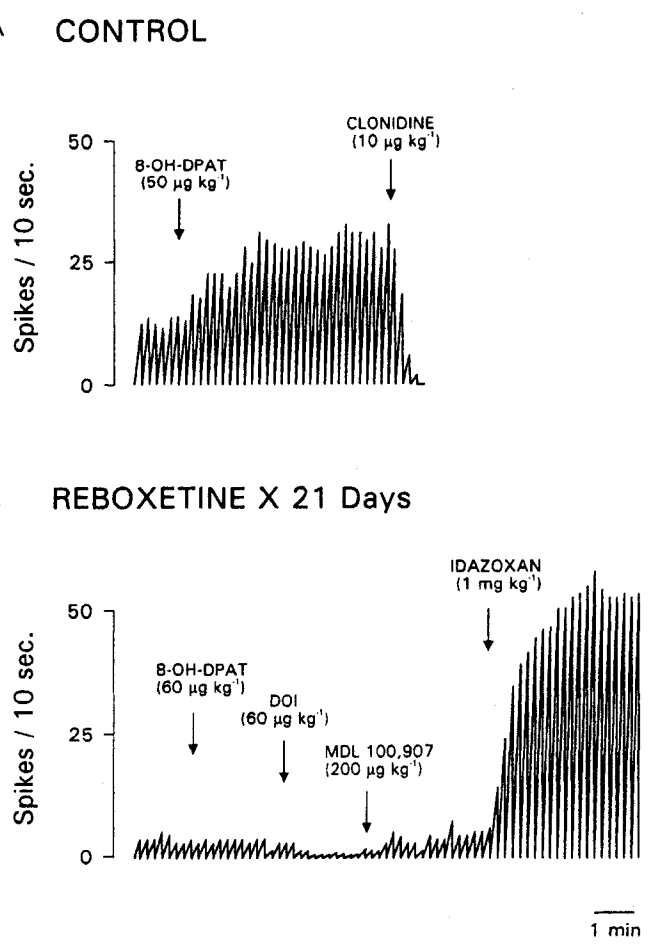


FIG. 8. (A) Integrated firing rate histogram of a locus coeruleus NA neuron illustrating the effects of intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT that produced an increase in the firing activity; subsequent injection of the α_2-adrenoceptor agonist clonidine reversed the effects in a control rat. (B) Integrated firing rate histogram of a locus coeruleus NA neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with reboxetine (2.5 mg/kg per day) for 21 days. Note that even during attenuated NA firing activity, a subsequent injection of the 5-HT_2 agonist DOI produced similar inhibition of firing when compared to controls (see Figs 6 and 7). The suppressant effects of DOI on NA firing was reversed by injection of the selective 5-HT_{2A} antagonist MDL 100 907. The identity of the NA neuron recorded in the reboxetine-treated rat was assessed by showing the excitatory effect of a subsequent intravenous administration of the α_2-adrenoceptor antagonist idazoxan.

significantly different from those of 2- and 7-day treatment regimens ($P < 0.05$).

Effect of sustained administration of reboxetine on the firing activity of dorsal raphe 5-HT neurons

Control groups treated with the saline vehicle resulted in no significant difference in dorsal raphe cell spontaneous firing activity when compared to each other and these data were, therefore, merged to make up one control group (range of firing, 0.4–2.6 Hz). Desipramine (10 mg/kg per day) did not alter the firing activity of dorsal raphe 5-HT neurons after 2-day treatment (Mongeau *et al.*, 1998). Systematic electrode descents into the dorsal raphe of rats treated with 10 mg/kg per day for 2 days (range of firing, 0.2–2.0 Hz) and 2.5 mg/kg per day for 21-days of reboxetine (range of firing, 0.4–2.3 Hz) did not differ significantly in the mean spontaneous firing activity of 5-HT neurons when compared to each other or control rat values, examples of which are provided in Fig. 5.

Effect of intravenous injection of the 5-HT agonists DOI and 8-OH-DPAT on locus coeruleus NA neurons in controls and reboxetine-treated rats

Treatment with DOI induces a suppression of NA firing activity that is reversed by a subsequent injection of the 5-$HT_{2A/2C}$ antagonist ritanserin ($n = 3$; Chiang & Aston-Jones, 1993; Szabo *et al.* 2000; Fig. 6A). It was recently reported that the inhibitory effects of DOI on locus coeruleus neuron firing activity is abolished upon prior systemic administration of the selective 5-HT_{2A} antagonist MDL 100 907 (200 μg/kg), thus, putting forth further evidence involving 5-HT_{2A} receptors (Szabo & Blier, 2000a). In 21-day citalopram (20 mg/kg per day), but not in desipramine (10 mg/kg per day) treated rats, the response to i.v. administration of DOI is shifted approximately three-fold to the right (Szabo *et al.*, 2000). In a rat treated with 2.5 mg/kg per day of reboxetine for 21 days, the response to DOI was blunted when compared to the control example (Fig. 6A and B). A subsequent injection of MDL 100 907 was able to reverse the effect of DOI ($n = 3$), while idazoxan further increased the attenuated NA neuron firing (Fig. 6A and B) to physiological levels (0.8–5.3 Hz, $n = 90$; Szabo *et al.*, 2000). A full dose–response relationship between the suppression of locus coeruleus firing activity and different doses of DOI showed a significant three-fold shift to the right in the reboxetine ($ED_{50} = 62 \pm 5.5$) treated rats as compared to controls ($ED_{50} = 20 \pm 0.5$; Fig. 7).

Systemic injection of the 5-HT_{1A} receptor agonist 8-OH-DPAT produced a dose-dependent increase in the firing activity of locus coeruleus NA neurons (Piercey *et al.*, 1993; Szabo *et al.*, 2000; Fig. 8A and yielded an ED_{50} of 15 μg/kg (Fig. 9). After long-term treatment with different classes of antidepressants (desipramine or citalopram), the excitatory response of NA firing activity to 8-OH-DPAT was abolished (Szabo *et al.* 2000). The enhancing effect on locus coeruleus NA firing activity of 8-OH-DPAT was also abolished in rats treated with 2.5 mg/kg per day of reboxetine for 21 days (Fig. 8B). In fact, even doses of 8-OH-DPAT of up to 120 μg/kg failed to alter the firing activity of NA neurons in long-term reboxetine-treated rats (Fig. 9). In the absence of a 8-OH-DPAT response, a subsequent injection of the 5-HT_2 receptor agonist DOI produced a suppression of firing and the selective 5-HT_{2A} receptor antagonist MDL 100 907 ($n = 2$) reversed that inhibitory effect, while a final injection of idazoxan ($n = 3$) was able to restore NA firing to the upper end of the physiological range of firing of NA neurons observed in control animals (Fig. 8B).

Effect of intravenous injection of clonidine on locus coeruleus NA and dorsal raphe 5-HT neurons in control and reboxetine-treated rats

The selective α_2-adrenoceptor agonist, clonidine, injected systemically, decreased NA neuronal firing activity, while 8-OH-DPAT subsequently enhanced it ($n = 2$), and a final injection of idazoxan ($n = 3$) reversed the effect of clonidine (Fig. 10A). This effect of idazoxan is consistent with previous studies showing that idazoxan is capable of reversing the inhibitory effects of clonidine on locus coeruleus NA firing activity (Goldstein *et al.*, 1983). In long-term reboxetine (2.5 mg/kg per day) treated rats, clonidine still produced the same inhibitory effect on NA neuron firing rates as in controls. The dose–response curve of clonidine, in the suppression of locus coeruleus firing activity, was not statistically different when comparing control to reboxetine-treated rats (Fig. 11A and B). The excitatory effect of 8-OH-DPAT was absent following an injection of

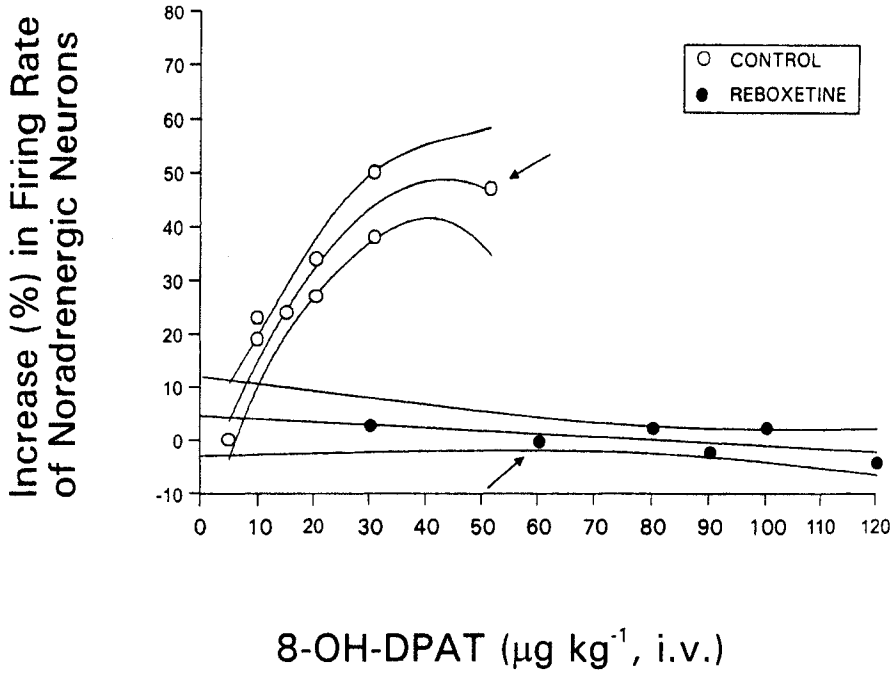


FIG. 9. Relationship between the degree of augmentation of locus coeruleus NA firing activity and doses of 8-OH-DPAT administered intravenously in controls and reboxetine-treated rats. Only the initial response of a single NA neuron to the first dose of 8-OH-DPAT in each rat was used to construct the curves. The arrows pointing to the data points on the dose–response curves correspond to the doses used in Fig. 8. Outer lines represent the standard error of the regression line.

clonidine and the subsequent administration of idazoxan (n = 3) increased the firing activity to the control range (Fig. 10B).

Systemic administration of clonidine decreases the firing activity of dorsal raphe 5-HT neurons with an ED_{50} of about 5 μg/kg (Freedman & Aghajanian, 1984; Mongeau *et al.*, 1993; Haddjeri *et al.*, 1997). While recording a dorsal raphe 5-HT neuron, clonidine at 8 μg/kg decreased the firing rate of the 5-HT neuron by approximately 70%, whereas, a subsequent injection of idazoxan reversed this effect (Fig. 12A). In rats treated with reboxetine for 21-days, two subsequent injections of 8 μg/kg of clonidine were ineffective in decreasing 5-HT neuron firing activity (Fig. 12B). In the same neuron, an additional injection of 12 μg/kg of clonidine (cumulative dose of 38 μg/kg) was able to induce a significant reduction in 5-HT neuronal firing. Administration of 10 μg/kg of the 5-HT autoreceptor agonist LSD (n = 5) shut down firing activity, this was reversed by a final injection of the selective 5-HT_{1A} antagonist WAY 100 635 (n = 3) in reboxetine-treated rats (Fig. 12B). The latter dose of LSD has consistently been found in our laboratory to be the ED_{100} value in control rats. In SSRI or 5-HT_{1A} agonist-treated animals, this dose of LSD is at least increased by two-fold. The full dose–response curves for clonidine, on the inhibition of 5-HT neuron firing activity in controls and reboxetine-treated rats, revealed that there was a significant shift to the right in the treatment group (Fig. 13).

Discussion

The results of the present study indicate that acute and sustained administration of reboxetine significantly reduced the spontaneous firing activity of locus coeruleus NA neurons. The enhancement of NA concentrations in the locus coeruleus, via reboxetine blockade of NA transporters, produced an overactivation of cell body α_2-adrenergic autoreceptors and suppressed NA neuron firing. The exact location of these NA transporters mediating the effects on locus coeruleus firing are likely on the cell body of NA neurons (Svensson *et al.*, 1975; Mateo *et al.*, 1998). There was, however, some striking differences when comparing the acute and sustained effects of reboxetine administration to those of the selective NA reuptake inhibitor desipramine. Desipramine, acutely administered, suppresses the firing activity of locus coeruleus neurons with an ED_{50} of about half (240 ± 54 μg/kg; Béïque *et al.*, 1999) as much as that of reboxetine (480 ± 14 μg/kg, Fig. 1), which is consistent with *in vitro* measurements of NA reuptake inhibition by these two drugs (Wong *et al.* 2000). However, a suppression of NA neuron firing activity by about 70% was achieved with a quarter of the dose of reboxetine as compared to that of desipramine after a 2-day treatment (2.5 vs. 10 mg/kg per day). Furthermore, when treatment duration with reboxetine (2.5 mg/kg per day) and desipramine (10 mg/kg per day) was increased from 2 to 21 days (Szabo *et al.*, 2000), locus coeruleus firing activity was further reduced with reboxetine (down to 83% of the control value), but not with desipramine.

A possible explanation for the marked difference in potency between acute and long-term reboxetine administration on attenuating NA firing activity is that reboxetine possesses a greater dissociation constant for the NA transporter. This possibility could be examined *in vitro* using dilution experiments rather than substrate displacement with another NA reuptake inhibitor. Previous experiments have documented that sustained administration of desipramine (10 mg/kg per day) produces a small but significant shift to the right of the clonidine dose–response curve in the suppression of locus coeruleus firing rate after a 14-day treatment (Lacroix *et al.*, 1991). In the present study, the inhibitory effect of clonidine on NA neuronal firing rate was similar in 21-day reboxetine and saline-treated rats which would explain the lack of recovery of the firing rate of NA neurons (Fig. 11). Although several studies have documented the possibility that presynaptic α_2-adrenoceptors become desensitized following long-term antidepressant treatments (Crews & Smith, 1978; Svensson & Usdin, 1978; Cohen *et al.*, 1980; McMillen *et al.*, 1980; Spyraki & Fibiger, 1980; Finberg & Tal, 1985; Szabo & Blier, 2000b), there are some studies that have not (Blier & de Montigny, 1985; Mateo *et al.*, 1998).

Desipramine at a dose of 10 mg/kg per day does not alter the firing activity of dorsal raphe 5-HT neurons after a 2-day treatment regimen

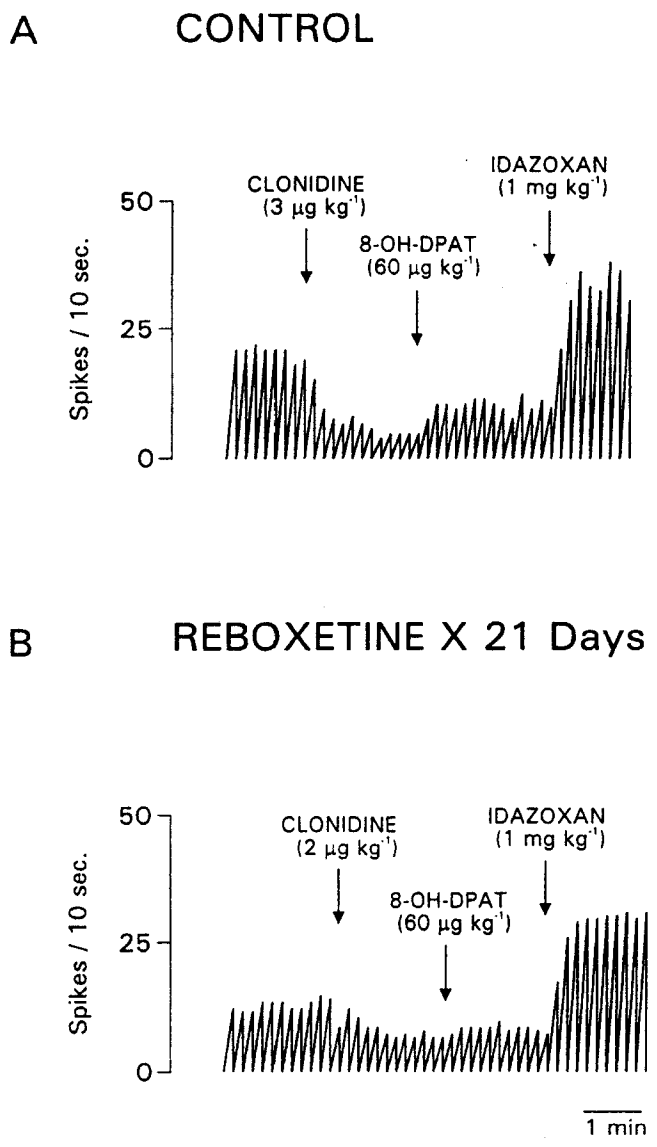


FIG. 10. (A) Integrated firing rate histograms of locus coeruleus NA neurons illustrating the suppressant effects of intravenous administration of the selective α_2-adrenoceptor agonist clonidine that produced suppression of the firing activity; a subsequent injection of the 5-HT_{1A} agonist 8-OH-DPAT increased the firing rate in a control rat. (B) Integrated firing rate histograms of locus coeruleus NA neurons illustrating the suppressant effects of intravenous administration of the selective α_2-adrenoceptor agonist clonidine that produced suppression of the firing activity; the augmentation effect normally produced by a subsequent injection of the 5-HT_{1A} agonist 8-OH-DPAT was abolished. The identity of the NA neuron recorded in the control and reboxetine (2.5 mg/kg per day)-treated rat was assessed by showing the excitatory effect of a subsequent intravenous administration of the α_2-adrenoceptor antagonist idazoxan.

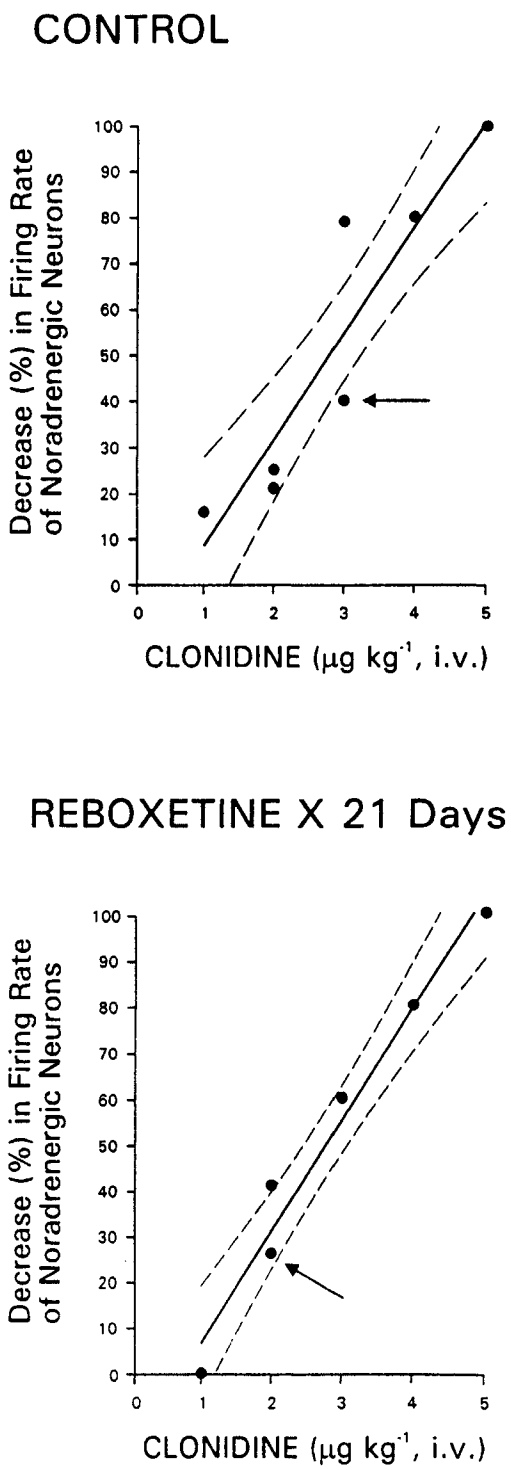


FIG. 11. Relationship between the degree of suppression of locus coeruleus NA firing activity and doses of clonidine administered intravenously in controls (A) and rats treated with reboxetine (2.5 mg/kg per day) for 21 days (B). Only, the initial response of a single NA neuron to the first dose of clonidine in each rat was used to construct the curve. The arrows pointing to the data points on the dose–response curves correspond to the doses used in Fig. 10. Outer lines represent the standard error of the regression line and the straight line represents the mean (SEM × 2) of the responses obtained in controls.

(Mongeau *et al.*, 1998). Reboxetine also displayed a similar lack of effect on the firing activity of dorsal raphe 5-HT neurons (Fig. 5). These results are quite surprising because the spontaneous firing rate of 5-HT neurons is highly dependent on a tonic activation of excitatory α_1-adrenoceptors in the dorsal raphe (Baraban & Aghajanian, 1980; Vandermaelen & Aghajanian, 1983). Thus, given that 5-HT neuronal activity remained unaltered, these data suggest that either NA reuptake blockade 'perfectly' compensated for the decreased impulse flow reaching NA terminals in the raphe, or that there was an increase of NA levels that triggered an adaptive mechanism which ultimately left unaltered the firing rate of 5-HT neurons. Indeed, acute reboxetine administration has been shown to

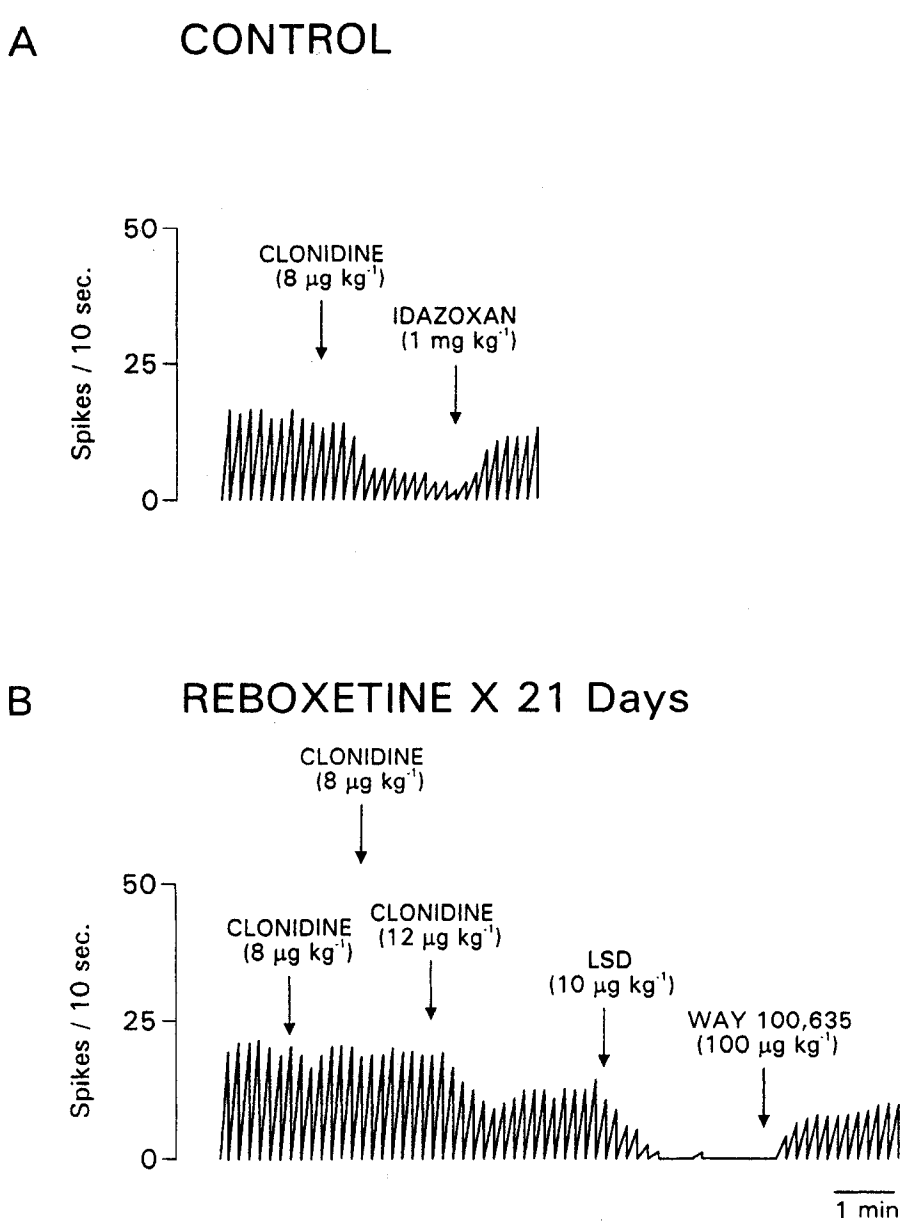


FIG. 12. (A) Integrated firing rate histogram of 5-HT neurons of the dorsal raphe nucleus showing the suppressant effects of intravenous administration of the α_2-adrenoceptor agonist clonidine; a subsequent injection of the α_2-adrenoceptor antagonist idazoxan (n = 2) reversed the effects. (B) Integrated firing rate histogram of a 5-HT neurons of the dorsal raphe illustrating the lack of responsiveness to two intravenous injections of 8 μg/kg clonidine in a rat treated with reboxetine (2.5 mg/kg per day) for 21 days. Note that a subsequent injection of 12 μg/kg of clonidine decreased the firing activity of the 5-HT neuron. The identity of the 5-HT neuron recorded was assessed by showing the suppressant effect of a subsequent injection of LSD.

increase the concentration of NA in the frontal cortex and hippocampus in microdialysis experiments and it may, therefore, also increase NA levels in the dorsal raphe (Sacchetti *et al.*, 1999). Nevertheless, in the presence of an unaltered 5-HT firing activity, the inhibitory effects of clonidine, on the firing activity of 5-HT neurons in 21-day reboxetine-treated rats, was attenuated, similar to results previously obtained with the monoamine oxidase inhibitor befloxatone (Haddjeri *et al.*, 1998). This altered clonidine response on 5-HT neuron firing activity most likely reflected the already decreased NA firing rate of locus coeruleus neurons on which clonidine had a proportionally smaller effect than in saline treated rats. Indeed, the mean firing activity of NA neurons was only of 0.4 Hz in 21-day reboxetine-treated rats instead of 2.4 Hz in the controls (Fig. 3). Consequently, this attenuated responsiveness of 5-HT neurons to clonidine cannot be interpreted as a desensitization of α_2-adrenoceptors.

It was previously reported that the incremental effect of systemic administration of the 5-HT_{1A} receptor agonist on locus coeruleus NA neuron firing activity is abolished by long-term desipramine (10 mg/kg per day) or citalopram (20 mg/kg per day) treatments (Szabo *et al.*, 2000). A reboxetine treatment for 21 days also had the same effect (Fig. 9). It thus appears that drugs which block either 5-HT or NA transporters produce a desensitization of the 5-HT_{1A} receptors controlling locus coeruleus firing activity after long-term administration. This phenomenon has been well documented for the desensitization of 5-HT_{1A} autoreceptors which impart a negative feedback influence on 5-HT neuron firing activity that occurs following long-term SSRI treatment, but not for drugs like desipramine (Blier & de Montigny, 1980; Blier & de Montigny, 1994) or reboxetine which block NA reuptake (Fig. 1). Unlike their postsynaptic counterparts in the hippocampus, the 5-HT_{1A} receptors which control locus coeruleus firing activity desensitize after long-term administration of SSRIs (Szabo *et al.*, 2000). However, it seems that desensitization of the 5-HT_{1A} receptor which controls locus coeruleus firing activity is common to all major classes of antidepressant drugs tested thus far and may represent an important

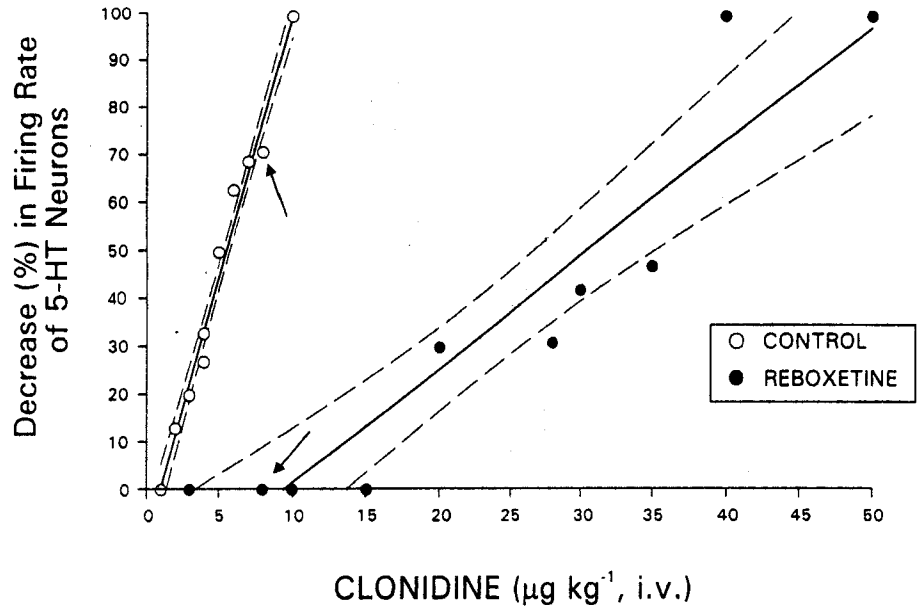


FIG. 13. Relationship between the degree of suppression of dorsal raphe 5-HT firing activity and doses of clonidine administered intravenously in controls and rats treated with reboxetine (2.5 mg/kg per day) for 21 days. Only, the initial response of a single 5-HT neuron to the first dose of clonidine in each rat was used to construct the curve. The arrows pointing to the data points on the dose–response curves correspond to the doses used in Fig. 12. Outer lines represent the standard error of the regression line and the straight line represents the mean (SEM × 2) of the responses obtained in controls. The shift to the right of the dose–response curve was significant ($P < 0.01$).

finding with respect to the treatment of anxiety and affective disorders.

The location of this 5-HT_{1A} receptor which exerts an excitatory influence on locus coeruleus firing activity is probably not the 5-HT_{1A} autoreceptor controlling dorsal raphe neuron firing activity. The selective 5-HT_{1A} antagonist WAY 100 635, at a dose of 0.1 mg/kg (i.v.), does not alter the firing rate of 5-HT neurons, but shuts off that of locus coeruleus neurons. In 5-HT-lesioned rats, this inhibitory effect of WAY 100 635 is abolished (Haddjeri *et al.*, 1997). Furthermore, it appears that an intact 5-HT neuronal system is also necessary to produce the augmentation effect of 8-OH-DPAT on locus coeruleus firing activity (Szabo & Blier, 2000a). Further research will be needed to determine the exact location of the 5-HT_{1A} receptor which, hypothetically, could be located on a projection neuron feeding onto a 5-HT terminal (Szabo & Blier, 2000a).

In vitro, DOI is a nonselective 5-HT_2 receptor agonist, however, *in vivo*, it acts as a preferential 5-HT_{2A} receptor agonist (Aulakh *et al.*, 1995; Mazzola-Pomietto *et al.*, 1995). It induces a dose-dependent decrease in the firing activity of locus coeruleus NA neurons (Chiang & Aston-Jones, 1993; Fig. 7). In addition, it was reported that this suppressing effect of DOI, on the firing activity of locus coeruleus NA neurons, is completely abolished upon prior administration of the selective 5-HT_{2A} receptor antagonist MDL 100 907 (Szabo & Blier, 2000a). When rats were treated with reboxetine for 21-days, the DOI dose–response curve on locus coeruleus firing activity was significantly shifted to the right. This is different from results previously obtained with desipramine whereby no significant difference was found between the DOI dose–response curve in long-term desipramine and control treated rats (Szabo *et al.* 2000). It is important to note, however, that due to the firing activity of reboxetine-treated rats being decreased by 83% after long-term treatment, this may explain why the DOI dose–response appeared to be different. Indeed, it may be expected that 5-HT_{2A} receptor responsiveness is more difficult to assess reliably when the firing rate is so low.

Recently, it was reported that long-term administration of SSRIs is able to attenuate the firing activity of locus coeruleus NA neurons (Szabo *et al.*, 1999, 2000). This decrease in NA neuronal firing is possibly due to desensitization of 5-HT_{1A} receptors resulting in an increase in 5-HT neurotransmission in the locus coeruleus impacting on 5-HT_{2A} receptors to mediate this final NA effect (Szabo & Blier, 2000a). It is, nevertheless, surprising that selective NA reuptake blockers, like desipramine and reboxetine, produce such an adaptive change of a 5-HT neuronal element which was also reported for the 5-HT_{1A} receptor inhibiting the forskolin stimulated production of cyclic AMP in the rat hippocampus (Newman & Lerer, 1988). The exact mechanism by which this occurs remains to be unveiled.

In conclusion, the present results indicate that reboxetine is a potent and selective NA reuptake blocker. However, when it was administered in a sustained fashion, its potency in suppressing the firing rate of NA neurons was enhanced, when compared to that of desipramine. As the firing of NA neurons did not recover following long-term administration of reboxetine, it remains to be determined what adaptative changes of 5-HT and NA receptor responsiveness will be induced in postsynaptic structures after long-term treatment and their impact on monoamine transmission. These experiments are presently underway in our laboratory.

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Abbreviations

NA, noradrenaline; SSRI, selective serotonin (5-HT) reuptake inhibitor.

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ARTICLE

Progressive attenuation of the firing activity of locus coeruleus noradrenergic neurons by sustained administration of selective serotonin reuptake inhibitors

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Abstract

Sustained administration of the selective serotonin (5-HT) reuptake inhibitors (SSRIs) citalopram for 2, 14, and 21 d, and paroxetine for 2 and 21 d (20 and 10 mg/kg.d, respectively, s.c. using osmotic minipumps) produced a gradual decrease in spontaneous firing activity of locus coeruleus (LC) noradrenergic neurons. In contrast, sustained desipramine administration for 2 and 21 d (10 mg/kg.d) robustly reduced LC firing activity, though only to the same extent, following these two treatment periods. The enhancement of the firing rate of LC neurons produced by the 5-HT_{1A} agonist 8-OH-DPAT (10–50 μg/kg, i.v.) in desipramine- and citalopram-treated rats was abolished, indicating a desensitization of 5-HT_{1A} receptors. However, the attenuation of the firing rate of LC neurons induced by the 5-HT_2 agonist DOI (5–50 μg/kg, i.v.) was decreased approx. 2-fold in citalopram-treated rats but not significantly altered in desipramine-treated rats. Since 5-HT neurons exert a tonic inhibitory effect on LC neurons, it appears that enhancing 5-HT neurotransmission by sustained SSRI administration leads to a reduction of the firing rate of noradrenergic neurons. In conclusion, SSRIs attenuate the activity of noradrenergic neurons with a delay that is consistent with their beneficial effect in depression and some anxiety disorders, such as panic, generalized and social anxiety disorders. However, given the hyperadrenergic state often observed in anxiogenic conditions the latter phenomenon is believed to contribute more to the anxiolytic effect of SSRIs than to their antidepressant action.

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Key words: Antidepressant, noradrenaline, serotonin, SSRI, major depression, panic disorder.

Introduction

The noradrenergic and 5-HT systems modulate the activity of various structures of the CNS. The biological functions in which 5-HT and noradrenaline participate are numerous, and disturbances associated with perturbations of these two monoaminergic systems are diverse. The noradrenergic and 5-HT systems have both been implicated in anxiety and affective disorders, with panic disorder being more closely linked to the former and major depression to the latter system. The exact pathophysiology of these two disorders, however, remains elusive. In contrast, more is known about the mechanisms of action of the antidepressant drugs used to treat these disorders (Blier and de Montigny, 1997). Selective 5-HT reuptake inhibitors (SSRIs) promptly prevent the reuptake of 5-HT, but they require at least 2 wk administration before exerting a significant therapeutic effect, similar to other antidepressant agents. Therefore, the rapid blockade of 5-HT reuptake by antidepressant drugs per se cannot account for their therapeutic effect in major depression and panic disorder. SSRIs, however, have been shown to enhance 5-HT neurotransmission in projecting brain areas by increasing 5-HT release as a result of a progressive desensitization of somatodendritic and terminal 5-HT autoreceptors, which normally exert a direct negative feedback influence on the firing rate of 5-HT neurons and on 5-HT release, respectively. In the treatment of panic disorder, when an SSRI or a tricyclic antidepressant (TCA), affecting the 5-HT and/or the noradrenergic reuptake process is administered at a starting dose equivalent to that utilized in the treatment of major depression, exacerbation of the symptoms often occurs (Taylor, 1995; Westenberg, 1996). Consequently, the

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Note: Some preliminary results were presented before Szabo et al. (1999).

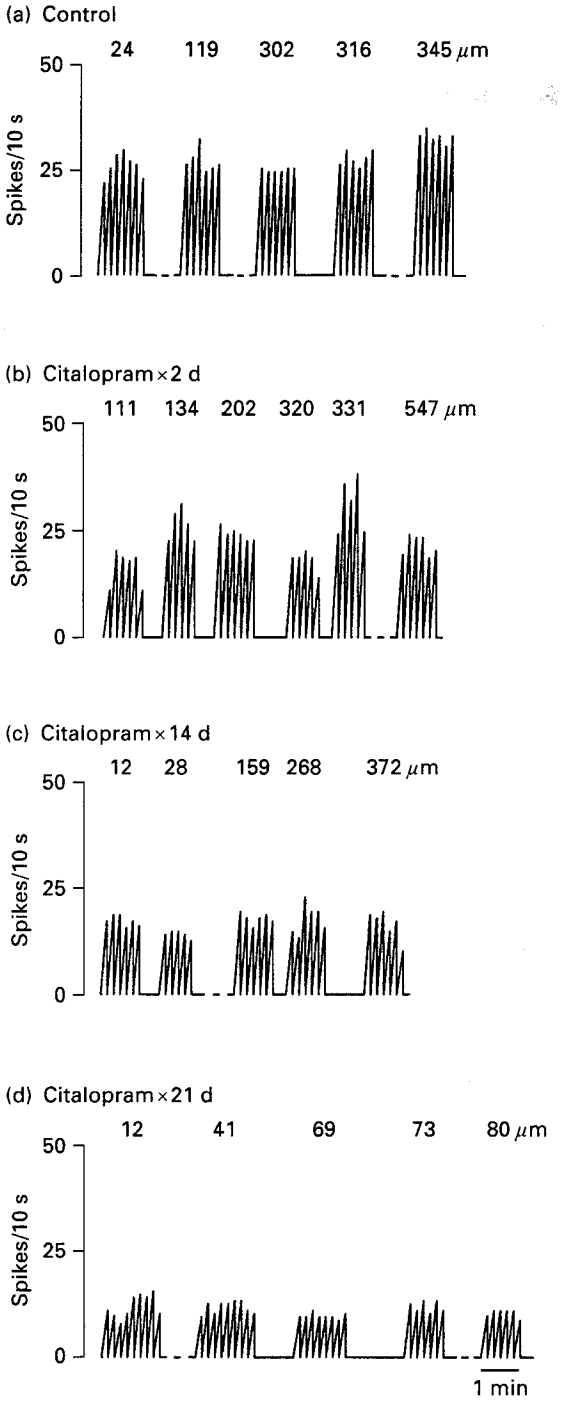

Figure 1. Integrated firing rate histograms of LC noradrenergic neurons, recorded in single electrode descents in the LC showing their spontaneous firing activity in (a) control; (b) 2-d citalopram treatment (20 mg/kg.d); (c) 14-d citalopram treatment (20 mg/kg.d); (d) 21-d citalopram treatment (20 mg/kg.d). The dotted lines in between neurons indicate approx. 5-min time lapses. The number above each neuron indicates the depth from the floor of the fourth ventricle at which it was recorded.

signal-to-noise ratio. A burr hole was drilled 1 mm posterior to lambda and 1 mm lateral to midline for recording of LC neurons. LC noradrenergic neurons were recorded with micropipettes lowered at −0.7 mm interaural and 1.1–1.4 mm lateral. Spontaneously active noradrenergic neurons of the LC were identified using the following criteria: regular firing rate (1–5 Hz) and positive action potential of long duration (0.8–1.2 ms) exhibiting a characteristic burst discharge in response to nociceptive pinch of the contralateral hind paw. Noradrenergic neurons were recorded for at least 1 min to establish basal firing rate. In order to determine possible changes of spontaneous firing activity of noradrenergic neurons in treated animals, 4–5 electrode descents were carried out through this nucleus in control and treated rats. Cell position within the LC did not appear to correlate with firing rate.

Dose–response curves for the alteration of LC neuron firing activity were obtained for systemic (i.v.) administration of the prototypical 5-HT_{1A} receptor agonist 8-OH-DPAT and the 5-HT_2 receptor agonist DOI which acts as a preferential 5-HT_{2A} agonist in vivo but not in vitro (Aulakh et al., 1995; Mazzola-Pomietto et al., 1995; Yamada et al., 1995). Changes in the firing activity are expressed as percentages of the baseline firing rate and were measured after administration of the agonists. After systemic injection of 8-OH-DPAT and DOI, the selective 5-HT_{1A} antagonist WAY-100635 and the 5-HT_2 receptor antagonist ritanserin were systemically administered in attempts to reverse the effects of each of the agonists, respectively. Dose–response curves of 8-OH-DPAT and DOI were constructed, where only one dose was injected to each rat to generate an effective dose 50 (ED_{50}).

Drugs

The following drugs were used: citalopram hydrobromide (H. Lundbeck A/S, Copenhagen, Denmark), desipramine HCl, DOI, 8-OH-DPAT, ritanserin, idazoxan, clonidine (RBI, Natick, MA, USA), WAY-1000635 (Wyeth Research, Berkshire, UK). The concentrations and the doses used for these compounds were chosen on the basis of previously successful experiments carried out in our and other laboratories. Drugs administered i.v. were all dissolved in distilled water while ritanserin was solubilized in acetic acid and then diluted with distilled water to the appropriate concentration.

Statistical comparisons

All results were expressed as mean (±S.E.M.) of single neuron values. Statistical comparisons of values obtained

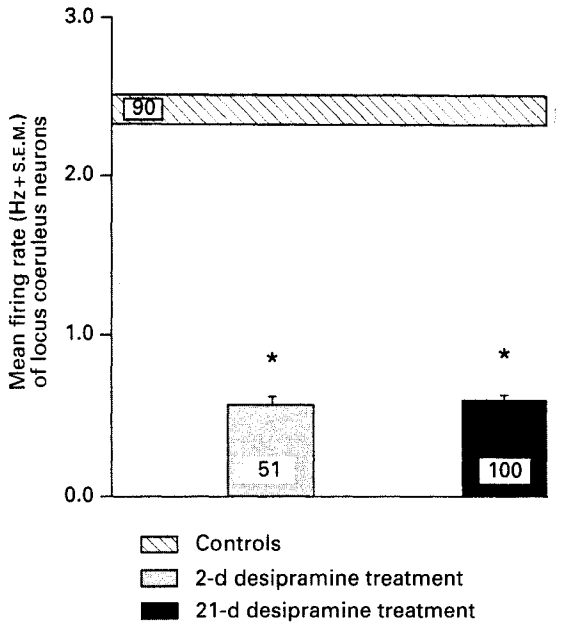

Figure 4. Effects of 2- and 21-d desipramine treatment (10 mg/kg . d) on the firing activity of LC neurons in 3 rats for each group. The *shaded area* represents the range (S.E.M. × 2) of the mean firing activity of neurons recorded in control rats ($n = 6$). * $p < 0.05$ (Dunn's method) when compared to the control value. The number of neurons recorded is displayed in each box.

Table 1. Firing activity of locus coeruleus noradrenergic neurons in controls and treated rats

	Average no. of noradrenergic neurons per descent	No. of descents
Control	2.7 ± 0.2	33
Paroxetine (10 mg/kg . d)		
2 d	2.8 ± 0.6	17
21 d	2.7 ± 0.2	39
Citalopram (20 mg/kg . d)		
2 d	3.1 ± 0.3	35
14 d	3.4 ± 0.4	18
21 d	3.4 ± 0.4	16
Desipramine (10 mg/kg . d)		
2 d	2.8 ± 0.4	18
21 d	4.8 ± 0.5*	21

* $p < 0.05$, when compared to the control group, using ANOVA followed by post-hoc Dunn's method.

the 21-d desipramine-treated rats was probably reflecting an increased proficiency in recording given the identical firing activity in the 2- and 21-day desipramine groups (Table 1).

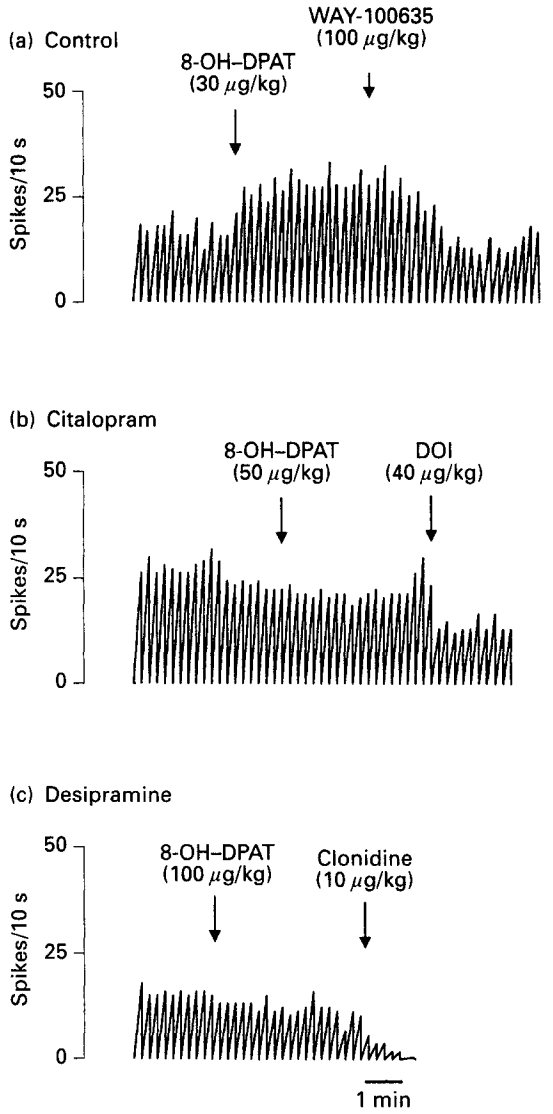

Figure 5. (a) Integrated firing rate histogram of a LC noradrenergic neuron illustrating the effects of intravenous administration of the 5-HT_{1A} agonist 8-OH-DPAT producing an increase in the firing activity, a subsequent injection the 5-HT_{1A} antagonist of WAY-100635 reversed the effects in a control rat. (b) Integrated firing rate histogram of a LC noradrenergic neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with citalopram (20 mg/kg . d, $n = 6$) for 21 d. Note that a subsequent injection of the 5-HT_2 agonist DOI produced a lesser inhibition of firing when compared to controls (see Figures 7 and 8). (c) Integrated firing rate histogram of a LC noradrenergic neuron showing its absence of response to intravenous 8-OH-DPAT in a rat treated with desipramine (10 mg/kg . d) for 21 d. The identity of the noradrenergic neuron recorded in the desipramine-treated rat was assessed by showing the suppressant effect of a subsequent intravenous administration of the α_2-adrenoceptor agonist clonidine.

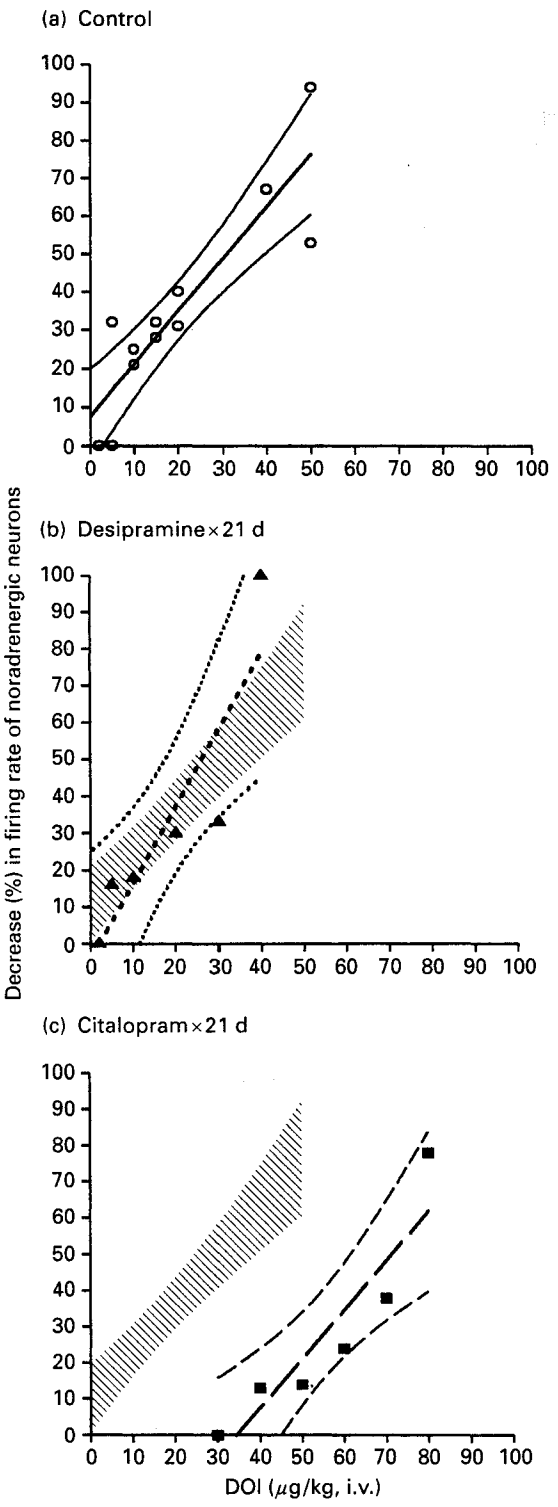

Figure 8. Relationship between the degree of suppression of LC noradrenergic firing activity and doses of DOI administered intravenously in (a) controls; (b) desipramine-treated rats (10 mg/kg.d) and (c) citalopram-treated rats (20 mg/kg.d). Only the initial response of a single noradrenergic neuron to the first dose of DOI in each rat was used to construct the curve. Outer lines represent the standard error of the regression line and the hatched area represents the mean (S.E.M. × 2) of the responses obtained in controls. The shift to the right of the dose–response curve was significant.

neurons in a time-dependent manner. Similarly, a 21-d but not a 2-d paroxetine treatment greatly reduced the spontaneous firing activity of LC noradrenergic neurons, indicating that this phenomenon is a class specific effect and not merely a drug-specific effect (Szabo et al., 1999). A 33% decrease of LC firing had also been previously reported using long-term sertraline administration. This effect did not, however, reach statistical significance, probably due to a small sample size as only 10 neurons were recorded (Valentino et al., 1990). In contrast, the acute and short-term administration of SSRI greatly reduces the firing activity of 5-HT neurons of the dorsal raphe nucleus in the rodent brain (de Montigny et al., 1981; Quinaux et al., 1982). Contrary to noradrenergic neurons, 5-HT neurons regain their normal firing rate after long-term treatment (Blier and de Montigny, 1983). This has been shown to be due to the desensitization of the somatodendritic 5-HT_{1A} autoreceptor which controls their firing activity (Blier and de Montigny, 1994).

Previous experiments have shown that systemic injection of the 5-HT_{1A} agonist 8-OH-DPAT produces a dose-dependent increase in LC firing activity with an ED_{50} similar to that reported here (Piercey et al., 1993). In an attempt to better understand the possible basis for the progressive decrease of LC firing activity following sustained citalopram treatment, the sensitivity of this 5-HT_{1A} receptor involved in enhancing noradrenergic firing activity was assessed in long-term 21-d citalopram-treated rats. The response to systemic 8-OH-DPAT after long-term 21-d citalopram treatment was abolished, indicating a marked desensitization of such 5-HT_{1A} receptors normally mediating an excitatory effect on LC neuronal firing. The desensitization of this 5-HT_{1A} receptor could thus account for the alteration of firing activity of LC neurons: an increase in 5-HT release per action potential, as documented for the ascending 5-HT pathway projecting to the forebrain (Blier and de Montigny, 1983), probably attributable to the fact that this 5-HT_{1A} receptor can no longer be activated following long-term SSRI treatment. This would thus explain the decreased firing rate of noradrenergic LC neurons following long-term administration of SSRIs. This pharmacological condition would then mimic that of the administration of the 5-HT_{1A} antagonist WAY-100635 which produces a suppression of firing of LC neurons (Haddjeri et al., 1997). Indeed, blocking a receptor or desensitizing it should have the same physiological consequence.

5-HT concentration in most postsynaptic structures (Fuller, 1994), but has little effect on noradrenergic neuronal firing rate. It is thus possible that increased symptoms upon SSRI treatment initiation may in fact be attributable to an increased activation of some subtypes of 5-HT receptors not counteracted by an attenuation of noradrenergic firing activity. In analogy, one may think of the 5-HT_3-mediated nausea sometimes produced upon initiation of an SSRI treatment in the absence of any antidepressant effect (Bailey et al., 1995; Bergeron and Blier, 1994). However, as the treatment is prolonged, 5-HT neurotransmission is further increased but noradrenergic neurotransmission would be progressively attenuated. The latter effect may contribute to the anxiolytic and anti-panic effect of SSRIs since an enhancement of noradrenergic firing and release achieved with the α_2-adrenoceptor antagonist yohimbine can produce anxiety in healthy volunteers and trigger panic attacks in patients with panic disorder (Charney et al., 1984). On the other hand, a recent study by Page and Abercrombie (1997) reported that while acute and chronic blockade of 5-HT reuptake did not alter basal extracellular levels of noradrenaline, chronic fluoxetine administration r7esulted in an increase of stress-induced noradrenaline efflux in the rat hippocampus. This may be explained by a lack of absolute selectivity of fluoxetine for the 5-HT transporter at higher doses, with noradrenaline reuptake being blocked to a significant extent in the presence of marked 5-HT reuptake blockade (K_i = 143 and 14 nM, respectively; Bolden-Watson and Richelson, 1993). Indeed, the affinity ratio of fluoxetine is one of the lowest among drugs considered as SSRIs (Hyttel, 1982; Owens et al., 1997; Stanford, 1996). This interpretation of the data would, however, not be compatible with an enhancement of extrasynaptic noradrenaline in the rat frontal cortex following long-term sertraline administration because this drug is highly selective for the 5-HT transporter (Thomas et al., 1998).

The decrease in firing activity of LC noradrenergic neurons combined with the increase in 5-HT neurotransmission may thus be an adaptive mechanism whereby SSRIs eventually exert their therapeutic effect in some anxiety disorders, such as panic and generalized as well as social anxiety disorders (Connor and Davidson, 1998; Davidson, 1998; Jefferson, 1998). In contrast, this putative attenuated noradrenergic tone could explain in part the fatigue and asthenia sometimes reported following long-term SSRI treatment in major depression (Montgomery et al., 1993). Indeed, these symptoms occasionally remain in the presence of markedly improved mood (Feighner and Boyer, 1991). In addition, this delayed reduction of LC neuronal firing may account for the lesser efficacy of SSRI than dual 5-HT/noradrenergic reuptake blockers in some depressed patients, although the latter agents often produce more side-effects (Danish University Antidepressant Group, 1986, 1990; Einarson et al., 1999; Poirier and Boyer, 1999; Silverstone and Ravindran, 1999). In fact, despite decreasing noradrenergic neuronal firing as SSRIs, drugs such as venlafaxine in addition block noradrenergic reuptake in projection areas (Béïque et al., 1999), which would enhance noradrenergic transmission.

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SPECIAL REPORT

Modulation of noradrenergic neuronal firing by selective serotonin reuptake blockers

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Using *in vivo* extracellular unitary recording, the effect of short term (2-day) and long-term (21-day) administration of the selective 5-HT reuptake inhibitor (SSRI) paroxetine (10 mg kg^{-1} day^{-1}, s.c. using osmotic minipumps) was examined on the spontaneous firing activity of locus coeruleus noradrenergic neurons. Long-term but not short-term treatment significantly decreased firing activity. Thus, it appears that enhancing 5-HT neurotransmission by sustained SSRI administration leads to a reduction of the firing rate of noradrenergic neurons. The SSRI paroxetine therefore alters the activity of noradrenergic neurons with a delay that is consistent with its therapeutic action in depression and panic disorder.

Keywords: Antidepressant; noradrenaline; major depression; panic disorder; serotonin (5-HT); selective serotonin reuptake inhibitor (SSRI)

Abbreviations: SSRI, selective serotonin reuptake inhibitor

Introduction The pathophysiology underlying major depression and panic disorder is poorly understood, however, more is known about the mechanisms of action of the antidepressant drugs used to treat these disorders (reviewed by Blier & de Montigny, 1997). For instance, selective 5-HT reuptake inhibitors (SSRIs) have been shown to enhance 5-HT neurotransmission in projecting brain areas by increasing 5-HT release as a result of a progressive desensitization of somatodendritic and terminal 5-HT autoreceptors which normally exert a negative feedback influence on the function of 5-HT neurons. Since SSRIs and other antidepressant drugs require an administration of about 2 weeks before exerting a detectable therapeutic effect, the blockade of 5-HT uptake *per se* cannot account for their therapeutic efficacy in major depression and panic disorder. In the treatment of panic disorder, when a SSRI is administered at a starting dose equivalent to that utilized in the treatment of major depression, an exacerbation of the symptoms often occurs (van Vilet *et al.*, 1996). Consequently, the starting dose is routinely decreased by at least half to avoid this deterioration and then it is progressively titrated to the upper range of the therapeutic window. These clinical observations suggest that panic disorder patients, contrary to depressed patients, might have an increased hypersensitivity of certain 5-HT receptor subtypes. The beneficial effects of the drugs in panic disorder occur gradually at about the same rate as for the treatment of major depression.

It is well established that noradrenergic neurons modulate the 5-HT system. Dorsal raphe 5-HT neurons receive noradrenergic projections from the locus coeruleus (Baraban & Aghajanian, 1980; Anderson *et al.*, 1977; Loizou, 1969), a nucleus which gives rise to more than 90% of noradrenergic innervation of the brain. The noradrenergic neurons located in the locus coeruleus modulate the activity of 5-HT neurons in the dorsal raphe nucleus *via* excitatory α_1-adrenoceptors (Baraban & Aghajanian, 1980). In turn, noradrenergic neurons of the locus coeruleus receive dense 5-HT projections which have revealed an inhibitory role of 5-HT using different experimental approaches (Vertes & Kocsis, 1994; Léger & Descarries, 1978; Cedarbaum & Aghajanian, 1978). This modulation is indicated by several lines of evidence. For instance, lesioning of 5-HT neurons with a selective 5-HT neurotoxin produces an elevation of firing rate of noradrenergic neurons (Haddjeri *et al.*, 1997). The noradrenergic system is in itself a neuronal system which has been implicated in the antidepressant response. Consequently, the therapeutic effect of drugs selective for the 5-HT system, like SSRIs, could in fact be mediated in part by a modification of the efficacy of 5-HT transmission in the locus coeruleus. Changes in noradrenergic function in various brain areas by antidepressant drugs may play a crucial role in controlling 5-HT output, and noradrenergic/5-HT interactions may ultimately be relevant to onset antidepressant efficacy and/or to their side effects. In the present study, electrophysiological experiments were performed in male rats undergoing short-term (2-day) and long-term (21-day) treatment with the SSRI paroxetine where the spontaneous neuronal firing rate of locus coeruleus noradrenergic neurons was determined since this parameter controls in large part the release of noradrenaline in the brain.

Methods The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing between 300-325 g, kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water *ad libitum*). Two groups of rats were treated with paroxetine (10 mg kg^{-1} day^{-1}) for either 3 weeks or 2 days and one group of rats was treated with citalopram (20 mg kg^{-1} day^{-1}) for 3 weeks delivered by osmotic minipumps (ALZA, Palo Alto, CA, U.S.A.) inserted subcutaneously. Two groups of rats were treated with a vehicle (a 50% v/v ethanol/water solution) for 3 weeks or 2 days *via* osmotic minipumps implanted subcutaneously to act as respective controls for the treated groups. The rats were tested with the minipumps in place. Electrophysiological experiments were performed on rats anaesthetized with chloral hydrate (400 mg kg^{-1}, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments). Supplemental doses (100 mg kg^{-1}, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37°C

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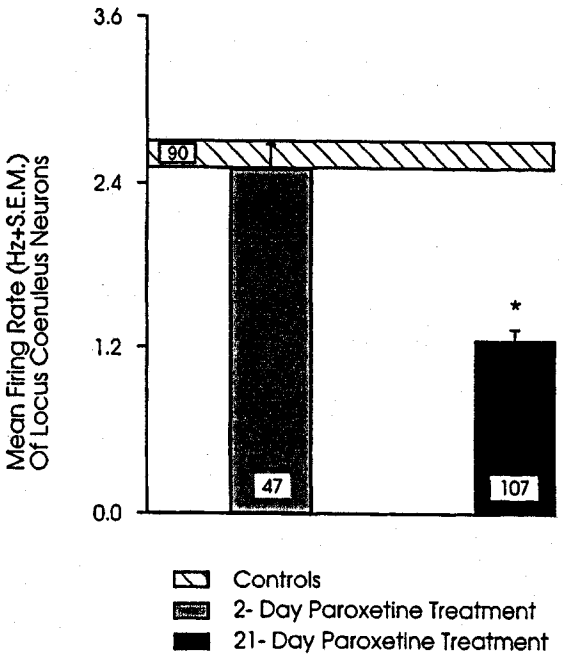

Figure 2 Effects of 2- and 21-day paroxetine treatments (10 mg kg^{-1} day^{-1}) on the firing activity of locus coeruleus neurons. The shaded area represents the range (s.e.mean × 2) of the mean firing activity of neurons recorded in control rats. $^*P<0.05$ (Tukey Test) when compared to the control value. The number of neurons recorded is displayed in each box.

The present findings are interesting when taken into the context of the time course needed for SSRIs to exert their therapeutic efficacy of major depression and panic disorder. The increase in 5-HT release resulting from long-term SSRI treatment would theoretically lead to an increased activation of 5-HT_{2A} receptors on noradrenergic locus coeruleus neurons (Haddjeri *et al.*, 1997). This would yield an increased inhibitory response and ultimately a decrease in firing activity of locus coeruleus noradrenergic neurons which is what we have observed. SSRIs thus decrease the locus coeruleus firing rate and may ultimately also attenuate noradrenaline release in projection areas. This in turn may have a profound impact on the α_2-adrenergic heteroreceptors on the 5-HT terminals, thus diminishing the inhibitory influence on these noradrenergic receptors and contributing to the increase of 5-HT neurotransmission by the SSRI.

The present findings might also be related to the initial exacerbation of panic disorder generally observed with usual starting doses of SSRI for major depression. The acute and short-term administration of SSRIs produces in general a small increase in extracellular 5-HT concentration in several postsynaptic structures (Romero *et al.*, 1996), but has no effect on noradrenergic neuronal firing rate (Béïque *et al.*, 1998). It is thus possible that increased symptoms upon SSRI treatment initiation symptoms may in fact be attributable to an increase in 5-HT synaptic availability not counteracted by an attenuation of noradrenergic firing activity. However, as the treatment is prolonged, 5-HT neurotransmission is further increased but noradrenergic neurotransmission is attenuated. The latter effect may contribute to the anxiolytic and antipanic effect of SSRI since an enhancement of noradrenergic firing and release achieved with the α_2-adrenoceptor antagonist yohimbine can produce anxiety in healthy volunteers and trigger panic attacks in patients with panic disorder (Charney *et al.*, 1984). The decrease in firing activity of locus coeruleus noradrenergic neurons combined with the increase in 5-HT neurotransmission may thus be the adaptive mechanisms whereby SSRIs eventually exert their therapeutic effect in some anxiety disorders. In contrast, this attenuated noradrenergic tone could explain in part the fatigue and asthenia sometimes reported following long-term SSRI treatment in major depression. Indeed, these symptoms occasionally remain in the presence of markedly improved mood (Feighner *et al.*, 1991).

This work was supported by the Medical Research Council of Canada (MRC; grants MA 6444 and MT 11014). S.T.S. is in receipt of a Max Stern Fellowship Award and P.B. of a MRC Scientist award.

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Effects of Chronic Antidepressant Drug Administration and Electroconvulsive Shock on Locus Coeruleus Electrophysiologic Activity

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ELSEVIER

Increased Tonic Activation of Rat Forebrain 5-HT_{1A} Receptors by Lithium Addition to Antidepressant Treatments

Nasser Haddjeri, Ph.D., Steven T. Szabo, B.Sc., Claude de Montigny, M.D., Ph.D., and Pierre Blier, M.D., Ph.D.

The present study was undertaken to determine whether lithium addition to long-term treatment with different classes of antidepressant drugs could induce a greater effect on the serotonin (5-HT) system than the drugs given alone. Because 5-HT_{1A} receptor activation hyperpolarizes and inhibits the firing activity of CA_3 pyramidal neurons in the dorsal hippocampus, the degree of disinhibition produced by the selective 5-HT_{1A} receptor antagonist WAY 100635 was determined using in vivo extracellular recordings. In controls, as well as in rats receiving a lithium diet for 3 days, the administration of WAY 100635 (25-100 μg/kg, IV) did not modify the firing activity of dorsal hippocampus CA_3 pyramidal neurons. When the tricyclic antidepressant imipramine (10 mg/kg/day, SC), the monoamine oxidase inhibitor tranylcypromine (2.5 mg/kg/day, SC) and the selective 5-HT reuptake inhibitor paroxetine (10 mg/kg/day, SC) were administered alone for 21 days, a dose of 50 μg/kg of WAY 100635 was needed to increase significantly the firing activity of these neurons. On the other hand, WAY 100635, at a dose of only 25 μg/kg, increased significantly the firing rate of CA_3 pyramidal neurons in rats receiving both a long-term antidepressant treatment and a short-term lithium diet. It is concluded that the addition of lithium to antidepressant treatments produced a greater disinhibition of dorsal hippocampus CA_3 pyramidal neurons than any treatments given alone. The present results support the notion that the addition of lithium to antidepressants may produce a therapeutic response in treatment-resistant depression by enhancing 5-HT neurotransmission. **[*Neuropsychopharmacology* 22:346–356, 2000]** © *2000 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

KEY WORDS: *Lithium; Imipramine; Tranylcypromine; Paroxetine; WAY 100635, 5-HT_{1A} receptors; Dorsal hippocampus*

Although the physiopathology of major depression is not fully defined, there is a growing body of evidence suggesting the implication of the serotonin (5-HT) system in the therapeutic effect of antidepressant treatments (Heninger and Charney 1987; Price et al. 1990a; Van Praag et al. 1990; Cummings 1993; Blier and de Montigny 1994; Maes and Meltzer 1995). For example, it has been shown that long-term tricyclic antidepressant (TCA) treatment and repeated electroconvulsive shock (ECS) administration lead to enhanced 5-HT neurotransmission via sensitization of the postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian 1978; de Montigny 1984; Welner et al. 1989; Nowak and Dulinski 1991; Stockmeier et al. 1992). Long-term treatment with either monoamine oxidase inhibitors (MAOIs) or selective 5-HT reuptake inhibitors (SSRIs) results in a desensitization of the somatodendritic 5-HT_{1A} autoreceptor of 5-HT neurons in the dorsal raphe nucleus, thereby allowing their firing rate to recover in

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the presence of the drugs (Blier et al. 1986; Chaput et al. 1986). In addition, long-term SSRI treatment also desensitizes terminal 5-HT_{1B} autoreceptors; whereas, long-term MAOI treatment desensitizes terminal α_2-adrenergic heteroreceptors located on 5-HT terminals (Blier and Bouchard 1994; Mongeau et al. 1994). The desensitization of the latter two receptors is thought to contribute to a greater release of 5-HT following SSRI and MAOI administration. Long-term treatment with the antidepressant mirtazapine, an α_2-adrenoceptor antagonist, increases 5-HT neurotransmission as a result of a sustained increase in the firing activity of 5-HT neurons in the presence of the decreased function of α_2-adrenergic heteroreceptors located on 5-HT terminals (Haddjeri et al. 1997). Finally, long-term treatment with 5-HT_{1A} receptor agonists, such as gepirone, desensitizes the 5-HT_{1A} autoreceptor on 5-HT neurons, but not the postsynaptic 5-HT_{1A} receptors located on CA_3 pyramidal neurons (Blier and de Montigny, 1987).

Recently, novel direct evidence of an enhanced 5-HT neurotransmission by antidepressant treatments has been provided (Haddjeri et al. 1998a). This study showed that long-term treatment with either the TCA imipramine, the SSRI paroxetine, the selective and reversible MAO-A inhbitor befloxatone, the α_2-adrenergic antagonist mirtazapine, the 5-HT_{1A} receptor agonist gepirone, as well as repeated ECS administration, enhanced the tonic activation of postsynaptic 5-HT_{1A} receptors in the dorsal hippocampus, as put into evidence by the enhanced disinhibition produced by the acute administration of the selective 5-HT_{1A} receptor antagonist WAY 100635 (Haddjeri et al. 1998a). Furthermore, no disinhibition was detectable in rats treated for 3 weeks with the neuroleptic chlorpromazine or in rats receiving only one ECS, two treatment modalities devoid of antidepressant effect. Finally, that 5-HT_{1A} receptors mediate the disinhibition induced by WAY 100635 is further supported by the observation that the inactivation of the $G_{i/o}$ proteins by pertussis toxin prevented the disinhibition in rats treated with repeated ECS (Haddjeri et al. 1998a). Finally, it was recently reported that WAY 100635 dishibits CA1 pyramidal neurons firing activity in naive, but not in 5-HT depleted, freely moving rats (Susuki et al. 1999).

Lithium remains a first-line approach for treatment of acute mania and the prophylactic management of manic-depressive illness (see Lenox et al. 1998, for review). Interestingly, lithium is also a useful augmentation strategy in the treatment of depression (de Montigny et al. 1981; Rouillon and Gorwood 1998). Although the underlying neurobiological mechanism remains as yet not fully defined, there is a considerable body of preclinical evidence reporting an increase in 5-HT neurotransmission following lithium administration (Baptista et al. 1990; Grahame-Smith and Green 1974; Treiser et al. 1981; Blier and de Montigny 1985; Goodwin et al. 1986; Goodwin 1989; Price et al. 1990b; Sangdee and Franz 1980; Sharp et al. 1991).

The present study was undertaken to determine whether an association of lithium with various types of antidepressant treatments could act in synergy to enhance 5-HT neurotransmission. Thus, the effects of short-term lithium diet and long-term treatment with the TCA imipramine, the type A and B MAOI tranylcypromine and the SSRI paroxetine, given alone and in combination, were assessed on the degree of activation of postsynaptic 5-HT_{1A} receptors, using an in vivo electrophysiological paradigm in the rat dorsal hippocampus.

METHOD

Animals and Treatments

Male Sprague–Dawley rats (Charles-River, Quebec, Canada) weighing 250–300 g on the day of the experiment were used. The animals were maintained on a 12:12 hours light:dark cycle with free access to food and water. Rats were treated for 21 days with either imipramine (10 mg/kg/day), paroxetine (10 mg/kg/day), or tranylcypromine (2.5 mg/kg/day), using osmotic minipumps (ALZA, Palo Alto, CA, USA) implanted subcutaneously under halothane anesthesia. These drugs were dissolved in a water/ethanol solution (50/50, v/v), and the rats received 0.75 ml of 95% ethanol over a period of 21 days. For each series of experiments, controls were implanted with a minipump filled with the same vehicle as the corresponding treated groups. All the experiments were carried out with the minipumps on board. Rats were fed lithium-containing chow (Ren's Feed & Supplies Ltd, Oakville, ON) for 72 h preceding the electrophysiological experiments. Plasma lithium levels were determined following all experiments by flame emission photometry and ranged from 0.4 to 1.1 mEq/l. None of these treatments altered the normal behavior of the animals in their cages or upon handling. The drug regimens have been chosen on the basis of previous experiments indicating effective doses (de Montigny and Aghajanian 1978; Blier and de Montigny 1985; Goodnough and Baker 1994; Piñeyro et al. 1994).

Electrophysiological Procedures

Rats were anesthetized with chloral hydrate (400 mg/kg, IP) and were mounted in a stereotaxic apparatus. Additional doses (100 mg/kg, IP) were given to maintain the anesthesia along the experiment.

Extracellular Recording and Microiontophoresis from Dorsal Hippocampus CA_3 Pyramidal Neurons. A hole was drilled 4.2 mm lateral and 4.2 mm anterior to

RESULTS

Effects of Long-Term Antidepressant Treatments on the Responsiveness of Dorsal Hippocampus CA_3 Pyramidal Neurons to 5-HT

It has been previously demonstrated that the microiontophoretic application of 5-HT onto rat dorsal hippocampus CA_3 pyramidal neurons produces a suppressant effect on their firing activity via the activation of postsynaptic 5-HT_{1A} receptors (Blier and de Montigny 1987; Chaput and de Montigny 1988). For all CA_3 pyramidal neurons tested, 5-HT (10 nA) induced a reduction of firing activity (Figure 1). This inhibitory effect of 5-HT occurred without any alteration of the action potential shape, thus ruling out current artifact in altering firing pattern. Long-term treatment with tranylcypromine or with paroxetine alone or in combination with a short-term treatment with lithium did not modify the suppressant effect of microiontophoretically applied 5-HT on the firing activity of CA_3 pyramidal neurons. On the other hand, long-term treatment with imipramine alone or in combination with lithium markedly enhanced the responsivity of CA_3 pyramidal neurons to microiontophoretically applied 5-HT: the mean $I{\cdot}T_{50}$ value for 5-HT was significantly lower in rats treated with imipramine alone or in combination with lithium than in controls or in rats having a short-term lithium diet (Figure 2A).

The mean RT_{50} value for 5-HT was increased by 78% in paroxetine-treated rats and by 57% in rats treated with paroxetine in combination with lithium, because of the blockade of the 5-HT uptake process (Figure 2B). No significant change in the RT_{50} value for 5-HT was observed in the other groups (Figure 2B).

As previously reported (Haddjeri et al. 1998a,b), the intravenous administration of the selective 5-HT_{1A} receptor antagonist WAY 100635 (100 μg/kg) significantly reduced the suppressant effect of 5-HT on CA_3 pyramidal neurons in control rats (Figure 1). WAY 100635 significantly reduced the suppressant effect of 5-HT on the firing activity of CA_3 pyramidal neurons by 73% in controls ($t = 10.38$, $df = 6$, $p < .001$), 71% in lithium-treated rats ($t = 6.72$, $df = 6$, $p < .001$), 80% in imipramine-treated rats ($t = 10$, $df = 5$, $p < .001$) and 76% in rats treated with imipramine followed by a lithium diet ($t = 9.62$, $df = 4$, $p < .001$).

Tonic Activation of the Postsynaptic 5-HT_{1A} Receptors on the Dorsal CA_3 Hippocampus Pyramidal Neurons by Antidepressants

As mentioned in the Materials and Methods section, the dorsal hippocampus CA_3 pyramidal neurons were activated by a leak or a small current of quisqualate. It is important to mention that none of the treatments used significantly modified the firing activity of the dorsal hippocampus CA_3 pyramidal neurons when compared to controls (−0.1 ± 0.5 nA of quisqualate resulted in a firing activity of 3.8 ± 0.6 Hz, $n = 9$; in rats having received a lithium diet, the application of 0.1 ± 0.6 nA of quisqualate resulted in a firing activity of 4.6 ± 0.7 Hz, $n = 7$; in imipramine-treated rats, 0.2 ± 0.5 nA of quisqualate resulted in a firing activity of 3.5 ± 0.6 Hz, $n = 6$; in rats treated with both imipramine and lithium, −0.3 ± 0.6 nA of quisqualate resulted in a firing activity of 3.6 ± 0.7 Hz, $n = 6$; data not shown for the other groups). This was expected, because the only pretreatment that altered this parameter was the destruction of 5-HT neurons (Blier and de Montigny 1987).

As illustrated in Figure 1, the prior injection of saline did not alter the firing activity of the dorsal hippocampus CA_3 pyramidal neurons in control and treated rats. The IV injection of WAY 100635 also did not modify the firing activity of CA_3 pyramidal neurons in control rats (Figures 1A and 3). In rats having a lithium diet for 3 days, the responsiveness of CA_3 pyramidal neurons to IV injections of WAY 100635 was similar to that of the control rats ($t = -0.2$, $df = 14$, $p = .84$; Figures 1B and 3). On the other hand, in rats treated for 21 days with either tranylcypromine or paroxetine, significant increases in the mean firing rate of CA_3 pyramidal neurons were observed in response to the IV injection of 50 μg/kg of WAY 100635, but not with a dose of 25 μg/kg. This increase was of 37% in rats treated with imipramine ($t = -2.2$, $df = 12$, $p < .05$; Figure 3), of 59% in rats treated with paroxetine ($t = -5.7$, $df = 12$, $p > .05$, Figure 3) and of 80% in rats treated with tranylcypromine ($t = -2.76$, $df = 12$, $p < .05$; Figure 3).

In contrast, in rats treated with imipramine for 21 days in combination with the lithium diet, a dose of only 25 μg/kg (IV) of WAY 100635 increased by 75% the firing activity of hippocampus CA_3 pyramidal neurons (Figure 3); hence, this firing rate was significantly greater than that obtained in controls or in imipramine-treated rats for a dose of 25 μg/kg of WAY 100635 ($t = -5.51$, $df = 13$, $p < .001$; Figure 3). Similarly, in rats treated with tranylcypromine for 21 days in combination with a lithium diet, a dose 25 μg/kg (IV) of WAY 100635 produced a robust increase of 182% of the firing activity of hippocampus CA_3 pyramidal neurons ($t = -5.47$, $df = 13$, $p < .001$; Figure 3); whereas, following the IV injection of a dose 25 μg/kg (IV) of WAY 100635 in rats treated with tranylcypromine alone, the firing rate was not significantly different than in controls ($t = -1.23$, $df = 13$, $p > .24$; Figure 3). Finally, and as previously reported (Besson et al. 1997), in rats treated with paroxetine for 21 days, the IV injection of a dose 25 μg/kg (IV) of WAY 100635 did not affect the firing activity of hippocampus CA_3 pyramidal neurons ($t = -0.41$, $df = 13$, $p > .68$; Figure 3). On the other hand, the same dose of WAY 100635

lambda. Extracellular recordings of dorsal hippocampus CA_3 pyramidal neurons and microiontophoretic applications of 5-HT were performed with five-barreled glass micropipettes pulled in the conventional manner to achieve a tip diameter of 10–15 μm. The central recording barrel was filled with a 2 M NaCl solution saturated with fast green dye. One of the side barrels was filled with quisqualic acid (1.5 mM in 400 mM NaCl, pH 8), because a leak or a small ejection current (+2 to −3 nA) of quisqualate was needed to activate the CA_3 pyramidal neurons within their physiological range, because they are not spontaneously active in anesthetized animals. These neurons were identified according to the criteria of Kandel and Spencer (1961): large amplitude and long duration complex spike discharges. A side-barrel was filled with 5-HT (2 mM in 200 mM NaCl, pH 4). A 10 nA ejection current of 5-HT was used, each ejection period lasting 50 seconds. One barrel was filled with 2 M NaCl and served as an automatic current balance.

To assess the effectiveness of the long-term treatment with paroxetine, the recovery time 50 (RT_{50}) method was used. The RT_{50} value has been shown to be a reliable index of the in vivo activity of the 5-HT reuptake process in the rat hippocampus. This value is obtained by calculating the time in seconds required for the neuron to recover 50% of its initial firing rate at the end of the microiontophoretic application of 5-HT onto the CA_3 pyramidal neuron. Thus, the blockade of the 5-HT transporter by an SSRI reveals a greater RT_{50} value than in controls (Piñeyro et al. 1994). The neuronal responsiveness to 5-HT was assessed using the $I{\cdot}T_{50}$ method. It is the product of the current (in nA) used to eject 5-HT from the micropipette and the time (in sec) required to obtain a 50% decrease from the baseline of the firing rate of the recorded neuron. The more sensitive a neuron is to 5-HT, the smaller will be the $I{\cdot}T_{50}$ value, because the number of molecules ejected is proportional to the charge (de Montigny and Aghajanian 1978).

The tonic activation of the postsynaptic 5-HT_{1A} receptors of the dorsal hippocampus CA_3 pyramidal neurons was assessed in the following manner. The firing rate of dorsal hippocampus CA_3 pyramidal neurons was determined before and after systemic injection of the selective 5-HT_{1A} receptor antagonist WAY 100635, via a cannula inserted in a lateral tail vein, in control and treated groups. It is well established that the suppression of the firing activity of CA_3 pyramidal neurons by microiontophoretic applied 5-HT is mediated through the activation of 5-HT_{1A} receptors (Chaput and de Montigny 1988; Blier et al. 1993). Thus, the blockade of these 5-HT_{1A} receptors by WAY100635 will disinhibit the CA_3 hippocampus pyramidal neurons resulting in an increase of their firing activity (Haddjeri et al. 1998a). In this series of experiments, the current of quisqualate was adjusted in order to obtain a firing rate around 4 Hz to allow the detection of enhancements in firing more readily following administration of WAY 100635. This baseline firing was recorded for at least 2 minutes before the IV injection of WAY 100635. An IV injection of saline preceded the first injection of WAY 100635 to eliminate any effect attributable to the injection by itself. Doses of WAY 100635 (25 μg/kg, IV) were administered at time intervals of 1 to 2 minutes, and only one neuron was studied in each rat. WAY 100635, administered IV, does not modify the firing rate of 5-HT neurons in the dorsal raphe nucleus of the anesthetized rats but restores 5-HT neuronal firing if it was reduced by 5-HT_{1A} autoreceptor activation (Forster et al. 1995; Gartside et al. 1995; Lejeune and Millan 1998). Therefore, in treated animals, where there would be increased extracellular levels of 5-HT in the raphe region, WAY 100635 would restore 5-HT neuronal firing activity. However, because WAY 100635 was given systemically, it would be simultaneously blocking the effects of 5-HT on postsynaptic neurons, thereby canceling out the effect of WAY 100635 on the somatodendritic autoreceptors. Indeed, if the action of WAY 100635 at the somatodendritic 5-HT_{1A} autoreceptors was influencing the activity of the hippocampal neurons, it would serve to inhibit further their firing due to an increased release of 5-HT into the target area. Thus, it can be assumed that any increment in the firing activity of hippocampus pyramidal neurons would reflect an increased level in the tonic activation of the postsynaptic 5-HT_{1A} receptors and the degree to which WAY 100635 disinhibits this firing would presumably be a direct measure of the tonic level of activation of 5-HT_{1A} receptors on CA_3 pyramidal neurons by extracellular 5-HT. However, because WAY 100635 is injected systemically, it cannot be ruled out that such an effect of this agent could be attributable to its antagonistic action on postsynaptic 5-HT_{1A} receptors projecting to the hippocampus. Nevertheless, an enhanced effect would still be a reflection of an increased 5-HT transmission by the treatments studied.

Statistical Analysis

Results are expressed as the mean ± SEM. Dunett's method multiple comparison test following one-way analysis of variance was employed in the RT_{50} and $I{\cdot}T_{50}$ methods and in the experiments with WAY 100635. The criterion for significance was taken as $p \leq .05$.

Drugs

Paroxetine was provided by SmithKline Beecham (Harlow, UK), imipramine by Ciba-Geigy (Montréal, Canada) and WAY100635 by Wyeth-Ayerst (Princeton, NJ, USA). Tranylcypromine, 5-HT creatinine sulfate and quisqualic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA).

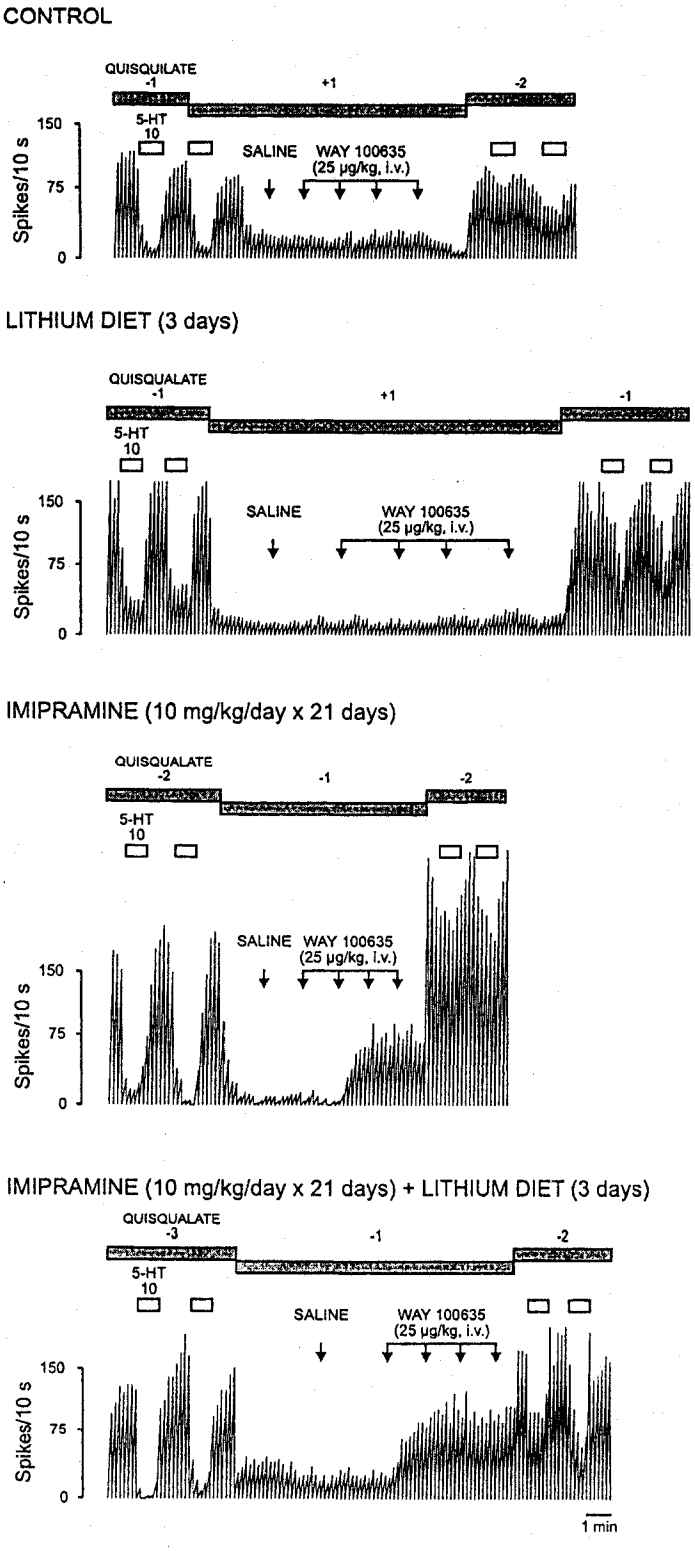

Figure 1. Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron, showing its responsiveness to microiontophoretic application of 5-HT in control **(A)**; a rat having received a lithium diet for 3 days **(B)**; a rat treated with imipramine for 21 days **(C)**; and a rat having both a treatment with imipramine and a lithium diet **(D)**. These neurons were activated with a quisqualate ejection current. Horizontal bars indicate the duration of the applications (current given in nanoAmperes). Note the altered effectiveness of 5-HT in suppressing firing activity after administration of WAY 100635 (4 × 0.25 mg/kg, IV) in control rat and treated rats.

increased by 104% the firing activity of hippocampus CA_3 pyramidal neurons in rats treated with paroxetine for 21 days in combination with a lithium diet ($t = -5.06$, $df = 14$, $p < .001$; Figure 3).

DISCUSSION

The main point of interest that emerges from the present electrophysiological experiments is that the en-

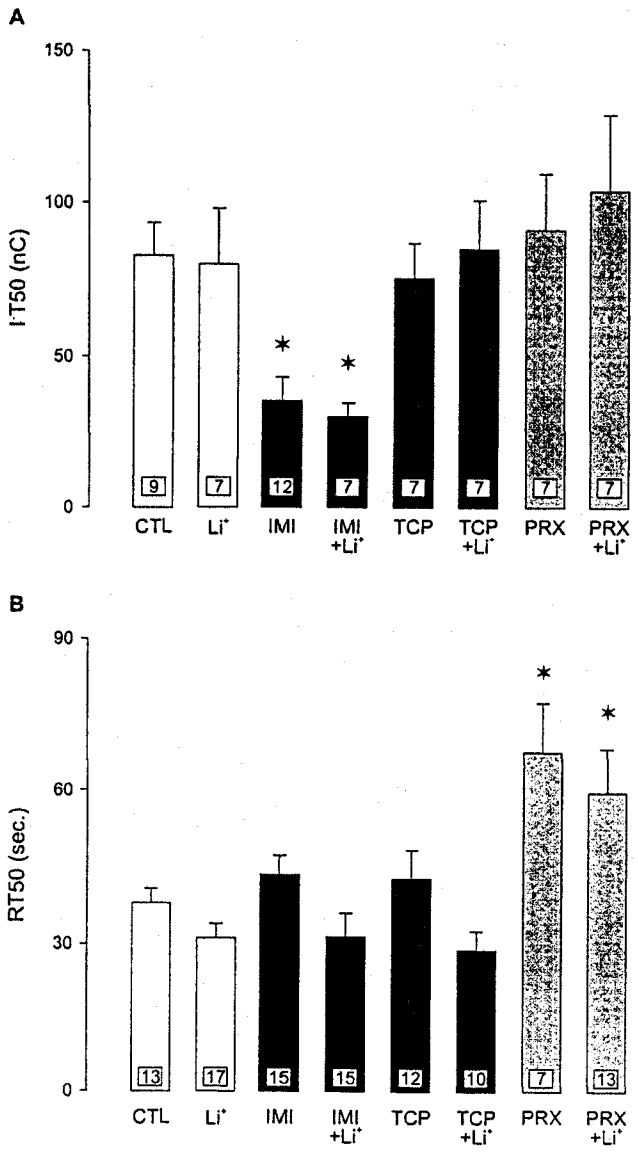

Figure 2. **(A)** Mean (± SEM) IT_{50} values (see the Method section) in control rats (CTL), rats having a lithium diet (Li^+), rats treated with imipramine for 21 days (10 mg/kg/day, SC, IMI), rats treated with imipramine and having a lithium diet (IMI + Li^+), rats treated with tranylcypromine for 21 days (2.5 mg/kg/day, SC, TCP), rats treated with tranylcypromine and having a lithium diet (TCP + Li^+), rats treated with paroxetine for 21 days (10 mg/kg/day, SC, PRX) and rats treated with paroxetine and having a lithium diet (PRX + Li^+). The number in the columns indicates the number of neurons tested. *$p < .05$ (unpaired Student's t test). **(B)** Recovery time, expressed as RT_{50} values (means ± SEM), of dorsal hippocampus CA_3 pyramidal neurons from the microiontophoretic application of 5-HT in control rats (CTL), rats having a lithium diet (Li^+), rats treated with imipramine for 21 days (10 mg/kg/day, SC, IMI), rats treated with imipramine and having a lithium diet (IMI + Li^+), rats treated with tranylcypromine for 21 days (2.5 mg/kg/day, SC, TCP), rats treated with tranylcypromine and having a lithium diet (TCP + Li^+), rats treated with paroxetine for 21 days (10 mg/kg/day, SC, PRX) and rats treated with paroxetine and having a lithium diet (PRX + Li^+). The number in the columns indicates the number of neurons tested. *$P < .05$ significantly different from control group by Dunett's test following ANOVA.

hanced tonic activation of postsynaptic 5-HT_{1A} receptors induced by long-term treatments with imipramine, tranylcypromine, and paroxetine was greater when a lithium diet was added; whereas, lithium alone did not modify this parameter. Hence, it is suggested that lithium addition in treatment-resistant depression might be attributable to a potentiation of 5-HT neurotransmission.

Among the 5-HT_{1A} receptor antagonists available, WAY 100635 is by far the most potent and selective antagonist at both pre- and postsynaptic 5-HT_{1A} receptors (Khawaja et al. 1994; Fletcher et al. 1996). The present results confirm that WAY 100635 is, indeed, an effective antagonist at postsynaptic 5-HT_{1A} receptors, because it reduced the suppressant effect of microiontophoretically applied 5-HT on the firing activity of CA_3 pyramidal neurons (Figure 1). If the tonic activation of postsynaptic 5-HT_{1A} receptors is increased, one could then expect that the blockade of these receptors by WAY100635 would result in an enhancement of the baseline firing activity of CA_3 pyramidal neurons. However, WAY 100635 (25 to 100 μg/kg, IV) did not produce any disinhibition on dorsal hippocampus CA_3 pyramidal neurons in control rats (Figures 1 and 3). This result indicates the lack of a tonic activation of postsynaptic 5-HT_{1A} receptors in the anesthetized untreated rats. It is also noteworthy that no disinhibition of CA_3 pyramidal neurons was detected following short-term treatments with various antidepressants (Besson et al. 1997). Similarly, in rats receiving a lithium diet for 3 days, WAY 100635 did not induce any significant disinhibition of CA_3 pyramidal neurons. The capacity of WAY 100635 to block the somatodendritic 5-HT_{1A} autoreceptor is not expected to alter its effectiveness in disinhibiting postsynaptic neurons in the paradigm used in the present study. Indeed, any interference of WAY 100635 at the somatodendritic 5-HT_{1A} autoreceptor would dampen this disinhibitory effect at postsynaptic 5-HT_{1A} receptors, because in the presence of an SSRI in freely moving cats (Fornal et al. 1996) and of befloxatone in anesthetized rats (Haddjeri et al. 1998b), WAY 100635 increases the firing of 5-HT neurons. As described in the Materials and Methods section, the CA_3 pyramidal neurons are activated by quisqualate, hence, it is important to mention that lithium can also affect glutamate neurotransmission. In fact, it has been shown that lithium blocks the uptake of glutamate from cerebral cortex slices of monkey and mouse (Dixon et al. 1994; Dixon and Hokin 1997). Moreover in the

evidence suggesting that this agent acts in bipolar disorder by affecting the levels of intracellular second messengers; that is, the activity of adenylate cyclases, the function of G protein, the activity of inositol monophosphatases, and the level or the function of protein kinases (Mørk 1993; Jope and Williams 1994; Manji et al. 1995; Mørk and Geisler 1995; Bitsch Jensen and Mørk 1997; Wang and Friedman 1999). Central 5-HT function has also been shown to be affected by lithium administration, although its effect on the 5-HT system differs according to the duration of treatment and the brain region studied (Goodwin 1989; Price et al. 1990b). Accordingly, it has been shown that acute, but not chronic, lithium administration increases rat brain 5-HT turnover (Grahame-Smith and Green 1974; Minegishi et al. 1981; Karoum et al. 1986). Acute treatment with lithium potentiates the tranylcypromine- plus l-tryptophan-induced 5-HT syndrome in the rat, a syndrome sensitive to stimulation of 5-HT synthesis (Grahame-Smith and Green 1974). Chronic lithium has been shown to increase 5-HT release from rat cortical, hippocampal, and hypothalamic brain slices, possibly resulting from down-regulation of 5-HT autoreceptors (Treiser et al. 1981; Wang and Friedman 1988). Moreover, Hotta and Yamawaki (1988) showed that the inhibitory effect of 5-HT on the KCl- or electrically evoked release of tritiated 5-HT, presumably mediated by presynaptic 5-HT autoreceptors, was attenuated in the hippocampus, but not frontal cortex, after a 3-day lithium treatment, and a significant increase in the release of 5-HT was observed in the hippocampus, but not in the frontal cortex. On the other hand, it has been shown that short-term lithium treatment does not affect dorsal raphe 5-HT neuronal firing rate but augments the endogenous release of 5-HT in the rat dorsal hippocampus induced by the electrical stimulation of the ascending 5-HT pathway, without affecting the sensitivity of terminal 5-HT autoreceptors (Blier and de Montigny 1985; Blier et al. 1987). This enhancing effect of lithium on 5-HT release has been proposed to be attributable to an increased amount of 5-HT released per impulse, because the responsiveness of hippocampal neurons to 5-HT (Figure 1) and to the 5-HT_{1A} receptor agonist 8-OH-DPAT, applied by microiontophoresis, was not modified by the short-term lithium treatment (Blier et al. 1987). Similarly, using microdialysis, the release of 5-HT in the hippocampus, evoked by electrical stimulation of the dorsal raphe nucleus, was markedly enhanced in treated rats with lithium for 3 days, but not 21 days. The same group showed in vitro that the depolarization (high potassium) -evoked release of endogenous 5-HT from the hippocampus was increased in lithium-treated rats after 3 days, but not 21 days (Sharp et al. 1991). Moreover, a reduced concentration of 5-HT in rat hippocampal dialysates has been observed after 21 days of treatment with lithium (Sharp et al. 1991). In contrast, Pei et al. (1995) have shown that long-term (21 days), but not short-term (3 days), lithium treatment enhances the stimulating effect on 5-HT efflux in vivo in the rat hippocampus (but not in striatum) induced by the potassium-channel blocking drug 4-aminopyridine. However, this group did not observe any changes in basal outflow of hippocampal 5-HT from lithium-treated rats versus controls (Pei et al. 1995). Taken together, all these experiments clearly show that lithium has the capacity to enhance 5-HT release, but some experimental conditions in laboratory animals may alter this potential.

Only a few preclinical studies have been undertaken to characterize the effects of lithium addition to antidepressant treatment on the 5-HT system. As initially proposed by Newman et al. (1990), Okamoto et al., (1996) have also recently suggested that the therapeutic action of lithium, when added to antidepressants in the treatment of refractory depression, may partly have its basis in a further activation of the 5-HT system. In fact, they showed, in vitro in the rat frontal cortex, that the addition of lithium for a short-term (5 days) to a long-term (19 days) period with the TCA clomipramine and the SSRI citalopram potentiated an increase in 5-HIAA level, but not that of 5-HT; whereas, lithium alone had no effect. In addition, they reported that binding parameters of 5-HT_{1A} receptors and 5-HT transporters, using rat cortical membranes, remain unchanged by such treatments; whereas, that of 5-HT_2 receptors were reduced by the clomipramine treatment alone and in combination with lithium. The efficacy of lithium augmentation of the antidepressant response, which is now supported by several placebo-controlled studies (see for review Rouillon and Gorwood 1998), may be at variance with some long-term studies not showing an enhanced 5-HT release. However, short-term lithium addition consistently produces an increase of 5-HT neurotransmission, which may be sufficient to obtain a clinical response. The latter discrepancy may not be so crucial, because it is not yet know how long 5-HT neurotransmission has to be potentiated in order to produce or maintain an antidepressant effect. Indeed, it was observed that some treatment-resistant patients maintained an antidepressant effect even if lithium addition was abruptly stopped immediately after obtaining a therapeutic response (de Montigny et al. 1983).

In summary, the present study showed that lithium addition to antidepressant drugs induced a greater enhancement of the tonic activation of postsynaptic 5-HT_{1A} receptors in rat dorsal hippocampus than any drug given alone. In the light of previous evidence of the key role of the postsynaptic 5-HT_{1A} receptor site in the therapeutic effects of antidepressant drugs (Blier and de Montigny 1994), it can be proposed from the present results that the addition of lithium to antidepressants potentiates the antidepressant response by further enhancing 5-HT neurotransmission.

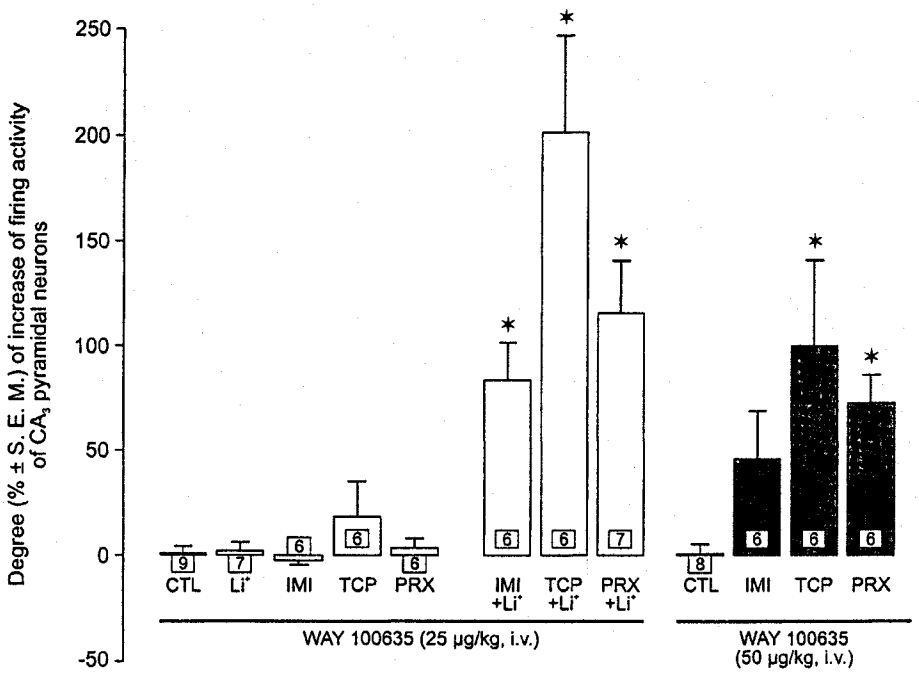

Figure 3. Changes (% ± SEM) of the firing activity of quisqualate-activated dorsal hippocampus CA_3 pyramidal neurons following intravenous injection of WAY 100635 (25 and 50 µg/kg) in control rats (CTL), in rats treated with imipramine (IMI), paroxetine (PRX), tranylcypromine (TCP), and in rats treated with both imipramine and lithium (IMI + Li^+), tranylcypromine and lithium (TCP + Li^+), or paroxetine and lithium (PRX + Li^+). One neuron per rat was tested and the number for each column indicates the number of neurons or rats tested. *$p < .05$ significantly different from control group by Dunett's test following ANOVA.

mouse, chronic treatment with lithium for 2 weeks upregulated synaptosomal uptake of glutamate (Dixon and Hokin 1998). Nevertheless, these effects of lithium most likely did not interfere with the present results, because the level of activation of hippocampus neurons induced by quisqualate was not different in any group of rats. Accordingly, it has been recently shown that a short-term lithium treatment (5 days) does not modify the neuronal activity, by assessing the cytochrome oxidase activity, in several brain regions, including the hippocampus (Lambert et al. 1999).

In the present study, WAY100635, at a dose of 50 µg/kg, induced a significant disinhibition of the firing activity of CA_3 pyramidal neurons in rats treated with tranylcypromine or paroxetine for 21 days. It is suggested that the tonic enhanced activation of postsynaptic 5-HT_{1A} receptors observed after the long-term treatment with paroxetine is accounted for by higher levels of 5-HT in the CA_3 region of the hippocampus, as a result of the recovery of the firing rate of 5-HT neurons associated with the blockade of 5-HT reuptake (Besson et al. 1997; Figure 2B). This assumption is consistent with previous studies showing increased extracellular concentrations of 5-HT in terminal brain areas (frontal cortex, hypothalamus) after long-term SSRI treatment (Bel and Artigas 1992; Rutter et al. 1994), resulting from the desensitization of 5-HT_{1A} and 5-HT_{1B} autoreceptors (Chaput et al. 1986, 1991; Rutter et al. 1994). The enhanced tonic activation of postsynaptic 5-HT_{1A} receptors observed after the long-term treatment with tranylcypromine can also be explained by increased levels of 5-HT in the CA_3 hippocampus resulting, as for other MAOIs, from the recovery of the firing rate of 5-HT neurons associated with the desensitization of α_2-adrenergic heteroreceptors located on the terminals of 5-HT neurons (Blier et al. 1986; Ferrer and Artigas 1994; Mongeau et al. 1994). Finally, it is suggested that the enhanced tonic activation of postsynaptic 5-HT_{1A} receptors observed after the long-term imipramine treatment occurred presumably through a sensitization of the postsynaptic 5-HT_{1A} receptors (de Montigny and Aghajanian 1978; Figure 2A). On the other hand, no disinhibition was detectable in rats treated for 3 weeks with the neuroleptic chlorpromazine or in rats receiving only one ECS, two treatment modalities devoid of antidepressant effect (Haddjeri et al. 1998a). However, in the dorsal hippocampus, 5-HT has been also shown to increase neuronal excitability via non-5-HT_{1A} receptors such as 5-HT_2/5-HT_4/5-HT_7 subtypes (Beck 1992; Torres et al. 1996; Beck and Bacon 1998). That 5-HT_{1A} receptors mediate the disinhibition induced by WAY 100635 is further supported by the observation that the inactivation of the $G_{i/o}$ proteins by pertussis toxin prevented the disinhibition in rats treated with repeated ECS (Haddjeri et al. 1998a). However, 5-HT receptors other than those of the 5-HT_{1A} subtype may also be involved in the antidepressant response. Indeed, it has been demonstrated that some postsynaptic 5-HT receptors, other than the 5-HT_{1A} subtype, become sensitized following long-term antidepressant treatments. For example, repeated TCA administration sensitizes postsynaptic 5-HT_2 receptors in the facial motor nucleus and a yet uncharacterized 5-HT receptor subtype in the amygdala (Menkes et al. 1980; Wang and Aghajanian 1980).

Although the mechanism of action of lithium is not yet fully defined, there is, however, a growing body of

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Reprints of Articles

ELSEVIER

Effects of the Selective Norepinephrine Reuptake Inhibitor Reboxetine on Norepinephrine and Serotonin Transmission in the Rat Hippocampus

Steven T. Szabo, B.Sc. and Pierre Blier, M.D., Ph.D.

Given that norepinephrine (NE) and serotonin (5-HT) neurons are implicated in the mechanisms of action of antidepressant drugs and both project to the hippocampus, the impact of acute and long-term administration of the selective NE inhibitor reboxetine was assessed on CA_3 pyramidal neuron firing in this postsynaptic structure. Cumulative injections of reboxetine (1–4 mg/kg, i.v.) dose-dependently increased the recovery time of the firing of these neurons following iontophoretic applications of NE, but not 5-HT. In rats treated with reboxetine for 2.5 mg/kg/day for 21 days, a robust increase in the recovery time following NE applications was observed, and a small but significant prolongation occurred following 5-HT applications. In controls and reboxetine-treated rats, 1 and 5 Hz stimulations of the afferent %-HT bundle to the hippocampus, which allows determination of terminal 5-HT_{1B} autoreceptor sensitivity, produced similar frequency-dependent decreases in pyramidal neuron firing in both groups. However, after low and high doses of clonidine (10 and 400 μg/kg, i.v.), which assesses α_2-adrenergic auto- and heteroreceptor sensitivity, respectively, only the effect of the high dose of clonidine was attenuated. Interestingly, administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 induced a 140% increase in basal pyramidal neuron firing in reboxetine as compared to saline-treated rats. This increase in tonic activation of postsynaptic 5-HT_{1A} receptors might be attributable in part to a desensitization of α_2-adrenergic heteroreceptors, presumably resulting from sustained NE reuptake inhibition. These results indicate that even a selective NE reuptake inhibitor can modulate 5-HT transmission.
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KEY WORDS: *Antidepressants; α_2-adrenoceptors; Clonidine; 5-HT_{1A} receptors; 5-HT_{1B} receptors; Locus coeruleus*

The major classes of antidepressant drugs, including the tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRIs), modify serotonin (5-HT) and/or norepinephrine (NE) neurotransmission through which they likely exert their therapeutic effects in anxiety and affective disorders (see Blier and de Montigny et al. 1999). It has been postulated that antidepressant drugs selective for either the 5-HT or NE system act via independent mechanisms. Furthermore, these "selective" drugs display side effect profiles indicative of their neurotransmitter specificity. This, however, does not preclude that antidepressant agents specific for one

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monoaminergic transporter, or receptor subtype, may exert their therapeutic action through interactions between the 5-HT and NE systems. For example, SSRIs induce a gradual decrease in the spontaneous firing activity of NE neurons after long-term administration (Szabo et al. 1999, 2000), via a complex neuronal circuitry (Szabo and Blier 2000a), which may contribute to their beneficial and/or side effects depending on the symptomatic profile of the patients (Blier 2000). On the other hand, the selective NE reuptake inhibitor desipramine increases the synaptic availability of NE but also alters 5-HT parameters after long-term administration, such as enhancing extracellular 5-HT concentrations and the responsiveness of 5-HT receptors in postsynaptic structures (de Montigny and Aghajanian 1978; Wang and Aghajanian 1980; Menkes et al. 1981; Yoshioka et al. 1995). This enhanced synaptic availability of NE may be due to a decreased sensitivity of α_2-adrenergic heteroreceptors located on 5-HT terminals that normally induce a negative feedback regulation on 5-HT release (Mongeau et al. 1993; Yoshioka et al. 1995). Interestingly, all TCA drugs, independent of their capacity to inhibit the reuptake of 5-HT and/or NE, progressively enhance the responsiveness of postsynaptic 5-HT_{1a} receptors with a time-course congruent to the delayed onset of action of these drugs in major depression (de Montigny and Aghajanian 1978; Heninger et al. 1984; Chaput et al. 1991). Due to the lack of effective antidepressant drugs selective for the NE transporter not belonging to the TCA family, it has been difficult to assess whether the effects of desipramine on 5-HT transmission is attributable to its TCA moiety (because at least one of these drugs does not block NE reuptake) or to NE blockade *per se*.

Reboxetine is not a TCA and it is a selective NE reuptake inhibitor. It is currently the only antidepressant agent of its kind in clinical use in Europe. Given that NE and 5-HT monoaminergic brainstem nuclei project to the hippocampus, the impact of acute and long-term administration of reboxetine was assessed on CA_3 pyramidal neuron firing in this brain region generally thought to be implicated in at least some aspects of depression. It is not currently known whether atrophy in the hippocampus trophy represent a depressive state or trait (Sheline et al. 1999; Bremner et al. 2000), however antidepressant treatments have been shown to induce adaptive changes in this structure (see Malberg et al. 2000 for review). The effect of reboxetine on NE transmission in this manuscript was not directly assessed as Sacchetti et al. (1999) already concluded that acute and sustained treatment with reboxetine leads to similar increases in extracellular levels of NE without producing any adaptive changes in α_2-autoreceptor sensitivity in the hippocampus. However, given the importance of receptors on 5-HT terminals in the hippocampus, which become altered after long-term antidepressant treatment, α-adrenergic heteroreceptor and 5-HT_{1B} autoreceptor function was assessed using electrical stimulation of the afferent 5-HT bundle to this postsynaptic structure.

MATERIALS AND METHODS

Animals and Treatments

The experiments were carried out in male Sprague Dawley rats (Charles River, St. Constant, Québec, Canada) weighing 300–325 g and were kept under standard laboratory conditions (12:12 light-dark cycle with access to food and water *ad libitum*, at a room temperature of 21 ± 2°C). Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and mounted in a stereotaxic apparatus (David Kopf Instruments). Supplemental doses (100 mg/kg, i.p.) were given to prevent any nociceptive reaction to pinching of the hind paw. Body temperature was maintained at 37°C throughout the experiments utilizing a thermistor-controlled heating pad (Seabrook Medical Instruments, Inc.). Prior to electrophysiological recording, a catheter was inserted in a lateral tail vein for systemic i.v. injection of drugs. All experiments were performed in compliance with NIH guidelines and the Canadian Council on Animal Care.

In sustained treatment regimens, rats were anesthetized with halothane containing a 2:1 O_2/N_2O mixture for subcutaneous implantation of osmotic Alzet 2ML4 minipumps (ALZA, Palo Alto, CA). The rats were tested with the minipumps in place in order to mimic the clinical condition whereby patients present an antidepressant response while taking their medication. Rats were treated with reboxetine (2.5 mg/kg/day) or the saline vehicle for 21 days delivered by osmotic minipumps. This dose was chosen because it produced a similar degree of attenuation of LC neuronal firing after a two-day treatment to that obtained with regimens of desipramine and MAOIs examined in long-term studies (Szabo et al.<Q3>; Blier and de Montigny 1985).

Recording from Dorsal Hippocampus CA_3 Pyramidal Neurons

Extracellular unitary recordings and microiontophoresis of drugs onto pyramidal neurons in the CA_3 region of the dorsal hippocampus were conducted with five-barreled micropipettes, pulled conventionally with the tips broken to a diameter of 9 to 12 μm under a microscopic control. The central barrel, used for recording, was filled with a 2 M NaCl solution. The side barrels contained the following solutions: 5-HT creatinine sulfate (5 mM in 200 mM NaCl, pH 4), NE bitartrate (20 mM in 200 mM NaCl, pH 4), quisqualate (1.5 mM in 200 mM NaCl, pH8), and a 2 M NaCl solution used for automatic current balancing. All drug solutions were in-

100,635 would be a reflection of the action of the antagonist at postsynaptic 5-HT_{1A} receptors. Thus, given that WAY 100,635 antagonizes the action of exogenous 5-HT at postsynaptic 5-HT_{1A} receptors, CA_3 pyramidal neuron activity would be a direct measure of the tonic level of activation of these receptors by extracellular 5-HT. Prior to the intravenous administration of four successive 25-μg/kg doses of WAY 100,635, the firing activity of the quisqualate-activated CA_3 pyramidal neurons was decreased to about 5 Hz in order to more readily allow the detection of enhancements in firing following administration of the antagonist in control and treated rats. After a steady baseline firing activity was established, an injection of saline always preceded the WAY 100,635 injections.

Drugs

The following drugs were used: reboxetine (Pharmacia UpJohn, Kalamazoo, MI, USA); clonidine and WAY 100,635 (RBI, Natick, MA, U. S. A.); 5-HT creatinine sulfate, NE bitartrate, and quisqualate were purchased from Sigma Chemical (St. Louis, MO, USA). The concentrations and the doses used for these compounds were chosen on the basis of previous successful experiments carried out in our laboratory and others. Drugs administered i.v. were all dissolved in distilled water and injected in a volume of less than 0.2 ml.

Statistical Analysis

Results were expressed as means ± SEM. The n refers to the number of neurons tested in the figures, however, in the results section n corresponds to the number of rats tested. The statistical significant difference between the means of iontophoretic application of NE and 5-HT on CA_3 pyramidal neurons firing activity to intravenous reboxetine injections and long-term reboxetine administration of RT_{50} values as well as the number of spikes suppressed was assessed with 2-way analysis of variance (ANOVA). The difference between the effects of 1 and 5 Hz stimulation frequencies of the 5-HT pathway on the duration of suppression of firing of CA_3 pyramidal neurons was assessed with the paired Student's *t*-test. Possible differences in the magnitude of the effects of 1 Hz electrical stimulation frequencies of the 5-HT pathway to intravenous clonidine injections in control and 21-day reboxetine-treated rats were assessed via 1-way ANOVA. Lastly, the effects of cumulative injections of reboxetine in control rats, and incremental doses of WAY 100,635 injections on the firing activity of CA_3 pyramidal neurons in control and 21-day reboxetine-treated rats were assessed using the 1-way repeated measures ANOVA. Post-hoc pairwise multiple comparison procedures were performed with the Tukey test and the Student-Newman-Keuls method for 1-way and 2-way ANOVAs, respectively.

RESULTS

Effects of Acute Reboxetine Injections on the Response of CA_3 Pyramidal Neurons to Microiontophoretic Applications of NE and 5-HT

The average number of spikes suppressed from baseline firing activity of hippocampus neurons during microiontophoretic applications of NE and 5-HT provides a reliable index of the sensitivity of postsynaptic α_2-adrenergic and 5-HT_{1A} receptors, respectively (Curet and de Montigny 1988a; Rueter et al. 1998). These values generated from microiontophoretic applications of NE and 5-HT on the firing activity of CA_3 pyramidal neurons was significant ($F_{2,122} = 15.4$ and $F_{2,120} = 11.3$, respectively; $p < .001$ for both monoamines) and current-dependent ($p < .05$ for both monoamines), meaning that increasing ejection currents enhanced the number of spikes suppressed following NE and 5-HT ejections from the micropipette, examples of which are provided in Figure 1. The recovery time necessary for pyramidal neurons to regain 50% of their firing rate (RT_{50} value) after microiontophoretic ejections of NE and 5-HT provides an index of the function of the reuptake transporters for these monoamines (de Montigny et al. 1980; Piñeyro et al. 1994). The RT_{50} values generated from microiontophoretic applications of NE and 5-HT on the firing activity of CA_3 pyramidal neurons were significantly prolonged ($F_{2,122} = 35.0$ and $F_{2,120} = 27.1$, respectively; $p < .001$ for both monoamines) and also current-dependent ($p < .05$ for both monoamines), examples of which are also provided in Figure 1.

Cumulative doses of 1,2 and 4 mg/kg of reboxetine were i.v. injected in succession and reduced the firing activity of CA_3 pyramidal neurons to 15%, 13%, and 23%, respectively, but did not significantly differ from baseline or each other ($F_{3,12} = 1.6$, $p = .248$; n = 4 rats), similar to desipramine (Curet et al. 1992). Reboxetine injections did not significantly influence the number of spikes suppressed by microiontophoretic applications of NE ($F_{3,121} = 0.1$, $p = <$**Q4**>1.0) and 5-HT ($F_{3,119} = 0.3$, $p = .8$). There was no statistically significant interaction between the different doses of reboxetine injected and current of NE and 5-HT ejected on the number of spikes suppressed ($F_{6,118} = 0.1$, $p = .990$ and $F_{6,116} = 0.4$, $p = .87$, respectively). In contrast, 1, 2, and 4 mg/kg injections of reboxetine prolonged the RT_{50} values across all of the currents employed on CA_3 pyramidal neurons firing for NE current ejections as compared to controls ($F_{3,122} = 58.4$, $p < .001$;, Figure 2), but not to that of 5-HT ($F_{3,119}$ 1.7, $p = .176$; Figure 2). The effect of reboxetine on

jected as cations and retained with a −10nA current between injections. Pyramidal neurons were identified by their large amplitude (0.5 mV to 1.2 mV) and long-duration (0.8 msec to 1.2 msec) simple spike alternating with complex spike discharges (Kandel and Spencer 1961). These characteristics readily allow the on-line differentiation of pyramidal neurons from interneurons. Since most hippocampus pyramidal neurons are not spontaneously active under chloral hydrate anesthesia, small ejection currents of quisqualate (0 to 5nA) were used to activate them within their physiological firing rate 8–15 Hz; (Ranck 1975). Furthermore, the level of cellular activation of the pyramidal neurons does not alter the estimates of neuronal responsiveness (Brunel and de Montigny 1987). To evaluate the effectiveness of reboxetine on the blockade of NE and 5-HT transporter reuptake, the recovery of the firing activity of pyramidal neurons following the microiontophoretic application of NE and 5-HT was assessed using the recovery time 50 (RT_{50}) value. The RT_{50} value is defined as the time in seconds required by the neurons to recover 50% of the initial firing frequency from termination of microiontophoretic application (de Montigny et al. 1980). The RT_{50} value has also been shown to be a reproducible measure and a reliable index of the *in vivo* activity of the 5-HT and NE reuptake process which is independent from postsynaptic neuronal responsiveness (de Montigny et al. 1980; Piñeyro et al. 1994). The neuronal responsiveness to microiontophoretic applications of NE and 5-HT was assessed and expressed as the number of spikes suppressed. This approach avoids an interference of the recovery of firing which is largely dependent on the activity of the reuptake transporters (Chaput et al. 1986). The sensitivity of neurons to NE or 5-HT was evaluated by counting the number of spikes suppressed during drug ejections.

Simulation of the 5-HT pathway

To activate the 5-HT projections originating from the dorsal and median raphe to the dorsal hippocampus (Hensler et al. 1994), a bipolar electrode (NE-100; David Kopf, Tujunga, CA) was implanted on the midline with a 10° backward angle in the ventromedial tegmentum, 1 mm anterior to lambda, and 8.3 mm below the cortical surface. A stimulator (S8800; Grass Instrument, Quincy, Mass; USA) delivered 200 square pulses of 0.5 ms at a frequency of 1 or 5 Hz at an intensity of 300 μA. The duration of suppression of pyramidal neurons firing activity produced by stimulation was measured on-line using an oscilloscope with memory (1201B; Hewlett Packard; Palo Alto, CA). The effect of the electrical stimulation of the ascending 5-HT pathway is due to the release of 5-HT into the synaptic cleft (Blier and de Montigny 1983, 1985; Chaput et al. 1986). In order to determine the function of the terminal 5-HT autoreceptors, two series of stimulations (1 and 5 Hz) were carried out, while recording the same neurons. Since it has been previously demonstrated that the activation of the terminal 5-HT autoreceptors decreases the release of 5-HT, thus increasing the frequency of stimulation from 1 to 5 Hz results in a greater activation of terminal 5-HT autoreceptors (Blier et al. 1989; Göthert et al. 1980). Also, the effect of 1 Hz stimulations was determined while recording from the same neurons before and after the successive intravenous injection of a low dose (10 μg/kg) and high dose (400 μg/kg) of clonidine. The effects of the electrical stimulation on CA_3 pyramidal neuron firing rate following low and high doses of clonidine allows for the assessment of the sensitivity of α_2-adrenergic auto- and heteroreceptors, respectively (Mongeau et al. 1994a). This is supported by previous experiments showing that in rats pretreated with the NE neurotoxin 6-hydroxydopamine, the inhibitory effects of the high dose of clonidine was abolished, but the enhancing action of the low dose did not change (Mongeau et al. 1993). Furthermore, prolonged treatment with the monoamine oxidase inhibitor befloxatone selectively attenuates the effect of the high dose of clonidine in intact rats but not in NE-lesion rats (Mongeau et al. 1994a).

Tonic Activation of 5-HT_{1A} Receptors on Dorsal Hippocampus CA_3 Pyramidal Neurons

The selective 5-HT_{1A} receptor antagonist WAY 100,635 (Fletcher et al. 1996) was used to assess the degree of 5-HT_{1A} receptor-mediated inhibition of CA_3 pyramidal neurons induced by the sustained administration of reboxetine for 21 days. The degree to which the antagonists could disinhibit the firing of hippocampal neurons has been determined to be a measure of the tonic activation of postsynaptic 5-HT_{1A} receptors (Haddjeri et al. 1998). In reboxetine-treated rats, if an increase in extracellular levels of 5-HT in the raphe region were present, WAY 100,635 would restore 5-HT neuron firing activity. However, this is probably not the case as it was previously documented that the firing activity of dorsal raphe 5-HT neurons, and the responsiveness of 5-HT_{1A} autoreceptors controlling these neurons, is not altered after a prolonged reboxetine administration (Szabo and Blier 2000). Nevertheless, because WAY 100,635 was given systemically, it would simultaneously be blocking the effects of 5-HT on postsynaptic neurons, thereby canceling out the effect of WAY 100,635 on the somatondendritic autoreceptors. Indeed, if the action of the antagonist at the somatodendritic 5-HT_{1A} autoreceptors were influencing the activity of hippocampus neurons, it would serve to further inhibit their firing rate due to an increased release of 5-HT into the target area. Therefore, it was assumed that any increases in firing observed during the administration of WAY

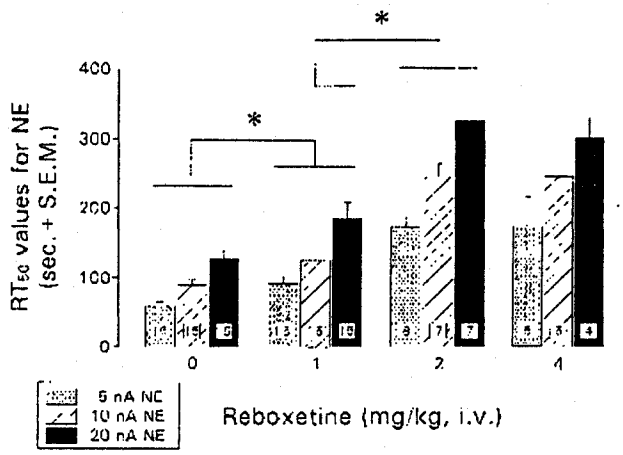

Figure 2. Histograms representing the recovery times (RT_{50}) of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of NE with varying currents in control rats receiving cumulative i.v. injections of reboxetine. The number of neurons tested is given at the bottom of each column. $^*P < 0.001$ when comparing microiontophoretic NE applications using 2-way ANOVA with Student-Newman-Keuls method.

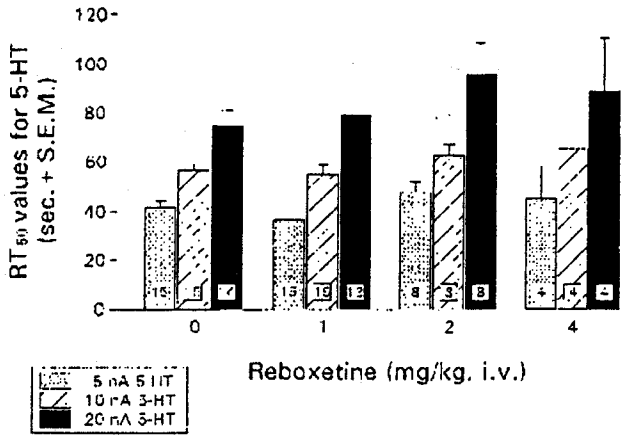

Figure 3. Histograms representing recovery time (RT_{50}) of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of 5-HT with varying currents in control rats receiving cumulative injections of reboxetine. The number of neurons tested is given at the bottom of each column. Note that reboxetine administration did not alter the current-dependent increases in the RT_{50} value as opposed to that for NE illustrated in Figure 2.

on the current used ($F_{2,152} = 2.6$, $p = .79$ and $F_{2,159} = 0.5$, $p = .59$, respectively).

Effect of Long-Term Reboxetine Treatment on the Effectiveness of Electrical Stimulation of the Afferent 5-HT Fibers to the Hippocampus

The net effect of long-term reboxetine treatment on 5-HT transmission was determined by stimulating the ascending 5-HT pathway at a frequency (1 Hz) similar to the spontaneous firing rate of 5-HT neurons (Vandermaelen and Aghajanian 1983). A brief suppression of firing of CA_3 neurons results from electrical stimulation of the 5-HT pathway due to the release of 5-HT mediated through postsynaptic 5-HT_{1A} receptors (Chaput et al. 1986; Chaput and de Montigny et al. 1988). The effectiveness of the electrical stimulation of the ascending 5-HT fibers at the level of the ventromedial tegmentum on the firing activity of the postsynaptic hippocampal CA_3 pyramidal neurons at a frequency of 1 Hz did not differ ($p = .74$) in control and reboxetine-treated rats (Figure 6). To assess the function of the terminal 5-HT_{1B} autoreceptors which control the amount of 5-HT released for each electrical impulse reaching 5-HT terminals, the ascending 5-HT pathway was subsequently stimulated at a frequency of 5 Hz while recording the firing activity of the same hippocampus CA_3 pyramidal neurons. In controls and reboxetine-treated rats, increasing the frequency of stimulation from 1 to 5 Hz induced the same 26% reduction of the duration of suppression of firing ($t_{17} = 11.87$ and $t_9 = 13.95$, respectively, $p < .001$ for both groups; Figure 6). Thus, at a frequency of 5 Hz, the effectiveness of the electrical stimulations in reboxetine-treated rats was not different from that of the controls ($p = .42$; Figure 6).

Effects of Long-Term Treatment with Reboxetine on the Sensitivity of α_2-Adrenergic Auto- and Heteroreceptors

Previous studies have shown that antidepressant treatments which increase the concentration of NE in the synaptic cleft desensitize the terminal α_2-adrenergic heteroreceptors located on 5-HT fibers in the dorsal hippocampus after long-term administration (Mongeau et al. 1994a,b; Yoshioka et al. 1995), however, leaving α_2-adrenergic autoreceptors normosensitive (Szabo and Blier 2000b; Sacchetti et al. 1999; Mateo et al. 1998; Mongeau et al. 1994a; Moret and Briley 1994). In control (n = 9 rats) and 21-day reboxetine (2.5 mg/kg/day; n = 7) treated rats, 1 Hz electrical stimulations of the ascending 5-HT pathway on the firing activity of dorsal hippocampus CA_3 pyramidal neurons produced similar suppressions of firing which did not statistically differ when compared to each other ($p = .27$; Figure 7). In addition, a low dose of clonidine (10 μg/kg), which accesses the sensitivity of terminal α_2-adrenergic autoreceptors, was able to significantly increase the effectiveness of a 1 Hz stimulation to a similar degree in control (34%) and reboxetine-treated (30%) rats ($p < .001$ for both; Figure 7). In contrast, a high dose of clonidine (400 μg/kg), which assesses the sensitivity of terminal α_2-adrenergic heteroreceptors, significantly reduced the effectiveness of 1 Hz electrical stimulations as compared to the stimulations without clonidine injection in controls (25%), but

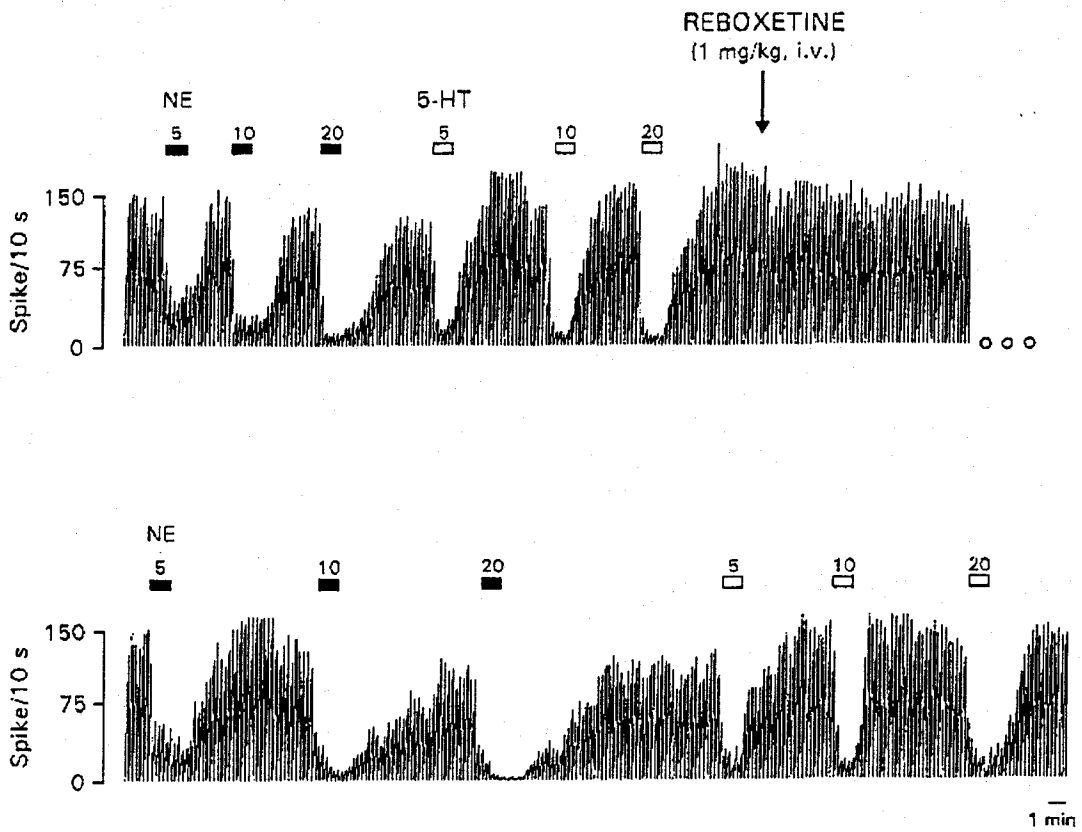

Figure 1. Integrated firing rate histograms illustrating the response of a CA_3 pyramidal neuron to microiontophoretic application of NE and 5-HT in a control rat before and after a reboxetine injection. The three circles at the bottom of the upper histogram indicate that it continues below. This neuron was activated with a quisqualate ejection current of −3 nA. Note that when compared to microiontophoretic drug application before reboxetine injection, the durations of CA_3 pyramidal neuron suppression with applications of NE are markedly increased and those for 5-HT are unchanged.

this parameter of NE was dose dependent and reached a plateau at the cumulative dose of 2 mg/kg ($p < .05$; Figure 3). Importantly, the lack of effect observed for 5-HT across reboxetine injections was unrelated to the current used ($F_{6,116} = 0.2$, $p = .97$).

Effect of 21-day Reboxetine Administration on the Response of CA_3 Pyramidal Neurons to Microiontophoretic Applications of NE and 5-HT

In reboxetine-treated rats, a significant number of spikes were suppressed for microiontophoretic application of NE and 5-HT ($F_{2,98} = 22.8$ and $F_{2,109} = 9.1$, respectively; $p < .001$ for both monoamines) on CA_3 pyramidal neurons. The effect of NE and 5-HT ejections on the number of spikes suppressed was current-dependent ($p < .05$) for both monoamines); however, it did not differ among controls and treated rats ($F_{1,99} = 3.2$, $p = .08$ and $F_{1,110} = 0.4$, $p = .55$, respectively; Figure 4). This lack of difference observed in reboxetine-treated rats as compared to controls on the number of spikes suppressed from NE and 5-HT current ejections were not due to the effects of reboxetine treatment ($F_{2,98} = 0.5$, $p = .95$ and $F_{2,109} = 0.9$ $p = .41$, respectively). Similarly, the RT_{50} value for NE and 5-HT applications on CA_3 neurons in reboxetine-treated rats also increased in a current-dependent manner (P 0.05 for both monoamines; Figure 5, Panel A and Panel B, respectively). In contrast to the number of spikes suppressed obtained for 5-HT ejections, where no difference was detected regardless of drug treatment, an enhancement resulted in the RT_{50} values to microiontophoretic applications of NE and 5-HT in reboxetine-treated rats on the firing activity of CA_3 neurons as compared to controls ($F_{2,152} = 79.7$ and $F_{2,158} = 58.7$, respectively, $p = .001$ for both monoamines). Furthermore, this difference was also present in the subset of neurons for which all currents were used: the RT_{50} values for NE and 5-HT were almost exactly the same and still significantly different from the control values ($F_{2,152} = 35.8$ and $F_{2,92} = 19.4$, respectively, $p = .001$). This prolongation of the effect of 5-HT ejections, as indicated by an enhanced RT_{50} value on CA_3 neuron firing, was much less robust than that observed for NE (Figure 5, Panels A and B), but was nevertheless statistically significant ($F_{1,159} = 46.8$, $p < .001$). In addition, this effect of NE or 5-HT on the RT_{50} value did not depend

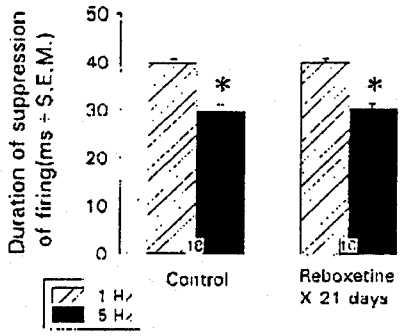

Figure 6. Effects of 1 and 5 Hz electrical stimulations of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons in 9 control and 8 reboxetine (2.5 mg/kg/day × 21 days) treated rates. $^*p <$ .001 using the paired Student's t test.

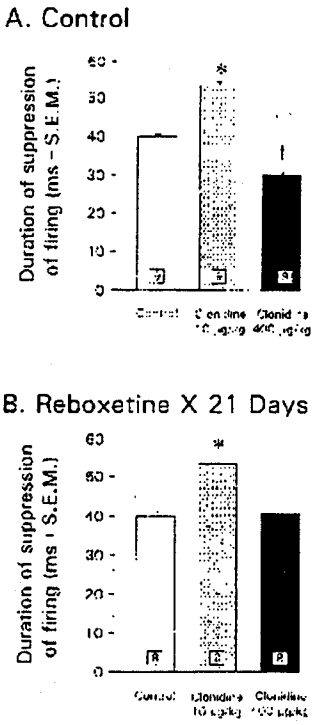

Figure 7. Histograms representing the effect of systemic injections of clonidine on the efficacy of the electrical stimulations of the ascending 5-HT pathway in suppressing the firing activity of dorsal hippocampus CA_3 pyramidal neurons in control and reboxetine-treated rats. *Corresponds to an enhancement and † to a decrease as compared to controls at a $p < .001$ significance level using a 1-way ANOVA with Tukey test.

NE is prolonged more than 2-fold (Figures 1 and 3). This specific effect of reboxetine on the RT_{50} values to application of NE and 5-HT on CA_3 pyramidal neuron firing is similar to results obtained with the TCA desipramine (Lacroix et al. 1991). These findings are also consistent with the results of microdialysis studies showing that acute reboxetine administration (15 mg/kg, i.p.) produces an increased level of NE in the dialysates in the frontal cortex and dorsal hippocampus without altering that of 5-HT in the striatum (Sacchetti et al. 1999). When considered together with biochemical binding data on the transporter (Wong et. al. 1999), such results confirm that acutely administered reboxetine selectively blocks the NE reuptake transporter *in vivo* without altering the function of the 5-HT reuptake transporter.

The potency of selective NE reuptake inhibitors or dual NE/5-HT reuptake inhibitors to suppress the firing activity of LC NE neurons by 50% (ED_{50} value) correlate well with the dose required to produce a maximal prolongation of the RT_{50} value for NE application on CA_3 pyramidal neuron firing. When comparing such ED_{50} and RT_{50} values for reboxetine (Szabo and Blier 2000b) to those previously obtained with desipramine (Béïque et al. 1998; Lacroix et al. 1991), a discrepancy between the potency of these two selective NE reuptake inhibitors is apparent in the hippocampus as compared to the LC. The ED_{50} value to suppress the firing activity of LC NE neurons for desipramine (0.24 ± 0.01 mg/kg; Béïque et al. 1998) is half that reported for reboxetine (0.48 ± 0.01 mg/kg; Szabo and Blier 2000b), whereas the effect of reboxetine to prolong the RT_{50} value to NE applications on CA_3 pyramidal neuron firing was three times greater than that of DMI: it reached a plateau at a cumulative dose of only 2 mg/kg (Figure 2) while that of desipramine produced a maximal effect after 6 mg/kg (Lacroix et al. 1991). In addition, reboxetine and duloxetine (a dual NE/5-HT reuptake inhibitor) possess nearly identical ED_{50} values on LC firing suppression, but reboxetine is five times more potent in prolonging the RT_{50} value to applications of NE on CA_3 pyramidal neurons as compared to duloxetine (Figure 2; Kasamo et al. 1996). These results indicate that reboxetine appears to be more potent on the NE reuptake process in the hippocampus than in the LC. The exact basis for this difference remains to be elucidated.

The sensitivity of postsynaptic α_2-adrenergicic and 5-HT_{1A} receptors was not altered in the hippocampus after long-term reboxetine administration, as indicated by the number of spikes suppressed from microiontophoretic applications of NE and 5-HT on CA_3 pyramidal neuron firing being similar (Figure 4). In contrast, it had been reported that 5-HT_{1A} receptors become supersensitive in postsynaptic structures to prolonged TCA treatment (de Montigny and Aghajanian 1978; <Q1>Menkes et al. 1980; Wang and Aghajanian 1980). Also, TCA drugs had been shown to alter adrenoceptor function, particularly to sensitize α_1-adrenoceptor in the facial motor nucleus and the lateral geniculate body and α_2-adrenoceptors in the amygdala after long-term treatment (Wang and Aghajanian 1980; Menkes et al. 1981; Menkes et al. 1983; Freedman and Aghajanian 1985). Prior to the advent of reboxetine, the lack of se-

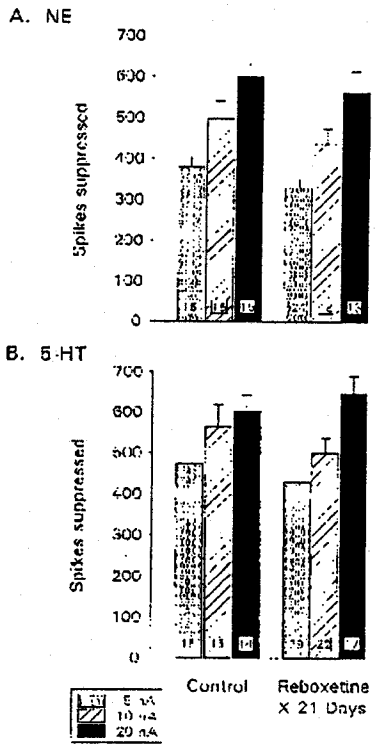

Figure 4. Histograms representing the number of spikes suppressed of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of NE (Panel A) and 5-HT (Panel B) with varying currents in control and 21-day reboxetine (2.5 mg/kg/day) treated rats. The number of neurons tested is given at the bottom of each column. All of the currents differed from each other on the number of spikes suppressed ($p < .05$); however, they did not differ when compared across treatment groups using the 2-way ANOVA with Student-Newman-Keuls method.

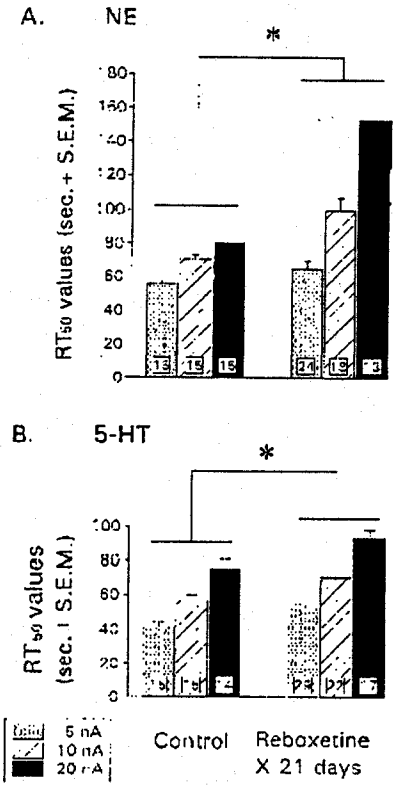

Figure 5. Histograms representing recovery times (RT_{50}) of dorsal hippocampus CA_3 pyramidal neurons from microiontophoretic applications of NE (Panel A) and 5-HT (Panel B) with varying currents in control and 21-day reboxetine (2.5 mg/kg/day) treated rats. The number of neurons tested is given at the bottom of each column. $^*p < .001$ using 2-way ANOVA with Student-Newman-Keuls method.

not in 21-day reboxetine-treated rats ($p < .001$ and $p = .79$, respectively; Figure 7).

Effect of Long-Term Treatment with Reboxetine on the Tonic Activation of 5-HT_{1A} Receptors Using WAY 100,635

Due to a recent finding that all major classes of antidepressants induce a tonic activation of postsynaptic 5-HT_{1A} receptors in the hippocampus (Haddjeri et al. 1998), the possibility that reboxetine could also influence this parameter was examined. Dorsal hippocampus CA_3 pyramidal neurons were activated by applying a small ejection current of quisqualate in controls and long-term reboxetine-treated rats (n = 5 rats for both groups); an example of each is provided in Figure 8. In control rats receiving saline for 21 days, the i.v. administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 (four successive doses of 25 μg/kg) did not modify the firing activity of dorsal hippocampus CA_3 pyramidal neurons ($p = .08$; Figure 8, Panel A). In contrast to rats receiving reboxetine for 21 days, i.v. administration of the second dose and subsequent doses of WAY 100,635 was able to induce a marked enhancement of the firing activity of CA_3 pyramidal neurons ($p < .001$ for all doses except the first; Figure 8, Panel B and Figure 9). The effect of a final injection of WAY 100,635 (cumulative dose of 100 μg/kg) increased the firing activity of CA_3 pyramidal neurons by 140% above the basal firing rates (Figure 9).

DISCUSSION

The electrophysiological results presented herein indicate that acute injections of reboxetine prolonged the duration of NE in the hippocampus via blockade of the NE transporter, as measured by a greater RT_{50} value for NE applications onto CA_3 pyramidal neurons (Figures 1 and 2). In contrast, reboxetine injections did not alter this parameter for 5-HT applications on the same neurons, demonstrating that acute injections of reboxetine do not impact upon the function of the 5-HT transporter in this postsynaptic structure when the effect of

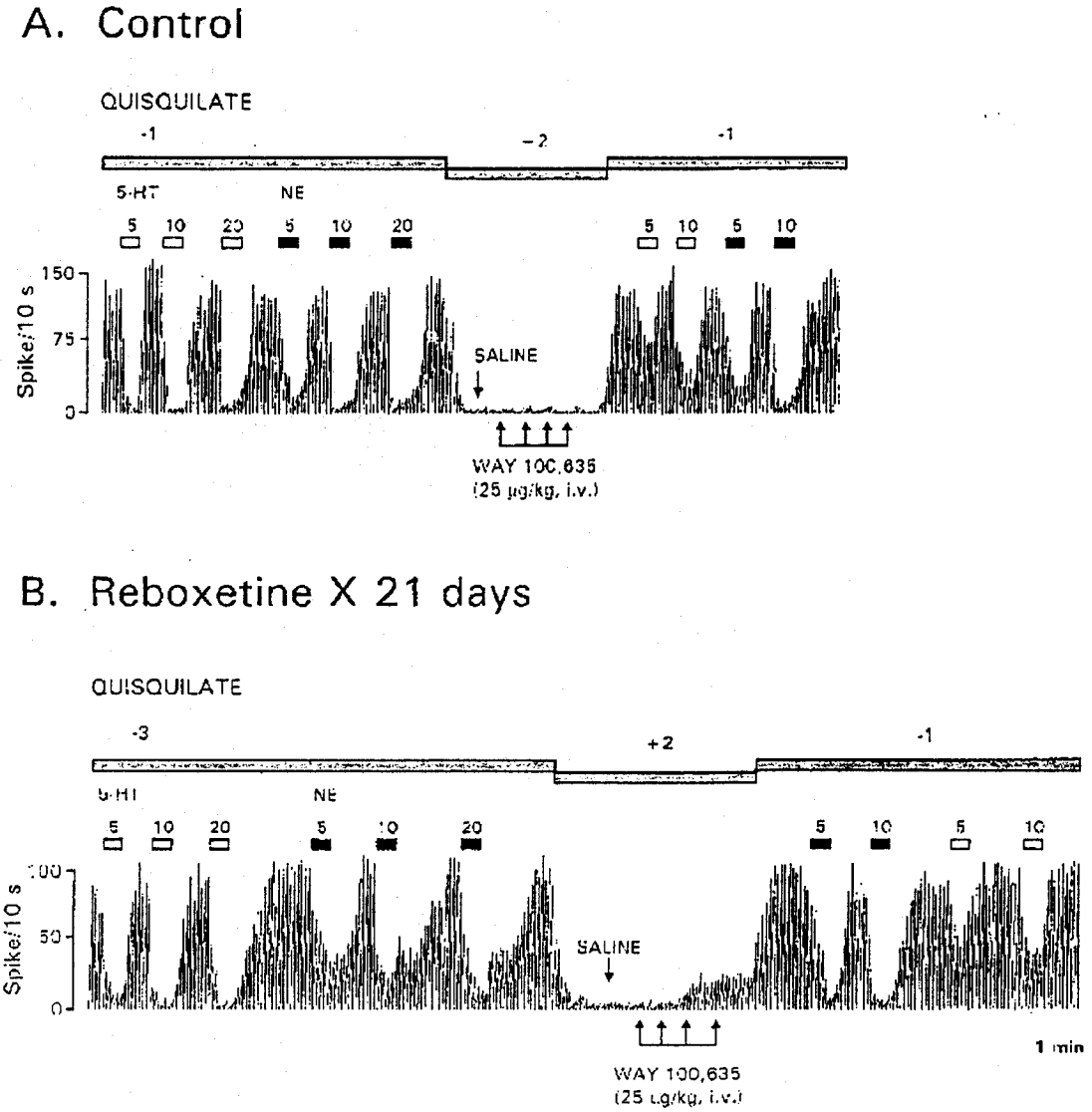

Figure 8. Integrated firing rate histogram of a dorsal hippocampus CA_3 pyramidal neuron, showing its responsiveness to microiontophoretic application of 5-HT before and after the intravenous injection of WAY 100,635 (25 µ/kg × 4) in a control (Panel A) and 21-day reboxetine (2.5 mg/kg/day) treated rat (Panel B). Horizontal bars indicate the duration of the applications (current given in nA). Note the altered effectiveness of 5-HT to suppress firing activity after administration of WAY 100,635 (100 µg/kg) in both histograms. Although the RT_{50} values to NE after WAY 100,635 in 21-day reboxetine rats are reduced, two other rats had slightly prolonged RT_{50} values.

lective NE reuptake blocking drugs used clinically without a TCA moiety made it impossible to rule out structural versus NE components of such drugs in delineating the clinical impact of these properties. The present results on reboxetine are thus similar to those reported by Lacroix et al. (1991), indicating that postsynaptic α_2 adrenoceptors remained normosensitive in the hippocampus after long-term desipramine (10 mg/kg/day) treatment, but stand in opposition to an increased 5-HT_{1A} receptors sensitivity (de Montigny and Aghajanian 1978). In contrast, both reboxetine and desipramine have been shown to reduce the sensitivity of 5-HT_{1A} receptors that control the firing activity of rat LC neurons after long-term treatment (Szabo and Blier 2000b). Yet, reboxetine but not desipramine attenuates the 5-HT_{2A}-receptor mediated inhibition of LC neurons firing (Szabo and Blier 2000b). These observations therefore clearly highlight that "similar" receptor subtypes located in various areas adapt differently to sustained treatment with antidepressants. Moreover, these differences among these two NE reuptake inhibitors may be due to the presence or absence of a tricyclic structure.

Consistent with the results obtained with acute reboxetine injections, rats treated with reboxetine for 21 days also presented an increase in the RT_{50} value to NE

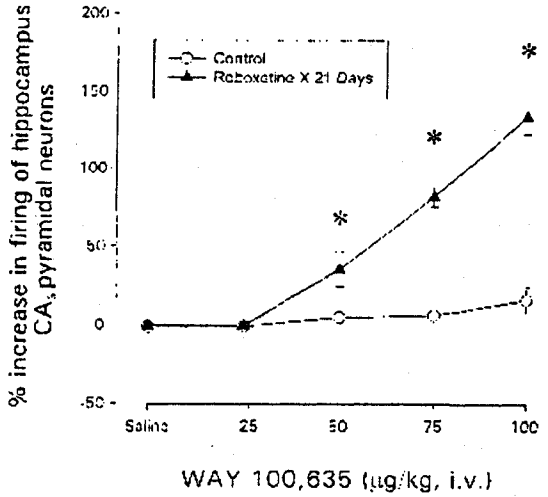

Figure 9. Effects of the intravenous injection of WAY 100,635 (25 μ/kg × 4) on the percent increase in the firing activity of dorsal hippocampus CA_3 pyramidal neurons in controls and 21-day reboxetine-treated rats. $^*p < 0.001$ when compared to controls and each other using 1-way repeated measures ANOVA and Tukey test.

application (Figure 5, Panel A). However, in contrast to results obtained with 5-HT in the acute reboxetine experiments, long-term reboxetine treatment also increased the RT_{50} value for 5-HT (Figure 5, Panel B). This slight increase in the RT_{50} value observed with 5-HT after prolonged administration does not detract from reboxetine being an effective NE reuptake blocker after acute and long-term administration, but it provides evidence that this agent may produce an alteration in 5-HT transporter activity (Figure 5, Panel B). In this regard, studies have shown that NE and 5-HT transporters are both regulated by protein kinase C (Miller and Hoffman 1994; Qian et al. 1997; Apparsundaram et al. 1998). Consequently, this small inhibitory action of reboxetine on 5-HT reuptake actually may result more from intracellular events than from a direct physical inhibitory interaction of reboxetine with the 5-HT reuptake binding sites. This alteration in 5-HT reuptake function may be germane to the observation of a small but significant NE reuptake inhibition produced after a sustained SSRI treatment with paroxetine (Owens et al. 2000). The functional significance of these phenomena, given their small magnitude, are not known and must be put into perspective when considering the impact of these selective agents on antidepressant drug efficacy.

The sensitivity of somatodendritic α_2-adrenergic autoreceptors which control the firing activity of LC NE neurons do not desensitize after a 21-day reboxetine administration in control rats, therefore explaining their sustained attenuation of firing activity (Szabo and Blier 2000b). Similarly, the terminal α_2-adrenergic autoreceptors mediating the release of NE in the hippocampus, remained normosensitive in 21-day reboxetine-treated rats (Figure 7). These results are fully consistent with the reports showing that the effect of clonidine on NE dialysates is unaltered in 14-day reboxetine-treated rats in the hippocampus, but attenuated in the frontal cortex (Invernizzi et al. 2000, 2001). However, drugs which increase the synaptic availability of NE desensitize α_2-adrenergic heteroreceptors located on 5-HT terminals in the hippocampus and may account for an increase in synaptic 5-HT availability in the same structure (Mongeau et al. 1994a; Yoshioka et al. 1995) and other postsynaptic structures (Blier and Bouchard 1994). It was thus decided to assess whether reboxetine desensitized the terminal α_2-adrenergic heteroreceptors located on 5-HT terminals, as previously reported with desipramine (Mongeau et al. 1993; Yoshioka et al. 1995). Reboxetine did desensitize terminal α_2-adrenergic heteroreceptors as determined by the attenuated response to the high dose of clonidine (400 μ/kg) on hippocampus neurons (Figure 7, Panel B). This effect is likely mediated by an increased availability of NE because the MAOI befloxatone could no longer desensitize this heteroreceptor in NE lesioned rats (Mongeau et al. 1994a).

The amount of 5-HT being released per impulse reaching 5-HT terminals was not altered in 21-day reboxetine as compared to saline-treated rats: there was no change in the efficacy of 5-HT fiber stimulation on hippocampus neuron firing as demonstrated in Figure 6. This paradigm assesses terminal 5-HT_{1B} autoreceptor sensitivity located on 5-HT neurons controlling 5-HT release. A change in sensitivity of this receptor subtype is usually indicative of antidepressants that potently block the reuptake of 5-HT (Mongeau et al. 1994a), such as citalopram (Chaput et al. 1991), fluvoxamine (Dong et al. 1991), fluoxetine (Blier et al. 1988), paroxetine (Chaput et al. 1991), and venlafaxine (Béïque et al. 2000). Clearly then, reboxetine does not belong to that category of antidepressant agents.

It is interesting that a drug like reboxetine which increases the synaptic availability of NE can also increase that of 5-HT in the hippocampus. As compared to controls, the 140% increase in CA_3 pyramidal neuron firing in response to i.v. administration of the selective 5-HT_{1A} receptor antagonist WAY 100,635 (Figure 8, Panel B, and Figure 9), is well within the range of that produced with other antidepressant treatments (Béïque et al. 2000; Rueter et al. 1998; Haddjeri et al. 1998), with lithium addition (Haddjeri et al. 2000) and even to a greater extent with the combination of mirtazapine and paroxetine than either drug alone (Besson et al. 2000). It is interesting to note that the latter strategies are endowed with a greater antidepressant efficacy (Rouillon and Gorwood 1998; Debonnel et al. 2000). These observations therefore provide considerable face validity for the use of the hippocampus as a brain structure relevant to antidepressant drug action. However, since the effectiveness of the stimulation of the afferent 5-HT

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pathway to the hippocampus is unaltered in 21-day reboxetine-treated rats, a small inhibition of 5-HT reuptake, combined with decreased α_2-adrenergic heteroreceptors sensitivity on 5-HT terminals (Figure 7, Panel B), may then be postulated to account for the increased tonic activation of 5-HT_{1A} receptors in the hippocampus of these animals.

In conclusion, it has been shown that NE availability in the hippocampus is enhanced by reboxetine treatment via blockade of the NE transporter (Sacchetti et al. 1999). Since both the acute and long-term administration of reboxetine produced the same degree of enhancement of NE in the extracellular milieu in the same experiments in the hippocampus, the relationship between the delayed clinical response to reboxetine with respect to hippocampal function is not clear. However, the hippocampus is endowed with excitatory β-adrenoceptors, inhibitory α_1-adrenergic and α_2-adrenergic receptors (Curet and de Montigny 1988a,b; Lacroix et al. 1991). Thus, it appears that a delayed desensitization of β-adrenoceptors, which is common to most antidepressant treatments, including desipramine, also occurs with reboxetine (Riva et al. 1989; Lacroix et al. 1991). This would lead to an overall increased inhibitory action of the treatment on the firing of pyramidal neurons mediated by a decreased function of excitatory β-adrenoceptors and an increased activation of normosensitive α-adrenergic and 5-HT_{1A} receptors. The increase in transmission t 5-HT_{1A} receptors in the hippocampus, also common to all antidepressant treatments, occurs for reboxetine, although not via their sensitization as is the case for TCA drugs, but rather via an increase in 5-HT availability. The latter effect of sustained reboxetine administration on the 5-HT system with respect to the antidepressant response is currently not known. A dietary tryptophan depletion in patients responding to reboxetine treatment for major depression could shed light on this issue.

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