

Chronic Deep Brain Stimulation and Pharmacotherapy for the Treatment of Depression: Effects on Neuroplasticity in Rats

by

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A thesis submitted in conformity with the requirements
for the degree of Master of Science.
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University of Toronto

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Abstract

Deep brain stimulation (DBS) is currently being investigated as a therapy for treatment-resistant depression, with promising results. However, it is not clear whether or not DBS works via the same mechanisms as those induced by antidepressant medications. Processes currently implicated in antidepressant effects include neuroplastic changes and promotion of neurogenesis. We investigated the effects of chronic treatment with three different classes of antidepressants and DBS on markers of neuroplasticity (brain-derived neurotrophic factor, (BDNF), and phosphorylated cyclic-AMP regulatory element binding protein, (pCREB)) and neurogenesis (Ki-67, bromodeoxyuridine (BrdU) and doublecortin) in the rat hippocampus. No clear treatment effects were seen on BDNF, pCREB and Ki-67 levels. However all treatments caused increased levels of BrdU (range: 46%-96%) and doublecortin (8%-61%), although these effects were statistically significant only for DBS and amitriptyline, respectively. This overall pattern of results may suggest that diverse antidepressant treatments could possibly share common mechanisms involving cell survival and neuronal differentiation. Potentiated effects of DBS on cell survival may underlie its efficacy in treatment-resistant depression.

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List of Abbreviations

5-HT	5-hydroxytryptamine, serotonin
BDNF	brain-derived neurotrophic factor
BrdU	5-bromo-2-deoxyuridine
cAMP	cyclic adenosine 3-5-monophosphate
CBT	cognitive-behavioural therapy
CMS	chronic mild stress
CREB	cAMP regulatory element binding protein
CRH	corticotropin releasing hormone
DAB	3,3'-diaminobenzidine
DBS	deep brain stimulation
DCX	doublecortin
ECS	electroconvulsive seizure
ECT	electroconvulsive therapy
FST	forced swim test
HDRS	17-item Hamilton Depression Rating Scale
HPA	hypothalamus-pituitary-adrenal
IPT	interpersonal therapy
LPT	long-term potentiation

MAM	methyloxymethanol
MAOI	monoamine-oxidase inhibitor
MRI	magnetic resonance imaging
NE	norepinephrine
PBS	phosphate-buffered saline
pCREB	phosphorylated CREB
PCR	polymerase chain reaction
PET	positron emission tomography
QIDS-SR	Quick Inventory of Depressive Symptomatology – Self-Report
SCG	subcallosal cingulate gyrus
SGZ	subgranular zone
SPECT	single photon emission computed tomography
SSC	saline sodium citrate buffer
SSRI	selective-serotonin reuptake inhibitor
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
T ₃	triiodothyronine
TCA	tricyclic antidepressant
TeCA	tetracyclic antidepressant
vmPFC	ventral-medial prefrontal cortex

Chapter 1

Introduction

1.1 Depression overview

Major depressive disorder is a widespread disorder affecting approximately 8% of Canadians, and 151.2 million individuals worldwide (Mathers and Ma Fat 2008). It is the highest cause of burden of disease globally, and up to 15% of depressed patients commit suicide (Guze and Robins 1970). There is also a gender bias, with women being approximately twice as likely as men to develop major depressive episodes at some point in their lives (Weissman, Bland et al. 1996). The average age of onset for major depressive disorder is in the mid-20s; however it may begin at any age (Weissman, Bland et al. 1996). The disease has a lifetime risk of 23.2% (Kessler, Berglund et al. 2005). Depressive episodes are generally recurrent: of individuals that seek treatment, approximately 80% subsequently have multiple episodes (Anderson, Ferrier et al. 2008).

Definition

As defined by the *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association 2000), major depressive disorder is characterized by depressed mood (feelings of sadness or emptiness), or loss of interest or pleasure in most activities for the better part of 2 weeks, plus 4 or more of the following accompanying symptoms: significant weight loss or gain, sleep disturbances, psychomotor agitation or retardation, loss of energy or fatigue, feelings of guilt or worthlessness, inability to concentrate or make decisions, recurrent thoughts of death or suicide, and/or decreased libido.

Although not part of the criteria for diagnosis, many depressed individuals also suffer from impaired cognitive function and memory impairment (Burt, Zembar et al. 1995; Zakzanis, Leach et al. 1998; Landro, Stiles et al. 2001), the latter being related to impaired hippocampal and anterior cingulate functioning (Bremner, Vythilingam et al. 2004).

Given the broad list of possible symptoms, depression is considered to be a heterogeneous disease, where any two patients may have largely different symptoms. It is thought that this heterogeneity may underlie differences in response to treatments.

Triggers

The main triggers for major depressive episodes are severe psychosocial stressors, such as the death of a family member or friend, or divorce (Post 1992; Mazure 1998; Kendler, Karkowski et al. 1999). It has been shown that there are genetic factors that also contribute to the development of this disorder: an individual is more likely to develop this disorder if a relative has been diagnosed with major depression.

Although much is known about depression, there is still much that is elusive. The causes of depression are still unclear, as are the exact underlying neurological mechanisms of its treatment. There is an array of different treatments with considerable efficacy, but exactly how these diverse treatments elicit antidepressant effects is still unclear. It is also perplexing that some people respond well to treatments, while others are treatment-resistant. Antidepressant treatments and treatment resistance shall be discussed, in light of a promising new therapy for the treatment-resistant population, deep brain stimulation (DBS). However, the mechanisms of this new treatment are still undetermined; therefore it would be pertinent to discover whether DBS shares any of the known features involved in pharmacotherapeutic effects on depression, including neuroplasticity and neurogenesis, or whether it mediates its antidepressant effects through entirely different mechanisms. This is a central issue of interest addressed in this thesis.

Discussed below is the known pathophysiology of depression and how these data coalesce into proposed mechanisms of disease.

1.2 Proposed mechanisms of depression

The causes of depression are currently unknown and specific brain mechanisms underlying the disease remain elusive. However, there are several distinct morphological, chemical and cellular effects observed in the depressed brain, which have lead to various theories about

depression's underlying mechanisms. These effects may not all be specific to depression, nor arise in all depressed patients, but they nevertheless provide information as to the pathophysiology and possible etiology of this disease, and suggest clues to mechanisms of antidepressant effects. Relevant data have been found with respect to brain imaging, detailing changes in regional metabolism, blood flow, and volume. Other relevant data have been found with respect to monoamine neurotransmitters and their receptors, stress and the HPA axis, and neuroplasticity. In the next few pages, some of the more pertinent findings are reviewed.

Evidence from imaging studies

Imaging studies can point to discrete regions where abnormalities exist that may underlie a given pathology. Various brain imaging studies using positron emission tomography (PET) and/or single photon emission computed tomography (SPECT) have pointed to distinct brain regions expressing reduced metabolism or blood flow in depressed patients. Metabolic activity markers such as glucose uptake and localized changes in blood flow can be used as indirect measures of regional brain activity, and magnetic resonance imaging (MRI) can be used to examine morphological alterations in the brain.

For most published studies, results establish overall decreased cerebral blood flow and regional metabolism in depressed patients over control subjects (Ketter, George et al. 1996; Mayberg 2003). Many of these studies also demonstrate a correlation between the degree of decrease in activity and the severity of depression (Baxter, Schwartz et al. 1989; O'Connell, Van Heertum et al. 1989; Schlegel, Aldenhoff et al. 1989; Kanaya and Yonekawa 1990; Kumar, Mozley et al. 1991; Austin, Dougall et al. 1992; Cohen, Gross et al. 1992; Drevets, Videen et al. 1992; Yazici, Kapucu et al. 1992; O'Connell, Van Heertum et al. 1995). This suggests a functional link between activity in distinct brain regions and the severity of depression. Although the exact pattern of change is not consistent among studies, metabolism is often found to be normalized following various treatment types, including treatment with selective-serotonin reuptake inhibitors (SSRIs), tricyclic antidepressants (TCAs), electroconvulsive therapy (ECT), interpersonal therapy (IPT), or transcranial magnetic stimulation used to treat depression (Brody, Saxena et al. 2001) (treatments are discussed in further detail in section 1.3 below). These results suggest that various

treatments have the capacity to induce changes in brain activity that are correlated with amelioration of depressive symptoms. This is indicative that a possible underlying mechanism of depression is a reduction in overall activity in these brain regions, especially the frontal and temporal lobes, including the hippocampus.

MRIs have also been employed to examine volumetric differences in various brain structures. Depressed patients are observed to have smaller hippocampi relative to non-depressed individuals (Campbell, Marriott et al. 2004; Videbech and Ravnkilde 2004). There has been a series of studies that suggests that this phenomenon of lowered hippocampal volume is caused *by* prolonged depressive illness, rather than being one of the triggers of depression. In this light, several studies have demonstrated that the degree of volume reduction is correlated with the total duration of the individual's depressive illness (Sheline, Wang et al. 1996; Sheline, Sanghavi et al. 1999). Therefore the longer an individual is affected by depression, the smaller their hippocampus is likely to be. Also, individuals suffering from their first depressive episode do not have reduced hippocampal volumes (MacQueen, Campbell et al. 2003), further supporting the notion that this symptom may not be a pre-existing condition or a precursor for the pathology. Shah et al. (1998) have extended these findings to behavioural effects. Reductions in left hippocampal grey matter density were correlated with reduced verbal learning and memory test scores in treatment-resistant depressed patients (Shah, Ebmeier et al. 1998). For patients that had recovered from depression, both of these scores were no different from those of control subjects, indicating that these parameters may normalize following successful treatment, and therefore that these changes may be reversible in the treatment-responsive population.

It should be noted, however, that reduced hippocampal volume is not unique to major depression, but has been observed in other disorders including post-traumatic stress disorder (Smith 2005), schizophrenia (Koolschijn, van Haren et al. 2010), Alzheimer's disease, Parkinson's disease, epilepsy, and obsessive-compulsive disorder (Geuze, Vermetten et al. 2005).

Volumetric differences are also not limited to the hippocampus, although this is the region that has been most implicated. The orbitofrontal and prefrontal cortices are also affected in depression, as they have been found to have reduced thickness, neuronal sizes, and neuronal

and glial cell densities in depressed patients (Rajkowska, Miguel-Hidalgo et al. 1999). Depression also causes reduced glial cell densities in the anterior cingulate cortex, and the dorsolateral prefrontal cortex (Cotter, Mackay et al. 2001; Cotter, Mackay et al. 2002).

In summary, reduced cerebral blood flow and regional metabolism have been found for depressed individuals, which have been correlated with the severity of depressive illness. Regional metabolism is normalized following various antidepressant treatment types. Volumetric studies have found reduced hippocampal volumes in depressed individuals, the degree of which has been correlated with the duration of illness. Studies suggest that volumetric changes are caused by prolonged depression, and are not a precursor to the disease. Lastly, these changes in hippocampal volume are associated with behavioural effects, as the degree of volume reduction has been correlated with reduction in verbal learning and memory capabilities, both of which are not observed in recovered patients.

The role of monoamines

The observations that several pharmacologically and chemically diverse agents not only possess antidepressant properties, but are also capable of increasing the synaptic concentrations of the monoamines norepinephrine (NE), serotonin (5-HT), and also dopamine, have led to the development of the monoamine hypothesis of depression (Iversen 2005). The first pharmacological agents found to have antidepressant properties were monoamine oxidase inhibitors (MAOIs). Soon after, tricyclic antidepressants (TCAs) were also discovered to possess antidepressant effects, and both of these classes of treatments primarily target both the 5-HT and NE systems in the brain. Given that, to date, most clinically effective antidepressant medications affect these neurotransmitter systems, it has been hypothesized that disruption of these pathways may be important in the pathophysiology of depression.

Depressed individuals have been observed to possess deficiencies in monoamine neurotransmitters. Lambert et al. found reductions in brain NE and dopamine levels in depressed individuals (Lambert, Johansson et al. 2000). Reduced 5-HT levels specific to depressive disorder have also been observed (Le Quan-Bui, Plaisant et al. 1984; Cleare 1997; Muck-Seler, Pivac et al. 2004). This suggests that the effects of antidepressant agents on

monoamines are a primary function of their antidepressant effect, and not just an epiphenomenon.

One way to directly investigate the roles of monoamines in mood regulation is by removing them from the brain. In studies conducted where monoamines are acutely depleted (Smith, Fairburn et al. 1997; Moreno, Gelenberg et al. 1999; Ruhe, Mason et al. 2007), depletion induced a decreased mood in healthy subjects with a family history of depression, as well as in patients currently in remission. Although this does not prove a causal link, it does demonstrate that these neurotransmitters can have a direct effect on mood in certain individuals.

It seems clear that monoamines are intimately involved in mood regulation. Monoamines are increased by antidepressant medications, and are reduced in untreated depressed individuals. On the other hand, an important and persisting issue refers to the fact that antidepressant drugs can increase neurotransmitter levels at the synapse quickly after first administration, but clinical improvement is usually not seen until after 10-14 days of daily exposure to the medication. It has been demonstrated that several monoamine receptors (5-HT₂, α ₂-adrenergic, β -adrenergic) are both up-regulated and exhibit increased sensitivity in depression (Mann, Stanley et al. 1986; Ordway, Widdowson et al. 1994; Drevets, Frank et al. 1999; Ordway, Schenk et al. 2003; Hirvonen, Karlsson et al. 2008). It is hypothesized that the enhanced sensitivity of monoamine receptors can underlie susceptibility to depression, and antidepressant treatments counteract this by down-regulating receptor expression (Charney, Menkes et al. 1981).

There is considerable evidence demonstrating the importance of these monoamine systems in mediating antidepressant response, but there are persisting issues related to the nature of this involvement. It is suggested that long-term neurochemical and structural changes are required to take place in order to induce the therapeutic effect, which may be initiated by the early monoamine responses. It is commonly thought that chronic antidepressant treatment leads to alterations in monoamine receptor density, sensitivity, and/or intracellular signaling. This can have a major impact on the hypothalamic-pituitary-adrenal (HPA) axis and the stress response.

The role of stress

It is well known that several of the symptoms of depression overlap with known effects of high cortisol levels (such as those that are present in Cushing's syndrome), and that psychosocial stressors can precipitate depressive episodes. These observations have led to the hypothesis that depression results from dysregulation of the HPA axis and the normal stress response. The HPA axis is a tightly controlled feedback loop regulating the production of circulating glucocorticoids that mediate the stress response. The fact that animal models of depressive-like symptoms invariably make use of stressors lends support to this hypothesis. As detailed below, elevated glucocorticoids can cause toxic effects that lead to reductions in cell signaling, as well as to atrophy and neuron loss within the hippocampus, which are associated with behavioural effects. There is also evidence that this pathway includes an important role for 5-HT regulation.

There is epidemiological evidence to support the role of dysregulated stress responses in depression: approximately 50% of depression patients exhibit elevated cortisol levels (hypercortisolemia) (Sachar, Hellman et al. 1970). Therefore hypercortisolemia does play a major role in the pathophysiology of this disease for many, but not all patients. The implication of this finding is that high circulating cortisol levels effect glucocorticoid receptor expression and contribute to disruption of feedback inhibition, leading to dysregulation of the stress response (Nemeroff, Widerlov et al. 1984; Sapolsky, Krey et al. 1984; Sapolsky, Krey et al. 1985; Nemeroff, Owens et al. 1988; Heuser, Yassouridis et al. 1994; Raadsheer, Hoogendijk et al. 1994; Smith 1996; Merali, Du et al. 2004). Several different antidepressants can reverse these effects (Nemeroff, Bissette et al. 1991; De Bellis, Gold et al. 1993; Kling, Geraciotti et al. 1994; Heuser, Bissette et al. 1998).

Glucocorticoids can cause cellular toxicity during metabolic insults. Glucocorticoids inhibit glucose uptake into neuronal and glial cells, which can cause neurotoxic effects during metabolic insults (Horner, Packan et al. 1990; Virgin, Ha et al. 1991). Glucocorticoids also increase synaptic glutamate levels, which cause excitotoxic effects in both neurons and glial cells, and may lead to cell death (Armanini, Hutchins et al. 1990; Virgin, Ha et al. 1991; Elliott and Sapolsky 1992; Tombaugh and Sapolsky 1992; Moghaddam 1993; Lawrence and Sapolsky 1994; Stein-Behrens, Lin et al. 1994).

Elevated glucocorticoids also have negative inter-cellular effects in the hippocampus. Effects include reduced cell signaling via reduced long-term potentiation and increased long-term depression (Diamond, Bennett et al. 1989; Pavlides, Watanabe et al. 1993; Diamond, Fleshner et al. 1994; Pavlides, Kimura et al. 1995). Excess glucocorticoids cause dendritic atrophy (Woolley, Gould et al. 1990; Watanabe, Gould et al. 1992) and neuron loss (Sapolsky, Krey et al. 1984; Sapolsky, Krey et al. 1985; Uno, Tarara et al. 1989; Sapolsky, Uno et al. 1990). Lastly, an increase in glucocorticoids is correlated with impaired cognitive performance, including spatial memory recall, and verbal memory recall (Issa, Rowe et al. 1990; Starkman, Gebarski et al. 1992; Watanabe, Gould et al. 1992; Watanabe, Gould et al. 1992; Luine, Villegas et al. 1994; Lupien, Lecours et al. 1994).

The HPA axis and 5-HT are reciprocally stimulatory. In a dysregulated state, overactive 5-HT neurons may over-stimulate corticotropin-releasing hormone (CRH) -producing neurons within the paraventricular nucleus (Merchenthaler, Vigh et al. 1983; Sawchenko, Swanson et al. 1983; Liposits, Phelix et al. 1987), leading to increased production and release of the stress hormones corticotropin, corticosterone and cortisol, in both rodents and humans (Gibbs and Vale 1983; Feldman, Conforti et al. 1987; Haleem, Kennett et al. 1989; Lesch, Rupprecht et al. 1989; Lesch, Mayer et al. 1990). Additionally, corticosterone has pronounced stimulatory effects on 5-HT production in the mid-brain and brain stem (Azmitia and McEwen 1969; Azmitia, Algeri et al. 1970; Azmitia and McEwen 1974; De Kloet, Kovacs et al. 1982).

It is thought that a dysregulated stress response contributes to depression. This correlates with the evidence for hypersensitive monoamine receptors in depression. Since cortisol and 5-HT are reciprocally stimulatory, an effect of hypersensitive 5-HT receptors could be contribution to an overactive stress response, which is known to lead to cellular toxicity, atrophy, neuron loss, and cognitive deficits. These effects are known to occur in major depression patients. It is possible that these negative effects also lead to the volume loss in the distinct brain regions discussed above, via reduced neuroplasticity.

Neuroplasticity

Neuroplasticity is the capacity of CNS neurons to adapt or change following stimulation. This may involve structural or functional plasticity. Structural plasticity denotes neuronal morphology, such as axonal and dendritic arborization, as well as cell proliferation (cytogenesis), differentiation, and survival. Functional plasticity refers to the efficacy of synaptic transmission between neurons, generally observed as long-term potentiation (LTP) or, conversely, long-term depression. All of these factors are regulated by experience. Neuroplasticity has functional consequences for learning and memory, as well as for survival of neurons and protection against neuronal damage.

Neurotrophic factors are required for neuronal cell growth, survival, and proper functioning. They increase cell survival by exerting inhibitory effects on cell death pathways (Duman, Malberg et al. 2000). Brain-derived neurotrophic factor (BDNF) has been the neurotrophic factor most consistently implicated in depression. Cyclic AMP regulatory element binding protein (CREB) is a transcription factor that is capable of regulating neurotrophin levels, and especially BDNF transcription (Conti, Cryan et al. 2002). Both BDNF and CREB appear to be intimately involved in depression, stress, antidepressant activity, and learning and memory processes, as outlined below. The effects of these proteins on both structural and functional neuroplasticity are also detailed.

BDNF is a member of the nerve growth family, and is found throughout the peripheral and central nervous systems. In the mammalian brain, it is widely distributed, but is most highly concentrated in the hippocampus, and also exists in high amounts in the cerebral cortex, as it is the most abundant neurotrophin in the brain (Ernfors, Wetmore et al. 1990; Hofer, Pagliusi et al. 1990; Wetmore, Ernfors et al. 1990).

In post-mortem brain tissue from depressed suicide victims, BDNF protein and mRNA levels were reduced in both the prefrontal cortex and in the hippocampus (Dwivedi, Rizavi et al. 2003; Karege, Vaudan et al. 2005).

Stress also impacts BDNF expression, as several different stress models reduce its expression (Roceri, Hendriks et al. 2002; Pizarro, Lumley et al. 2004). Surprisingly, both intact and adrenalectomized rats exhibit reduced BDNF mRNA levels in the hippocampus following

both acute and chronic stress paradigms (Smith, Makino et al. 1995; Murakami, Imbe et al. 2005). Although adrenalectomy had no observed effect on BDNF mRNA, the stress paradigms did produce a considerable effect, indicating a role for elevated glucocorticoids, but not reduced levels. Therefore, reductions in BDNF are likely to be at least partially mediated by glucocorticoids. It is postulated that stress interferes with normal neuronal functioning via decreased availability of BDNF.

Reductions of BDNF in depression are reversed by antidepressant treatment (Chen, Dowlatshahi et al. 2001), and BDNF mRNA is increased by chronic antidepressant administration of various drug classes (Nibuya, Morinobu et al. 1995). This neurotrophic factor seems to be important for antidepressant effect: conditional forebrain-BDNF knockout mice exhibit reduced antidepressant-like effects following desipramine treatment (Monteggia, Barrot et al. 2004; Monteggia, Luikart et al. 2007). BDNF also has antidepressant properties of its own, as demonstrated in a rat model where BDNF was locally infused into the dentate gyrus of the hippocampus to cause antidepressant-like effects after 3-10 days (Shirayama, Chen et al. 2002). Similar effects were seen after BDNF infusion into the midbrain, which produced antidepressant-like effects in two different rat models of depression (Siuciak, Lewis et al. 1997).

The functions of BDNF within the brain have been extensively studied, and have been found to involve both structural and functional neuroplastic effects. Structural neuroplastic effects include its importance for the survival of new neurons following neurogenesis (Sairanen, Lucas et al. 2005). BDNF is also important for cell survival in both cortical neurons and nigral dopaminergic neurons, and for both survival and differentiation within the hippocampus (Hyman, Hofer et al. 1991; Ghosh, Carnahan et al. 1994; Lindholm, Carroll et al. 1996; Lowenstein and Arsenault 1996). BDNF has demonstrated trophic effects specifically on 5-HT neurons in the adult rat brain by stimulating sprouting in both intact and lesioned cortical axons (Mamounas, Altar et al. 2000). This neurotrophic factor protects neurons from both metabolic and excitotoxic insults, including glutamate neurotoxicity, which are thought to mediate some of the negative effects of stress and glucocorticoid release, as discussed above (Cheng and Mattson 1994). This is mediated through attenuation of the internal Ca^{2+} responses to insult. It should be noted that BDNF is not the only neurotrophic factor implicated in these functions, as neurotrophin-3 and nerve-growth factor

are also neuroprotective. However, they are regulated by different mechanisms and do not share the same patterns of expression (Ernfors, Bengzon et al. 1991; Isackson, Huntsman et al. 1991; Rocamora, Palacios et al. 1992; Barbany and Persson 1993; Cheng and Mattson 1994).

Functional neuroplastic effects of BDNF include those involved in memory processes. BDNF is important for both induction of hippocampal LTP and hippocampus-dependent learning (Korte, Carroll et al. 1995; Monteggia, Barrot et al. 2004). Additionally, BDNF mRNA levels are increased by LTP (Patterson, Grover et al. 1992; Castren, Pitkanen et al. 1993). BDNF promotes synaptic innervation, and hypertrophy of cell bodies and axons (Causing, Gloster et al. 1997).

There is evidence that BDNF is decreased in depression and by stress, and it is also increased by several different antidepressants. It seems therefore reasonable to hypothesize that it may be part of a common antidepressant pathway engaged by all clinically effective antidepressant interventions. The functions of BDNF in depression may include both structural and functional neuroplastic effects, with behavioural implications for learning and memory. One transcription factor that is known to regulate BDNF transcription is CREB, which has also been widely associated with depression and antidepressant effect.

CREB is a transcription factor that initiates transcription of several genes, including BDNF. By regulating gene transcription, CREB participates in the cellular responses to stimuli and in initiating adaptations. CREB is activated by phosphorylation (pCREB) before binding to DNA regulatory elements. An important study used CREB knockout mice to demonstrate that CREB is required for antidepressant-induced upregulation of BDNF transcription, but not for behavioural or endocrine (corticosterone) antidepressant effects (Conti, Cryan et al. 2002).

Most studies of post-mortem brain found CREB to be decreased in depressed individuals. CREB expression is decreased in the temporal cortex (including hippocampus) for untreated depressed individuals, and increased in depressed patients that were treated with antidepressants (Dowlathahi, MacQueen et al. 1998; Dwivedi, Rao et al. 2003). Similar results have been demonstrated for both CREB and pCREB in the orbitofrontal cortex

(Yamada, Yamamoto et al. 2003). However a different post-mortem brain study reported both CREB and pCREB to be increased in drug-free and untreated depressed suicides, but not in antidepressant-treated depressed suicides (Odagaki, Garcia-Sevilla et al. 2001).

Antidepressant treatments can regulate levels of the intracellular proteins involved in the cAMP signaling cascade. Chronic administration of various classes of antidepressants increases CREB mRNA, cAMP response element-mediated gene transcription and also the phosphorylation of the CREB protein in the cortex, hippocampus, amygdala and hypothalamus in rodents (Nibuya, Nestler et al. 1996; Thome, Sakai et al. 2000). As well, over-expression of CREB in the dentate gyrus of mice has produced an antidepressant-like effect in two different models (Chen, Shirayama et al. 2001). These effects proved to be region-specific for the dentate gyrus.

CREB also has important structural neuroplastic effects, especially increased cytogenesis within the hippocampus. Increased CREB phosphorylation leads to increased cell proliferation, and CREB knockout mice demonstrate reduced cell proliferation (Nakagawa, Kim et al. 2002). As well, upregulation of cAMP leads to increased survival of new neurons (Nakagawa, Kim et al. 2002). With respect to functional neuroplasticity, CREB has been implicated in memory formation, via transcription of related proteins. CREB-knockout mice have displayed deficient long-term memory in several different models, while short-term memory has remained unaffected (Bourtchuladze, Frenguelli et al. 1994; Kogan, Frankland et al. 2000). Reversible inhibition of CREB in the dorsal hippocampus also disrupts spatial memory (Pittenger, Huang et al. 2002).

As well as BDNF, CREB is thus decreased in depression and is decreased by stress, and it is increased by many different antidepressants. Therefore we suggest that it may also be involved in a common antidepressant pathway engaged by all effective antidepressant treatments, and likely partakes in some of the same pathways as BDNF in regulating its expression in the same regions. CREB, like BDNF, has also demonstrated both structural and functional neuroplastic effects, with behavioural implications for learning and memory.

Neurogenesis

The mammalian subgranular zone of the dentate gyrus is rather unusual in that it is one of only two regions of the brain, along with the olfactory bulb, where neurons continue to divide well into adulthood and throughout life. Stem cells within the subgranular zone of the dentate gyrus divide to produce progenitor cells. These cells then must differentiate into immature neurons (or glia), then migrate ventrally towards the molecular layer of the dentate gyrus where they extend projections and forms synapses, before maturing to become adult granule neurons (Perera, Park et al. 2008).

It is suggested that suppressed adult hippocampal neurogenesis can lead to vulnerability to depression. This is based on the following findings: depressed individuals express reduced hippocampal volumes; neurogenesis is reduced following stress; antidepressant treatment increases neurogenesis; and blocking cytotogenesis prevents antidepressant effects following treatment.

First, as noted above, depressed patients often have reduced hippocampal volumes, the degree of which has been correlated with the duration of major depression (Sheline, Wang et al. 1996). This may be caused by hippocampal atrophy and/or reduced cytotogenesis. Stressed animals demonstrate reduced hippocampal volume and cell proliferation, which is prevented by antidepressant treatment (Czeh, Michaelis et al. 2001).

Second, stress leads to reduced hippocampal neurogenesis. Adrenalectomy in rats induces increases in rates of cell birth, which is conversely suppressed by corticosterone treatment (Gould, Cameron et al. 1992). Behavioural stress paradigms in rats, including chronic mild stress and learned helplessness, have also demonstrated the same effect (Malberg and Duman 2003; Jayatissa, Bisgaard et al. 2006; Mineur, Belzung et al. 2007; Ho and Wang 2010). Similarly, stress reduces dentate gyrus cell proliferation in adult monkeys (Gould, Tanapat et al. 1998). With respect to human beings, as discussed above, stress is a risk factor for individuals predisposed to depression, and mediates many other effects previously discussed.

Antidepressants of various classes, along with ECT, have demonstrated hippocampal cytotogenic capabilities in both rats and in monkeys, and reverse the inhibitory effects of stress (Malberg, Eisch et al. 2000; Jayatissa, Bisgaard et al. 2006; Perera, Coplan et al. 2007). This

is a dose-dependent effect when cytogenesis is stimulated by electroconvulsive seizures in the rat (Madsen, Treschow et al. 2000). Additionally, physical exercise, which is known to have antidepressant effects, increases cytogenesis, spatial learning, and long-term potentiation in the dentate gyrus of rats (van Praag, Christie et al. 1999). As well, administration of a synthetic cannabinoid causes cytogenic, antidepressant- and anxiolytic-like effects in rats (Jiang, Zhang et al. 2005). In this study, preventing hippocampal cytogenesis by means of x-irradiation blocked all the aforementioned effects.

Finally, blocking hippocampal cytogenesis can prevent antidepressant effects. In mice, x-irradiation of the hippocampus prevents some of the behavioural antidepressant-like effects of both fluoxetine (an SSRI) and imipramine (a TCA) treatment, using several different behavioural paradigms (Santarelli, Saxe et al. 2003; Surget, Saxe et al. 2008; Wang, David et al. 2008; David, Samuels et al. 2009). An important finding of one of these studies was that the antidepressant effects of a CRH 1 antagonist and of a vasopressin 1b antagonist were not prevented by blocking cytogenesis (Surget, Saxe et al. 2008). Therefore it appears that cytogenesis may be a requirement for antidepressant effect only for monoaminergic antidepressants.

Neurogenesis also has important implications for functional neuroplasticity. An important characteristic of new cells is that they possess membrane properties which are similar to those of neurons in the developing nervous system, and which are distinct from those of mature neurons. These properties facilitate long-term potentiation and synaptic plasticity (Schmidt-Hieber, Jonas et al. 2004; Ge, Yang et al. 2007). Ablation of hippocampal cytogenesis also eliminates long-term potentiation in the dentate gyrus (Saxe, Battaglia et al. 2006).

It has been demonstrated that hippocampal cytogenesis is important for hippocampus-dependent learning. Reduced cytogenesis causes impaired hippocampus-dependent memory-formation, impairs learning with respect to contextual fear conditioning and also spatial long-term memory (Shors, Miesegaes et al. 2001; Snyder, Hong et al. 2005; Saxe, Battaglia et al. 2006). There is evidence to support the notion that new neurons in the dentate gyrus are preferentially incorporated over mature neurons into spatial memory networks, which could denote at least one role that these new neurons may perform (Ramirez-Amaya, Marrone et al. 2006; Kee, Teixeira et al. 2007).

In summary, there is considerable evidence that structural and functional neuroplasticity are interdependent features of the mammalian brain that play an important role in cellular integrity, proliferation, connectivity, and learning and memory. All these functions are known to be impaired in depressed patients. Although these impaired functions do not reflect all of the features of depression, they do represent common targets of antidepressant treatments. One of the mediators of these processes is the cAMP signaling system, involving the previously described proteins CREB and BDNF. Volumetric studies demonstrating reduced volumes in depression provide further evidence of relationships between depression and reduced cellular integrity, increased atrophy and decreased proliferation, which are likely to be at least partially mediated via the cAMP signaling system and neurotrophic activity.

The cAMP signaling system and neurotrophic activity are influenced by stress and the HPA axis. Stress and the HPA axis are intimately involved with monoamine systems within the brain, all of which are affected in depression and are modulated by antidepressants. Although there are many factors influencing hippocampal volume, cognitive abilities including learning and memory processes, the stress response, and monoaminergic systems, neuroplasticity is presented here as one of the mediators involved in all of the above processes, and a common target of the very diverse repertoire of antidepressant treatments.

1.3 Treatments and treatment resistance

The goal of treatment is not simply *response to therapy*, where some symptoms improve but other residual symptoms may remain. This leads to poor outcome and increased relapse risk. Even though the majority of treated individuals achieve complete remission of symptoms, 20-30% of patients only attain partial remission, where some depressive symptoms remain (American-Psychiatric-Association 2000). Instead, treatment aims to achieve complete remission, thereby resulting in a relatively asymptomatic state (Fava 2003). Adequate therapy consists of one or more trials of antidepressant treatments with established efficacy, at a dose considered to be effective (by controlled clinical trials) for at least 12 weeks of duration (Quitkin, Rabkin et al. 1986; Fava 2003). Several forms of therapies are currently used, including psychotherapy, pharmacotherapy, and electroconvulsive therapy.

Psychotherapy

The first line of treatment for mild to moderate depression is psychotherapy, which can also be used as an adjunct to pharmacotherapy to treat moderate to severely depressed individuals. There are two main types of psychotherapy for the treatment of depression: cognitive-behavioural therapy (CBT) and interpersonal therapy (IPT). CBT aims to teach new ways of thinking and behaving to replace patterns that may have been contributing to the patient's disorder. IPT helps people to understand and deal with personal relationships that may have contributed to their depression. Either type of therapy usually requires 8-16 weeks for adequate results.

Pharmacotherapy

The pharmacological treatment of depression began in the 1950s with the introduction of the first monoamine oxidase inhibitors (MAOIs) followed by tricyclic antidepressants (TCAs) (Lopez-Munoz, Alamo et al. 2007). MAOIs, as the name suggests, inhibit monoamine oxidase. This has the effect of preventing the breakdown of the monoamines serotonin (5-HT), norepinephrine (NE), and dopamine in the presynaptic neuron, thereby increasing their availability for release through the synapse to the postsynaptic neuron. The main mechanism of action of TCAs involves the non-selective inhibition of both 5-HT and NE reuptake into presynaptic cells, thereby inducing higher concentrations of these agents within the synapse. In general, TCAs also possess antihistaminergic, antimuscarinic and α -adrenergic blocking abilities. The large side effect profiles for both of these classes of antidepressants led to the development of newer treatments with greater selectivity. SSRIs are specific blockers of the 5-HT transporter, which would otherwise remove 5-HT from the synapse, similar to TCAs. Other specific antidepressants include norepinephrine-dopamine reuptake inhibitors, and serotonin-norepinephrine reuptake inhibitors. Despite the availability of newer classes of antidepressive agents with fewer side effects, MAOIs and TCAs tend to have greater efficacy, and therefore are still widely used for second-line treatment.

It has been demonstrated that although some clinical actions of antidepressants are noticeable within a few days of initial treatment, significant improvement in mood is not generally seen before at least two weeks of treatment (Katz, Tekell et al. 2004).

Electroconvulsive therapy (ECT)

ECT is usually employed when other methods of treatment have been exhausted and the depressed patient has still not achieved remission. Upon undergoing brief anesthesia and taking muscle relaxants, the patient has bilateral surface electrodes attached to the skin over the medial temporal lobe, to induce a seizure with duration of at least 20 seconds of physical effect (Rami, Bernardo et al. 2004). This procedure is conducted several times per week, while patients continue to take their antidepressant medications. Treatment frequency is gradually decreased to once per month for up to 1 year, if necessary, although some individuals will only require a few sessions of ECT, and it is discontinued once depression symptoms no longer persist. Short-term side effects include confusion, disorientation, and memory loss (Rami, Bernardo et al. 2004).

The clinical effectiveness of ECT was investigated in a large-scale study conducted by Prudic et al. (2004). ECT was used to treat a large sample of major depression patients in diverse community settings to determine its overall clinical effectiveness. This group found that 30% of patients achieved remission, and 64% of patients had an incomplete response to treatment (Prudic, Olfson et al. 2004). Sadly, of the 30% of patients that remitted, 64% relapsed during the subsequent 6 months. Therefore, only about 10% of the original sample managed to sustain remission over the course of the study.

Treatment-resistance

It has been found that 5-10% of treated individuals do not ever achieve remission. At least 60% of individuals with major depressive disorder follow their initial depressive episode with a second episode. Subsequently, 70% of individuals who have had two episodes go on to suffer a third, and 90% of individuals who have had three episodes go on to suffer a fourth (American-Psychiatric-Association 2000).

Treatment-resistance has been defined as “an inadequate response after adequate antidepressant therapy among patients suffering from unipolar depressive disorders” (Fava 2003). Therefore, treatment-resistant individuals are those that have failed to achieve remission.

The five standard stages of treatment are detailed in Table 1 below:

Table 1: The Five Stages of Antidepressant Treatment

Stage:	Treatment Response:
0	No adequate trial of medication has been implemented
1	Patient fails to respond to first adequate trial of 1 medication
2	Patient fails to respond to 2 different monotherapy trials of medications with different pharmacological profiles
3	Stage 2 plus failure to respond to augmentation of 1 of the monotherapies
4	Stage 3 plus failure of a second augmentation strategy
5	Stage 4 plus failure to respond to ECT

Adapted from: Hauptman, DeSalles et al. 2008. (Adapted from: Thase and Rush 1997).

Stage 1 involves the initial trial of a single antidepressant agent, for a minimum of 2-4 weeks before symptoms are re-assessed and a different medication is considered if necessary. Stage 2 involves the administration of a second monotherapy trial, which can involve a switch to a different antidepressant within the same class, or a switch to an antidepressant of a different class (Thase, Friedman et al. 2007). Stage 3 is when augmentation is introduced. This approach involves coadministering an augmenting agent along with an antidepressant agent for individuals that are unresponsive or only partially responsive to that antidepressant alone. There are several common augmenting agents. Lithium tends to be rather effective and is commonly prescribed as an augmenting agent with monoamine reuptake inhibitors, or alternatively triiodothyronine (T₃) is used with TCAs (Aronson, Offman et al. 1996; Anderson, Ferrier et al. 2008). Bupropion, which is both a norepinephrine reuptake inhibitor and a nicotinic acetylcholine receptor antagonist, is also commonly used (Witkin and Li 2009). The antipsychotics olanzapine (in combination with fluoxetine) and aripiprazole are

also used as augmenting agents (Berman, Fava et al. 2009; DeBattista and Hawkins 2009; Trivedi, Thase et al. 2009; Witkin and Li 2009). The 4th stage of treatment involves a second augmentation trial, where a different combination of antidepressant therapy with an augmenting agent is used. The 5th and final stage is electroconvulsive therapy, as described above.

The STAR*D Study

The Sequenced Treatment Alternatives to Relieve Depression (STAR*D) trial is a large-scale study that was conducted to investigate treatment efficacy in depressed individuals in a more accurate manner. It is the largest study conducted of its kind, which aimed to accurately reflect the population of individuals seeking treatment as well as reflecting the sequential treatment strategies used, in order to achieve “real-world” results. In order to establish this, patients were recruited passively upon seeking treatment for depression from their physician or psychiatrist in the United States, instead of by advertisement. This was to ensure an accurate representation of the target population. From 2001 to 2004, 2876 patients were included in this open-label study, the outcome of which was evaluated by blinded assessors. The treatment approach was sequenced, similar to the levels described above: substitution, augmentation, and combination strategies were used. There were 4 different treatment levels (as ECT was not used for treatment), and the primary outcome of remission was measured using the 17-item Hamilton Depression Rating Scale (HDRS). Cumulative remission rates were measured using the Quick Inventory of Depressive Symptomatology – Self-Report (QIDS-SR). Each treatment level can be broken down as follows (see also Table 2 on page 21):

Level 1: Flexible doses of citalopram (an SSRI) were administered for up to 14 weeks, where 28% of patients achieved remission (Trivedi, Rush et al. 2006). The cumulative remission achieved was 37%.

Level 2: Those not achieving remission in level 1 were randomized into level 2-a or 2-b for 12 weeks. Level 2-a consisted of one of three different augmentation strategies of bupropion, buspirone, or CBT added to their existing citalopram treatment. The remission rates were: 30%, 30%, and 23%, respectively (Trivedi, Fava et al. 2006; Thase, Friedman et al. 2007).

Level 2-b consisted of one of four switch strategies, in which the antidepressant administered was changed to either: bupropion, sertraline (an SSRI), venlafaxine or CBT. The remission rates were: 21%, 18%, 25% and 25% respectively (Rush, Trivedi et al. 2006; Thase, Friedman et al. 2007). The cumulative remission achieved was 56%.

Level 3: Those still not achieving remission up to this point were randomized into level 3-a or 3-b for 12 weeks. Level 3-a consisted of a different augmentation strategy, where either lithium was administered, after which 16% of patients achieved remission, or where T₃ was administered, where 25% of patients achieved remission (Nierenberg, Fava et al. 2006). Level 3-b consisted of a second switch strategy of either mirtazapine (a tetracyclic antidepressant, TeCA), where 12% of patients achieved remission, or nortriptyline (a TCA), where 20% of patients achieved remission (Fava, Rush et al. 2006). The cumulative remission achieved was 62%.

Level 4: Patients that had failed to achieve remission in level 3 were randomized to either tranylcypromine (a MAOI), where 7% remission was achieved, or to the combination of venlafaxine (a serotonin-norepinephrine reuptake inhibitor) and mirtazapine, where 14% of patients achieved remission (McGrath, Stewart et al. 2006). This level of treatment was also 12 weeks in duration. Lastly, the cumulative remission achieved was 67%.

An important finding in this study was that there were no statistically significant differences in remission rates, response rates or times to remission, or responses between different treatments within the same treatment level (Warden, 2007). Also notable was that after four separate levels of treatment, only 67% of patients achieved remission, meaning that 33% of patients still remain treatment-resistant. This is a significant amount of people. Using the numbers quoted in the opening paragraph, given that over 150 million people worldwide suffer from depression, then more than 50 million people would remain treatment-resistant. Clearly, there is a need for effective treatment for this resistant population.

Table 2: Summary of the STAR*D Trial and Results

Level:	Treatment approach:	Duration:	% remission achieved (HDRS):	Cumulative % remission achieved (QIDS-SR):
1	Flexible doses of citalopram	12-14 weeks	28%	37%
2 – A	Augmentation (citalopram + 1 of: bupropion, buspirone, or CBT)	12-14 weeks	30%, 30%, 23%	
2 – B	Switch: change in antidepressant to bupropion, sertraline, venlafaxine, or CBT	12-14 weeks	21%, 18%, 25%, 25%	A + B = 56%
3 – A	Different augmentation: lithium or T ₃	12-14 weeks	16%, 25%	
3 – B	Switch: mirtazapine or nortriptyline	12-14 weeks	12%, 20%	A + B = 62%
4 – A	Switch: tranylcypromine	12-14 weeks	7%	
4 – B	Switch: venlafaxine + mirtazapine	12-14 weeks	14%	A + B = 67%

Adapted from: (Fava, Rush et al. 2006; McGrath, Stewart et al. 2006; Nierenberg, Fava et al. 2006; Rush, Trivedi et al. 2006; Trivedi, Rush et al. 2006; Thase, Friedman et al. 2007; Sinyor, Schaffer et al. 2010).

1.4 Deep brain stimulation

Deep brain stimulation (DBS) is a treatment that has only recently been employed for treatment-resistant depression patients, for whom all other treatment options have been exhausted. It involves a neurosurgical procedure to directly implant bilateral stimulating electrodes into localized brain regions. Magnetic resonance images are used to achieve accurate placement. These electrodes are connected to a pacemaker-like programmable pulse-generator that is fixed subcutaneously to the chest. This device can be manipulated to vary the stimulation parameters, but is generally set for ongoing high-frequency stimulation

(100+ Hz). As opposed to other surgical treatments, DBS has the distinct advantage of being adjustable and reversible, as well as acting locally.

DBS has mainly been used to treat Parkinson's disease, but recently has been applied for other diseases including treatment of depression, obsessive-compulsive disorder (Stein and Denys 2009), Gilles de la Tourette syndrome (Servello, Porta et al. 2008), dystonia (Vidailhet, Vercueil et al. 2005), Huntington's disease (Moro, Lang et al. 2004), and most recently even for Alzheimer's disease (Laxton, Tang-Wai et al. 2010). The possibility of these broad applications owes to the fact that DBS electrodes can be placed in local brain targets that are specific for each disease.

DBS for depression

Currently, the most promising target for DBS treatment of depression is the subcallosal cingulate gyrus (SCG) (see Figure 1). The SCG is a region that is involved in mood regulation, that has been associated with increased activity in depressed patients, and decreased activity following successful treatment (Mayberg 2003).

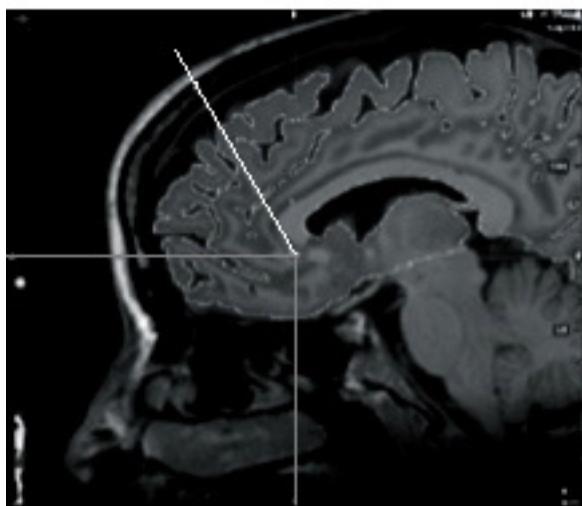


Figure 1. DBS in a Human Depression Patient. A diffusion tensor and stereotaxic image indicating electrode placement into the subcallosal cingulate gyrus (SCG), adapted from: (Hauptman, DeSalles et al. 2008). The thicker white angled line represents the insertion of the electrode (diameter is not to scale), and the grey lines represent the stereotaxic location of the electrode tip.

Although it is still at an investigational stage, Lozano et al. used SCG DBS to treat 20 patients suffering from major depressive disorder who had failed multiple trials of pharmacotherapy and psychotherapy (Lozano, Mayberg et al. 2008). Immediate behavioural effects included calmness, improved mood, and increased interest and motivation. Stimulation parameters were set to maximize these effects, with a range of 3.5 V to 5.0 V, with pulse width at 90 microseconds and frequency of 130 Hz.

Within the first week, 40% of patients had responded to treatment, and 1 had achieved remission, with progressive improvement continuing for 6 months. By this time, 60% of patients had responded to the new treatment, and 35% remission rates were achieved in these individuals that were otherwise treatment-resistant (Lozano, Mayberg et al. 2008). These benefits were for the most part maintained over the course of the study that lasted for 12 months following the initiation of treatment.

Improvements were seen in each of the mood, anxiety, somatic and sleep subcomponents of the 17-item Hamilton Rating Scale for Depression. These improvements were gradual over the course of 3-6 months. It has been suggested that the clusters of symptoms that respond at different times during treatment may be regulated by different substrates of the brain (Lozano, Mayberg et al. 2008). This has been demonstrated with various symptoms of obsessive-compulsive disorder, where different symptom clusters activate distinct areas of the brain (Mataix-Cols, Wooderson et al. 2004).

As well as the long-term effects of DBS, acute effects included calmness, improved mood, and increased interest and motivation. Reported side effects included: wound infections, headache or pain associated with the surgery itself, perioperative seizure, and worsening of mood or irritability (Lozano, Mayberg et al. 2008). Lastly, neuropsychological testing confirmed that there were no adverse cognitive effects of stimulation.

Interestingly, upon analyzing PET scan data, this group also found that SCG DBS therapy produced changes in regional glucose metabolism in many of the same brain regions as other antidepressant treatments, including antidepressant medications, cognitive behavioural therapy, and electroconvulsive therapy (Goldapple, Segal et al. 2004; Lozano, Mayberg et al. 2008). These regions include the SCG, hippocampus, and dorsal, ventral and medial frontal

cortices, ventrolateral prefrontal and anterior cingulate cortices, as described above. This suggests that these very diverse treatments may exert their antidepressant effects through neural substrates that are common to all treatments.

DBS mechanisms in depression

DBS has been shown to mimic the effects of ablative surgery of the thalamic nucleus ventralis intermedius for treatment of Parkinson's disease (Benabid, Pollak et al. 1987). This led to the hypothesis that DBS creates a reversible lesion by inhibiting the neurons undergoing stimulation (Giacobbe, Mayberg et al. 2009). However, given the evidence of time course, this seems to be an incomplete answer at best. It also cannot be assumed that mechanisms of DBS in the thalamic nucleus for Parkinson's disease are similar to mechanisms in the SCG for depression. It is understandably difficult to examine possible mechanisms of SCG DBS in human subjects. However a rat model of this treatment has recently been formulated to better understand its mechanisms.

A rat model of DBS

Neural and chemical mechanisms underlying DBS are still unknown. In order to investigate this further, Hamani et al. devised a rat model of clinical DBS by stimulating the ventral-medial prefrontal cortex (vmPFC), and using similar stimulation parameters as used in the clinical setting (Hamani, Diwan et al. 2010b). Based on anatomical connections and known functions, the infralimbic region of the vmPFC is considered to be the rat analog of the SCG (Gabbott, Warner et al. 2003; Hamani, Diwan et al. 2010b). Antidepressant-like effects of vmPFC DBS were assessed using the forced swim test (FST), a well-established tool for investigating antidepressant activity. DBS-treated rats showed a 45% decrease in immobility (Hamani, Diwan et al. 2010b). This effect was confirmed in another model, the novelty-suppressed feeding test. However vmPFC DBS did not have an effect on the learned helplessness paradigm. More recently, two weeks of daily DBS reverted anhedonia-like behaviour induced by chronic mild stress (Hamani et al, in submission).

An investigation into the possibility of local inactivation as the mechanism of DBS effect was pursued. Local axons and neuronal cell bodies of the vmPFC were ablated using radiofrequency lesions, and ibotenic acid lesions were applied in the same area to target cell

bodies only. Local muscimol injections were used for functional inactivation, however none of these three procedures produced a significant effect (Hamani, Diwan et al. 2010b). Therefore, it can be ruled out that antidepressant-like behaviours in the FST after stimulation could have been caused by local inactivation.

To investigate the roles of monoamines in the observed DBS effects, rats were monoamine depleted. First, rats were injected with 5, 7-dihydroxytryptamine into the raphe nuclei to selectively deplete 5-HT neurons, which abolished DBS effects (Hamani, Diwan et al. 2010b). 5-HT release in the hippocampus was also increased fourfold by 1 hour of DBS stimulation, as measured by microdialysis (Hamani, Diwan et al. 2010b). Next, rats were injected with *N*-(2-chloroethyl)-*N*-ethyl-2-bromobenzylamine to selectively deplete NE, but this did not demonstrate an effect on FST results (Hamani, Diwan et al. 2010b). Therefore, for vmPFC DBS effects on the FST for rats, it is apparent that the 5-HT system is required for effect, but the NE system does not appear to be involved with the antidepressant-like effects.

In summary, DBS of the SCG shows promise for treatment-resistant major depression patients. The mechanisms for its effect are still unknown, but imaging studies have confirmed that this treatment produces regional glucose metabolism changes in the same regions as do pharmacotherapy, CBT, and ECT. This suggests that there are common pathways to all of these very diverse antidepressant treatments. A rat model of SCG DBS has been created and verified using several widely accepted behavioural measures. There are, however, a number of questions to be answered. It has still not yet been addressed as to whether or how DBS overlaps with any other antidepressant treatment with respect to underlying brain changes, or whether it involves unique brain mechanisms. This general question provides the impetus for the current research. Although it is still unclear as to exactly how antidepressants cause their effects, it has been repeatedly demonstrated that neuroplasticity plays an important role. Cytogenesis can be required for antidepressant effect, and its reduction in depression could be an underlying factor for the gross morphological changes that are observed in imaging studies including metabolic and volumetric reductions. Both CREB and BDNF, as mediators of neuroplasticity, are also considered to be intimately involved in antidepressant effect, as they are each up-regulated

by antidepressant treatments, and further, simply over-expressing either protein leads to antidepressant effects.

Does DBS for depression, as other antidepressant treatments have demonstrated, involve up-regulation of neurogenesis, BDNF and/or CREB?

1.5 Summary

Major depression is a prevalent disease with no known cause. Although treatments are available, it is not entirely clear how they mediate their antidepressant effect, or why some individuals are resistant to certain treatments.

An exploration into the mechanisms underlying depression reveals several interconnected systems. Imaging studies have revealed robust metabolic and volumetric effects within the brain in depression. The importance of monoamine systems in mood regulation has been well established, and an important role for monoamine receptors in mediating antidepressant response is clear. The serotonin system is reciprocally stimulatory with the HPA axis, and the stress response is known to precipitate depression and mediate many of its symptoms. It is thought that stress leads to reduced hippocampal volume, as demonstrated in depression, via neurotoxicity and reduced cytotogenesis, both of which are reversed by antidepressant stimulation of neuroplastic effects. BDNF and CREB are known to mediate neuroplasticity, are both increased by antidepressant treatment, and over expression of each of these proteins is sufficient to induce an antidepressant response. Cytogenesis can be required for antidepressant effect. The roles of cytotogenesis, BDNF and CREB in mediating antidepressant response have been demonstrated for various antidepressant treatments and to date represent common targets of antidepressant treatment.

Treatments may range from psychotherapy, to pharmacotherapy, and even surgical treatments, which all share common antidepressant effects. It is unknown why all these very diverse treatments are effective against the same disease, or whether they share any common mechanisms. Resistance to treatment is a common occurrence, and for these patients a new treatment has been developed: deep brain stimulation. It is yet unknown how DBS mediates

its antidepressant effects, or whether it shares any of the same targets as other treatments. Given that it alters glucose metabolism in the same brain regions as other antidepressant treatments, it is suggested that they all share common targets that mediate their effects.

1.6 Objectives and hypotheses

The objective of this study was to determine whether DBS elicits its antidepressant effects through mechanisms common to all antidepressant treatments, if they exist, or whether instead DBS is a qualitatively different treatment with entirely different mechanisms. To address this, we examined the effects of DBS and three clinically relevant antidepressants (fluoxetine, amitriptyline, and moclobemide) of different classes on markers of neuroplasticity in the rat brain. We focused on their effects on neuroplasticity, which appear to be common to antidepressants of various classes. The most widely implicated mediators of the neuroplastic effects of antidepressants are BDNF and CREB, which are important to neurogenesis, which involves several stages including cytodifferentiation, cell survival and neuronal differentiation.

Hypothesis 1: All effective antidepressant treatments, including DBS, increase BDNF transcription.

Based on the findings by Nibuya et al. (1995), it was hypothesized here that DBS, as has been demonstrated for pharmacological antidepressants and ECT, also increases BDNF transcription. This may potentially be one of the pathways common to all treatments studied.

CREB is another important mediator of neuroplasticity. It is required for antidepressant-induced upregulation of BDNF, and CREB constitutively regulates BDNF transcription. CREB is decreased in depression, and its over expression is sufficient to induce antidepressant-like effects. CREB is activated to its functional form by phosphorylation, where it initiates the transcription of a variety of genes.

Hypothesis 2: All effective antidepressant treatments, including DBS, increase CREB phosphorylation.

It has been demonstrated that both CREB mRNA and phosphorylation of CREB protein are increased by a variety of antidepressants, and it is hypothesized here that DBS also induces the same effects.

Neurogenesis is an important component of structural plasticity. The importance and functions of new neurons are varied, as discussed above. Hippocampal volumes are often reduced in depressed individuals, and it is postulated that reduced cytotogenesis is a contributing factor. Cytogenesis is reduced by stress, and has been demonstrated to be required for antidepressant effects by several different antidepressant treatments.

Hypothesis 3: All effective antidepressant treatments, including DBS, increase cytotogenesis within the dentate gyrus of the hippocampus.

Cytogenesis is increased by a variety of antidepressant treatments, as well as by exercise (which is known to produce antidepressant effects), and also causes increased synaptic potentiation and memory formation.

BDNF and trophic factors are among several contributors to cell survival following proliferation. Stress has been well documented to increase cellular atrophy within the hippocampus and decrease cell survival, and cellular atrophy may be a contributing factor to decreased hippocampal volume in depression. In order for newly proliferated cells to gain functional importance and contribute to hippocampal connectivity, they must resist atrophy and survive long enough to differentiate and incorporate into existing circuitry, such as into hippocampus-dependent memory circuits, as described above.

Hypothesis 4: All effective antidepressant treatments, including DBS, increase proliferated cell survival and neuronal differentiation within the dentate gyrus of the hippocampus.

An increase in new neurons is induced by antidepressants, and represents a major functional implication of increased cytotogenesis. BDNF is known to increase cell survival, pCREB increases BDNF expression, and increased cytotogenesis increases opportunities for greater numbers of surviving cells. In order for these new cells to be incorporated into existing circuits, they must differentiate to become neurons. All of these factors are known to support

hippocampal learning and memory circuits, and incorporation of new neurons into memory circuits requires cell survival and neuronal differentiation.

1.7 Overall study strategy

The aim of this study was thus to compare effects of chronic DBS with those of various antidepressant drugs in pursuit of similarities in mechanisms. As such, several different classes of antidepressants with different pharmacological profiles were selected.

Rats were treated daily with antidepressant treatment or with an appropriate control condition for 21 days (see Table 3 on page 33). The antidepressant treatments were: fluoxetine, amitriptyline, moclobemide and DBS and the control conditions were saline-injections or surgery-control, respectively.

Fluoxetine was selected as it is the most widely studied antidepressant agent, and SSRIs are commonly used as first-line treatment. The tricyclic amitriptyline was selected as it is still widely prescribed and is known to be very effective against depression. It is a common second-line treatment. The reversible and selective MAOI moclobemide was also chosen for its efficacy and clinical relevance (Hollingworth, Burgess et al. 2010).

BDNF mRNA and pCREB were quantified as two measures of neuroplasticity. Cytogenesis was quantified using the Ki-67 protein as a marker (see Figure 2 on page 30). This protein is expressed only during cell division, and is not detectable in post-mitotic cells. Its validity for use as a cytogenesis marker has been well-established (Wojtowicz and Kee 2006). The survival of proliferated cells was quantified using the thymidine analog 5-bromo-2-deoxyuridine (BrdU) as a marker. The exogenous thymidine analog BrdU was injected on treatment day 14, which is 7 days prior to sacrifice, to mark survival of labeled progenitors and their progeny from post mitotic day 7 until sacrifice (Figure 2). The number of immature neurons was quantified as a measure of neuronal cell differentiation. This was achieved by staining for the endogenous protein doublecortin, which is a microtubule associated protein involved in differentiation and migration of young neuronal cells, and is expressed most strongly from post-mitotic cell day 7 to 14, but is faintly detectable from birth to beyond cell

day 21 (Brown, Couillard-Despres et al. 2003; McDonald and Wojtowicz 2005). Therefore, doublecortin staining measures the differentiation of proliferated neuronal cells, mainly between post-mitotic days 7 and 14 (Figure 2).

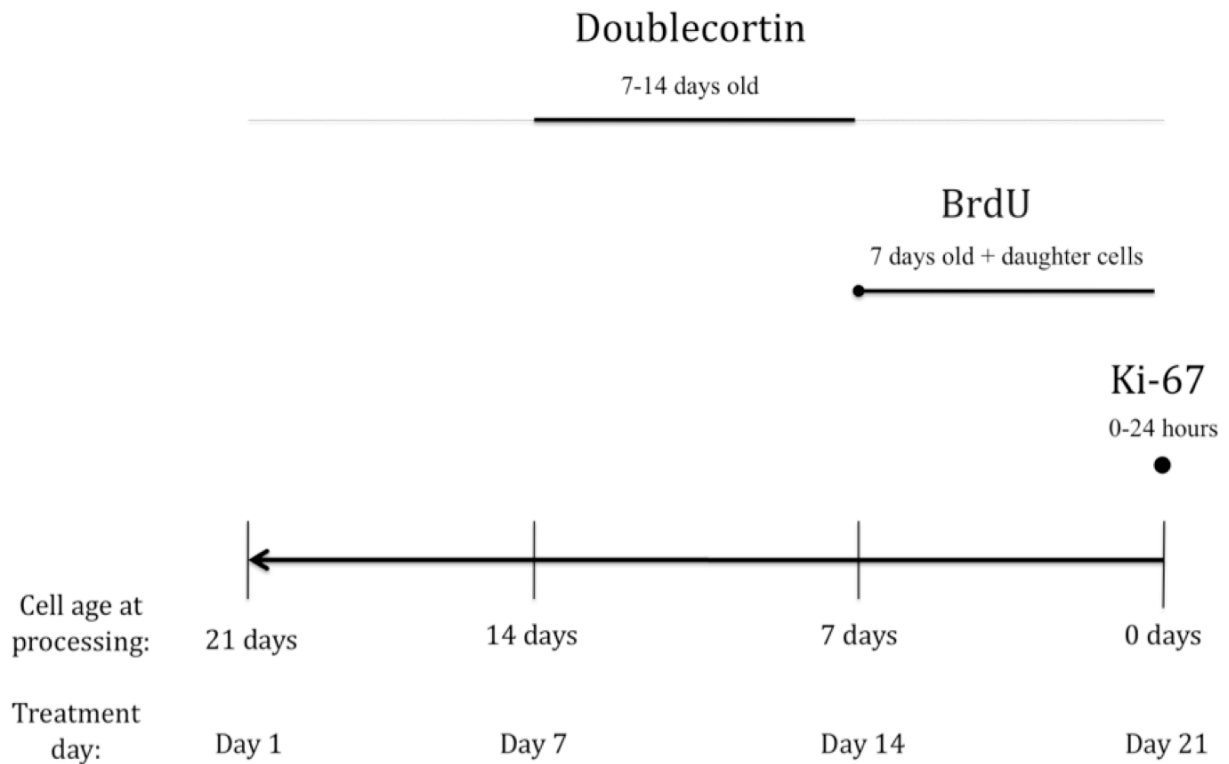


Figure 2. Neurogenesis Timeline. Ki-67 is a marker for cytogenesis that has occurred 0-24 hours from sacrifice. BrdU was injected on treatment day 14, and marks surviving cells proliferated on that day plus any surviving daughter cells. Doublecortin marks immature granule neurons, therefore cells that have undergone neuronal differentiation. The doublecortin signal is expressed most strongly from post-mitotic cell day 7 to day 14, but can be detectable from birth to beyond cell day 21.

Chapter 2

Materials and Methods

Procedures conformed to the recommendations of the Canadian Council on Animal Care and the Centre for Addiction and Mental Health Animal Care Committee approved the protocol. Experiments were performed on 59 adult male Sprague-Dawley rats (250-300g, Charles River, Wilmington MA).

2.1 Surgical procedures

Sterile surgery was performed on twelve DBS rats, under anesthesia induced and maintained with ketamine / xylazine (100/7.5 mg/kg i.p.). They were also injected with 0.9% saline (1 mL) for fluid loading. Effective anesthesia was determined by abolition of pedal withdrawal reflex. Rats were then shaved over the skull, and were fixed onto a stereotaxic frame, ensuring that the skull was parallel to the table. Towels were used to maintain an appropriate body temperature, and saline was periodically dropped onto the eyes to prevent them from drying. The skin over the skull was cleaned and lidocaine hydrochloride was injected (0.2 mL) as a local anesthetic.

The skin over the skull was opened using a surgical blade, and held using forceps in a position to allow access to the skull. Any blood was cleared and holes were drilled into the skull over where the electrodes were to be inserted. Surrounding this area, four epidural screws were secured into the skull to anchor the cap and electrodes. Two insulated stainless steel stimulating electrodes (250 μ m diameter with 0.5 mm of exposed surface) were bilaterally implanted into the ventral-medial prefrontal cortex, with the following stereotaxic coordinates: anteroposterior +3.0 mm, lateral +/- 0.5 mm, and depth 5.6 mm (Paxinos and Watson 1997). An insulated stainless steel electrode (125 μ m diameter) was attached to the epidural screws and used for grounding. Electrodes were fixed into a plastic pedestal (Plastics One) and onto both the skull and epidural screws using dental acrylic cement. The

pedestal was used to connect the stimulator to the electrodes during stimulation. Once the dental cement had dried, the skin was sutured together surrounding the plastic pedestal protruding from the skull. A topical antibiotic was administered, and the rats were returned to their home cages, to be monitored for the next several hours until the anesthetic wore off and rats returned to normal behaviour. Rats were given seven days of post-surgical recovery before beginning twenty-one days of stimulation, as described below.

Twelve surgical-control rats underwent similar surgical procedures, had holes drilled into the same area of the skull, but electrodes were not implanted. The skin over the skull was sutured back into place, and rats were allowed to recover as above.

2.2 Antidepressant administration, electrical stimulation, and BrdU administration

All rats were individually housed with ad libitum access to food and water in a room maintained at a constant temperature (20–22°C) on a 12 hour : 12 hour light–dark cycle, where lights were turned on at 8 a.m. Ten saline-treated rats were injected once daily with 2.5 mL/kg IP of 0.9% saline. Fluoxetine-hydrochloride (Spectrum Chemical Mfg. Corp., New Brunswick NJ) was dissolved in 0.9% saline and administered as 10 mg/kg IP injections to 10 rats at a volume of 2.5 mL/kg once daily (Qi, Lin et al. 2008; Reines, Cereseto et al. 2008). Amitriptyline-hydrochloride (Sigma-Aldrich, Oakville ON) was dissolved in 0.9% saline and administered once daily in 10 mg/kg IP injections to 9 rats at a volume of 2.5 mL/kg (Jain and Subhedar 1993). Lastly, moclobemide (AmplaChem, Carmel IN) was dissolved in sterile H₂O and also given in 10 mg/kg IP injections to 10 rats at a volume of 2.5 mL/kg once daily (Vilpoux, Carpentier et al. 2002) (see Table 3). All doses selected were based on published doses required to induce behavioural antidepressant response in rats.

DBS rats underwent daily stimulation in their home cages, for 4 hours at approximately the same time each day (from about 9:00 am to 1:00 pm). Stimulation was conducted using a stimulator (ANS, model 3510, Plano, TX) that was connected to the animals through extension cables and a multi-channel commutator (Plastics One, Roanoke, VA). Rats were allowed to roam freely in their cage, as normal. Given that the radius of the electrodes used

was 0.125 mm, and the height of the exposed surface was approximately 0.5 mm, the area of the exposed surface was approximately 0.4 mm² for each electrode. Based on this area, current and frequency of stimulation were set at 100 μ A and 130 Hz, respectively, with a pulse width of 90 μ s, using the optimal values determined by Hamani et al. (Hamani, Diwan et al. 2010a) which approximate the settings used in patients (Lozano, Mayberg et al. 2008). Surgery-control rats were handled daily in the same manner as the DBS-treated rats during the 21 day period, but were not connected to the stimulator.

BrdU was administered to rats of all treatment groups to label the number of surviving cells from proliferation on day 14 of the 21 treatment days, as well as the number of daughter cells that survived following treatment day 14 until treatment day 21 (see Figure 2 on page 30). All rats were administered 2 x 200 mg/kg i.p. injections of BrdU at a volume of 10 mL/kg on day 14 of the 21 treatment days (McDonald and Wojtowicz 2005). The 2 injections were administered 5 hours apart, for maximal BrdU absorption.

Table 3: Treatment Groups, Doses, Duration, and Final Group Sizes

Group	Dose	Duration	n
Saline	0.9%, 2.5 mL/kg IP	1 injection daily / 21 days	10
Fluoxetine	10 mg/kg, 2.5 mL/kg IP	1 injection daily / 21 days	10
Amitriptyline	10 mg/kg, 2.5 mL/kg IP	1 injection daily / 21 days	9
Moclobemide	10 mg/kg, 2.5 mL/kg IP	1 injection daily / 21 days	10
Surgery-control	None	21 days	12
DBS	100 μ A, 130 Hz	4 hours daily / 21 days	8

2.3 Sacrifice and histology

Twenty-four hours after the final antidepressant treatment, rats were deeply anesthetized with an IP injection of sodium pentobarbital, and once the pedal withdrawal reflex had been abolished, they were transcardially perfused with 0.9% saline at 4°C containing 2mL/L heparin, for five minutes.

This was followed by sacrifice via decapitation using a guillotine. Brains were immediately removed from the skull, while not disrupting proper morphology, then were cut in half by a sagittal slice down the midline. One half of the brain was post-fixed overnight in 4% paraformaldehyde solution for immunohistochemical staining, and the other half was immediately frozen at -80°C for in situ hybridization. The paraformaldehyde solution contained 4% paraformaldehyde that was heated to 60°C to dissolve in phosphate-buffered saline (PBS), which was then filtered, and lastly the pH of the solution was adjusted to 7.4 with NaOH.

Following overnight fixation, the half-brains were transferred to phosphate buffered solutions containing increasing sucrose concentrations of 10%, 20%, then finally 30%, for cryoprotection. The phosphate buffer contained: 0.01% thimerosal, 2.1% $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.5% NaOH, dissolved in dH_2O . Brains were transferred to the higher concentration once saturated, and after 30% sucrose saturation they were frozen at -80°C until sectioning.

The post-fixed halves of all the brains were sectioned in 40 μm coronal sections on a cryostat, and equivalent sets containing every eighth hippocampal section were prepared for: (a) immunohistochemistry for pCREB, (b) immunohistochemistry for Ki-67, (c) immunohistochemistry for BrdU, (d) immunohistochemistry for doublecortin (DCX), and (e) histological verification of electrode placements. Sections for immunohistochemistry were placed free-floating into a cryoprotectant solution, and stored at -30°C until stained. The cryoprotectant contained 45% phosphate buffer (same as above), 30% ethylene glycol, and 25% glycerol.

The unfixed halves of all the brains, which were immediately frozen at -80°C following sacrifice, were sectioned in $20\text{ }\mu\text{m}$ coronal sections on a cryostat and thaw-mounted onto Superfrost/Plus slides (Fisher, Toronto ON) and kept at -80°C until further processing.

2.4 In situ hybridization for BDNF

Prehybridization rinses were performed in an RNase-free environment. Slides were rinsed in 4% paraformaldehyde (in PBS; pH 7.3), rinsed twice in PBS, and then incubated in triethanolamine hydrochloride solution (1.86 % triethanolamine hydrochloride in water; pH 8.0) for 5 minutes. Slides were then incubated in acetic anhydride solution (0.25% acetic anhydride in triethanolamine hydrochloride solution) for 10 minutes, and rinsed twice in 2x saline sodium citrate buffer (SSC, 1x = 0.441 % tri-sodium citrate, 0.875% sodium chloride in water; pH 7.0). Slides were dehydrated in graded alcohols at 70%, 80%, 95% and 100% ethanol, incubated in chloroform for 5 minutes, and rinsed in 100% followed by 95% ethanol.

^{35}S -UTP-labeled riboprobes were prepared from primers complementary to GenBank # NM_012513.3 (bases 900-919 and bases 1254-1235). Left and right primers were reconstituted in 10mM Tris-HCl (0.12% Tris; pH 7.6). Primer mixes were added to PCR supermix and cDNA of rat hippocampus, and incubated in a thermal cycler to undergo polymerase chain reaction (PCR).

The radioactive probe was put together as follows. The PCR product from above was added to transcription buffer, ATP mix, CTP mix, GTP mix, radioactive [^{35}S] UTP, and RNA Polymerase, and incubated for 1 hour at 37°C . DNase I was then added, and the mix was incubated at 37°C for 15 minutes. Lastly, 0.5M EDTA was added. The radioactive RNA transcript was purified using a spin column (NucAway, Ambion, Austin, TX).

Slides were hybridized overnight at 60°C in slide mailers containing the ^{35}S -UTP-labeled riboprobe, 50% formamide, 35% Denhardt's solution, 10% dextran sulfate, 0.1x SSC, salmon sperm DNA (300 $\mu\text{g}/\text{mL}$), yeast tRNA (100 $\mu\text{g}/\text{mL}$), and dithiothreitol (40 μM).

Slides were rinsed following hybridization to remove excess radioactivity thereby ensuring the specificity of the signal. Slides were rinsed twice in 4x SSC (containing 1g sodium thiosulfate) at 60°C, followed by rinsing in RNase A solution (50% NaCl, 0.1% EDTA, 1% tris-HCl (pH 8.0), 0.2% RNase A) at 45°C. Slides were then rinsed twice in 2x SSC at room temperature, followed by 0.5x SSC at 60°C. Lastly, slides were rinsed in 0.1x SSC first at 60°C, then at room temperature, dipped in water, and finally dipped in 70% ethanol. Upon drying, slides were exposed to Kodak Biomax film for 6 days at 4°C before development.

2.5 Hybridization image acquisition and analysis

Autoradiograms of hippocampal sections were used to quantify BDNF mRNA. Using calibrated radioactive standards, the optical density on film was converted to amount of radioactivity per gram of tissue, which reflects the quantity of BDNF mRNA present. The CA1, the CA3 and the dentate gyrus hippocampal subregions were examined using image analysis software (MCID Basic, InterFocus, Inc., Leiton, UK) under blind conditions regarding the identity of the sections. Signal was averaged for all slices within each brain, and averaged again for all subjects in each treatment group.

2.6 Immunohistochemistry for pCREB, Ki-67, DCX and BrdU

For detection of cells expressing the pCREB antigen, tissue sections were first incubated in citrate buffer for 30 minutes at 80°C, followed by 0.3% H₂O₂ for 30 minutes, then in blocking solution (3% goat serum/1% BSA/0.3% Triton X-100 in PBS) for 2 hours at room temperature. Sections were then incubated for 48 hours in rabbit monoclonal anti-pCREB antibody (PhosphoSolutions, Aurora, CO) at a concentration of 1:1000 (in blocking solution) at 4°C. After washing, sections were incubated with biotinylated goat anti-rabbit secondary antibody (Vectastain, Vector Laboratories, Burlington ON) for 2 hours, followed by incubation in horseradish peroxidase avidin-biotin complex solution, diluted in PBS/0.5% Triton X, for 1 hour at room temperature. The horseradish peroxidase reaction was detected using a nickel-intensified diaminobenzidine (DAB) reaction (0.05% 3,3'-diaminobenzidine)

in the presence of 0.01% H_2O_2 and nickel chloride (Vector Laboratories, Burlington ON). The sections were mounted onto glass slides and air-dried. They were dehydrated in graded ethanol solutions of 50%, 75%, 95% and 100% for 5 minutes in each solution, followed by rinsing in xylene for 5 minutes, and cover-slipping using Eukitt as a mounting medium.

Ki-67 immunohistochemistry was performed identically to pCREB immunohistochemistry, instead incubating for 48 hours in a rabbit monoclonal anti-Ki-67 antibody (Vector Laboratories, Burlington ON) at a concentration of 1:200 (in blocking solution) at 4°C.

Doublecortin immunohistochemistry was performed to stain for immature granule neurons, using the same procedure as pCREB and Ki-67, but instead incubating for 48 hours in rabbit monoclonal anti-DCX antibody (Sigma-Aldrich, Oakville ON) at a concentration of 1:2000 (in blocking solution) at 4°C.

For detection of cells expressing the thymidine analog BrdU, sections were first incubated in 2N HCl for 45 minutes at 60°C. Sections were then rinsed in 0.1 M borate buffer (0.62 % boric acid; pH 8.3) followed by phosphate buffered saline. Slices were incubated in blocking solution (10% horse serum, 1% BSA, 0.3% Triton X-100 in PBS) for 60 minutes at room temperature, followed by incubation for 48 hours in mouse anti-BrdU antibody (Roche) at a concentration of 1:200 (in blocking solution) at 4°C. After washing, sections were incubated with biotinylated horse anti-mouse secondary antibody (Vectastain, Vector Laboratories, Burlington ON) for 2 hours, followed by incubation in horseradish peroxidase avidin-biotin complex solution, which was diluted in PBS/0.5% Triton X, for 1 hour at room temperature. The horseradish peroxidase reaction was detected using a nickel-intensified DAB reaction (0.05% 3,3'-diaminobenzidine) in the presence of 0.01% H_2O_2 and nickel chloride (Vector Laboratories, Burlington ON). The sections were mounted onto glass slides and air-dried. They were dehydrated in graded ethanol solutions of 50%, 75%, 95% and 100% for 5 minutes in each solution, followed by rinsing in xylene for 5 minutes, and cover-slipping using Eukitt as a mounting medium.

2.7 Immunoreactivity image acquisition and analysis

pCREB, Ki-67, BrdU, and DCX immunoreactivity was measured without knowledge of the identity of the brain sections, using a light microscope (Nikon eclipse E600), and image analysis software (MCID, InterFocus, Inc., Leiton, UK). Every eighth hippocampal section was counted. For pCREB, the pyramidal cell layers of CA1 and CA3, and the granular cell layer and hilus of the dentate gyrus were counted, where the density of the signal was measured.

For Ki-67, DCX, and BrdU, immunopositive cells were counted, and for Ki-67 cell clusters were also counted. The subgranular zone is defined as a 2-cell body-wide layer along the base of the granular cell layer. Ki-67-positive cell clusters are defined as three or more Ki-67-positive cells within a close proximity to each other (approximately 5 μm).

The number of labeled cells was expressed as the mean number of immunopositive cells for each marker or immunopositive cell clusters (for Ki-67) in the identified region in each section. The counts and density units were averaged for all slices within each brain, and averaged again for all subjects in each treatment group. Final values were expressed as percentage of the control mean.

2.8 Statistical analyses

For all markers, statistical analyses were performed using ratios of individual brain values to the mean of the appropriate control group. One-way ANOVAs were performed with Tukey post hoc comparisons, followed by Student's *t* tests. DBS rats were compared to sham surgery controls using the Student's *t* test. Statistical significance was set at $p \leq 0.05$.

2.9 Verification of electrode placements

DBS brain slices were stained with cresyl violet to assess electrode placement. Sections were mounted onto glass slides, and fixed overnight in formalin vapour at 60°C. Sections were dehydrated in graded ethanol solutions (70%, 90% and 100%), then rinsed in xylene for 3 minutes in each solution. This was followed by re-hydration in graded ethanol solutions (100%, 90% and 70%) and rinsing in deionized water for 3 minutes in each solution. Sections were stained in 0.2% cresyl violet with glacial acetic acid added to the solution, for 2 minutes. Sections were then dipped in deionized water to remove any excess stain, and dehydrated in graded ethanol solutions (70%, 90% and 100% 1 minute each) before a final rinsing in xylene for 10 minutes. Slides were then cover-slipped using Eukitt as a mounting medium. Electrode placement was verified using a light microscope (Nikon eclipse E600). Only animals with electrodes placed bilaterally in the infralimbic or prelimbic cortices of the vmPFC were included in the analyses.

Chapter 3

Results

3.1 Electrode placements

Cresyl violet staining was performed on sections from DBS-treated rat prefrontal cortices to determine electrode placement. The placements of electrodes are shown in Figure 3, where the placements range in distance between 3.2 mm and 2.2 mm anterior to bregma. The depth of the electrodes ranged from approximately 3.4 mm to 5.4 mm from the top of the brain. All electrodes were located within the left and right prelimbic and/or infralimbic cortices. Eight of twelve rats had electrodes correctly placed bilaterally in the infralimbic or prelimbic cortices of the vmPFC, and only these eight were included in the analyses.

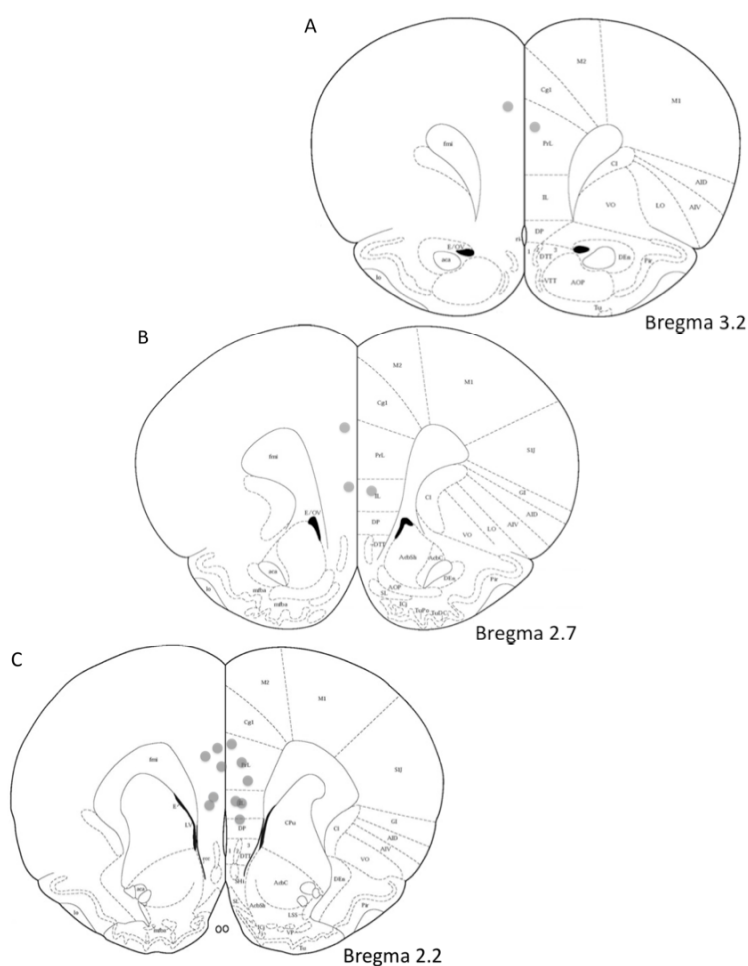


Figure 3. Electrode Placements. The grey circles indicate the locations of the tips of the electrodes for DBS-stimulated animals. Also shown are the respective distances anterior to bregma (Paxinos and Watson 1997).

3.2 BDNF mRNA

As illustrated in Figure 4, BDNF mRNA distribution in control brain slices agreed well with previously reported patterns, with high levels in the cortex, hippocampus and amygdala, and much lower levels in the thalamus. Within the hippocampus pyramidal cell layer, differences can be seen between CA1 and CA3, with the highest levels occurring in the granular layer of the dentate gyrus. These were the hippocampal regions included in the analyses. Results are shown in Figure 5 and are summarized in Table 4 (page 52). Chronic treatment with amitriptyline caused a significant 28% increase in BDNF mRNA in the dentate gyrus ($P = 0.039$), and a 16% increase in the CA1 that failed to reach significance ($P = 0.055$). DBS treatment led to a 21% decrease in BDNF mRNA within the CA1 ($P = 0.035$), and non-significant decreases in the CA3 and the dentate gyrus. Neither fluoxetine nor moclobemide treatment induced any significant changes in BDNF mRNA. No significant changes were observed in the CA3.

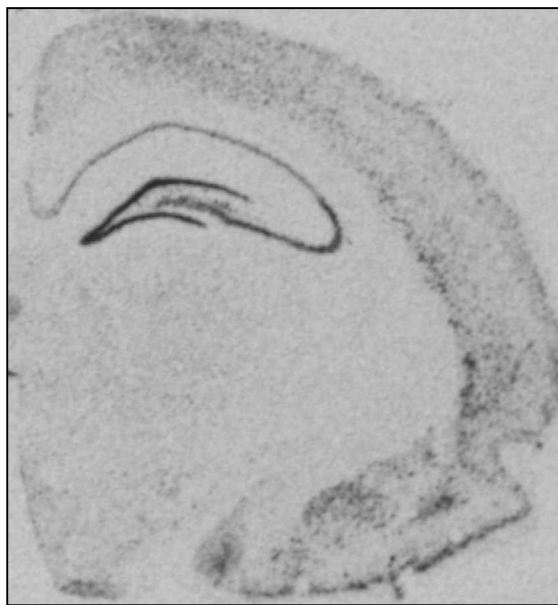


Figure 4. BDNF mRNA In Situ Hybridization.

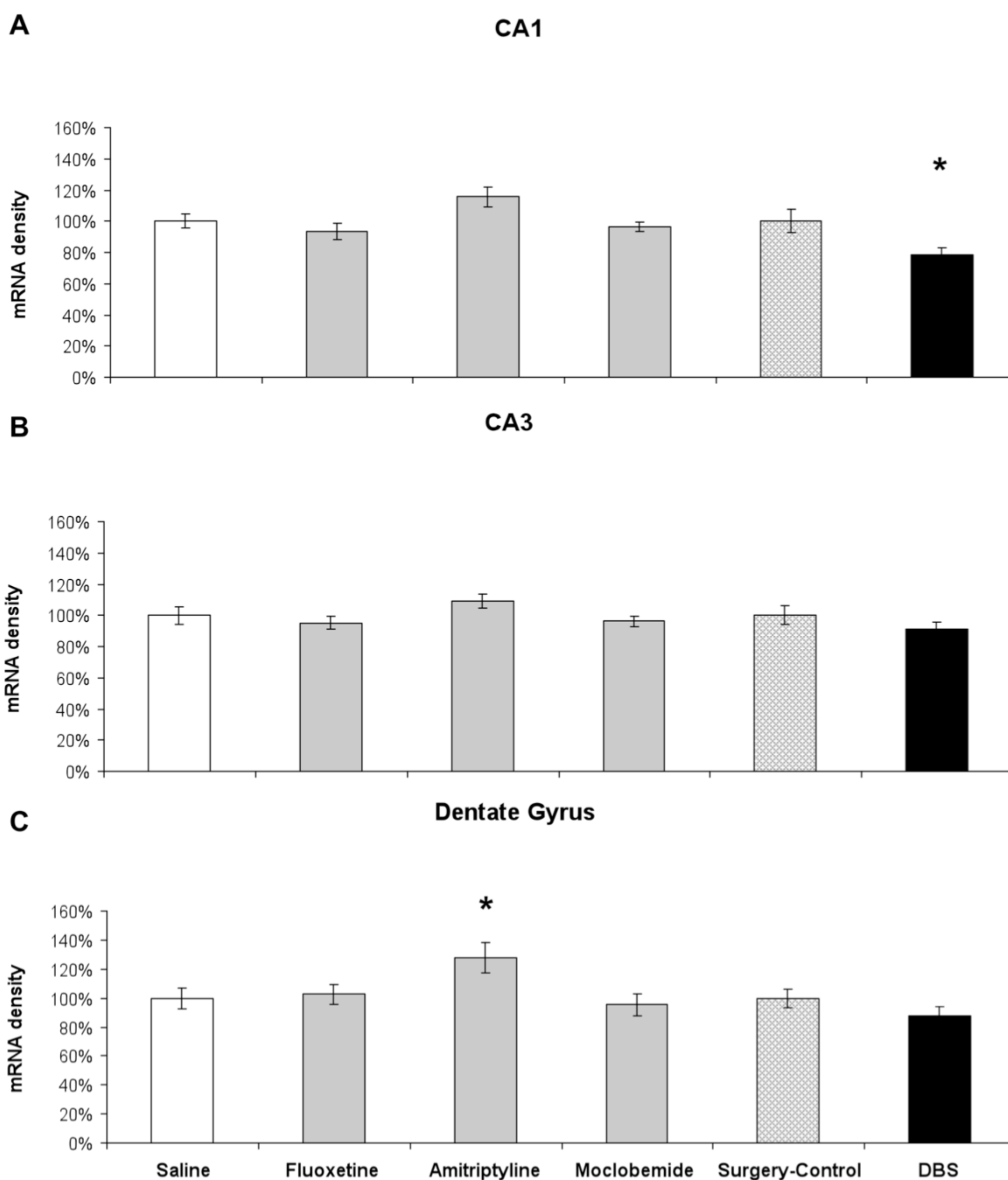


Figure 5. BDNF mRNA Effects. Values are ratios of treatment to appropriate control values (saline vehicle for drug treatments and sham surgery for DBS). Error bars indicate standard error. In CA1 (panel A) a decrease in mRNA density (21%) was observed following chronic DBS treatment ($P = 0.035$). An increase that tended towards significance (16%) was observed following chronic amitriptyline treatment ($P = 0.055$). No significant changes in BDNF mRNA levels were observed in CA3 (panel B). In the dentate gyrus (panel C) an increase (28%) was observed following chronic amitriptyline treatment ($P = 0.039$). Chronic treatment with fluoxetine or moclobemide did not produce significant changes in BDNF mRNA within any region tested. * = $P < 0.05$

3.3 Phosphorylated CREB

Figure 6 illustrates the typical appearance of pCREB-positive cells in untreated control hippocampal slices, with the subregions of interest indicated. Results are shown in Figure 7 and are summarized in Table 4. Although decreases were observed in all four regions after either amitriptyline or moclobemide treatment, and although increases were apparent in most regions after fluoxetine or DBS treatment, none of these effects was statistically significant.

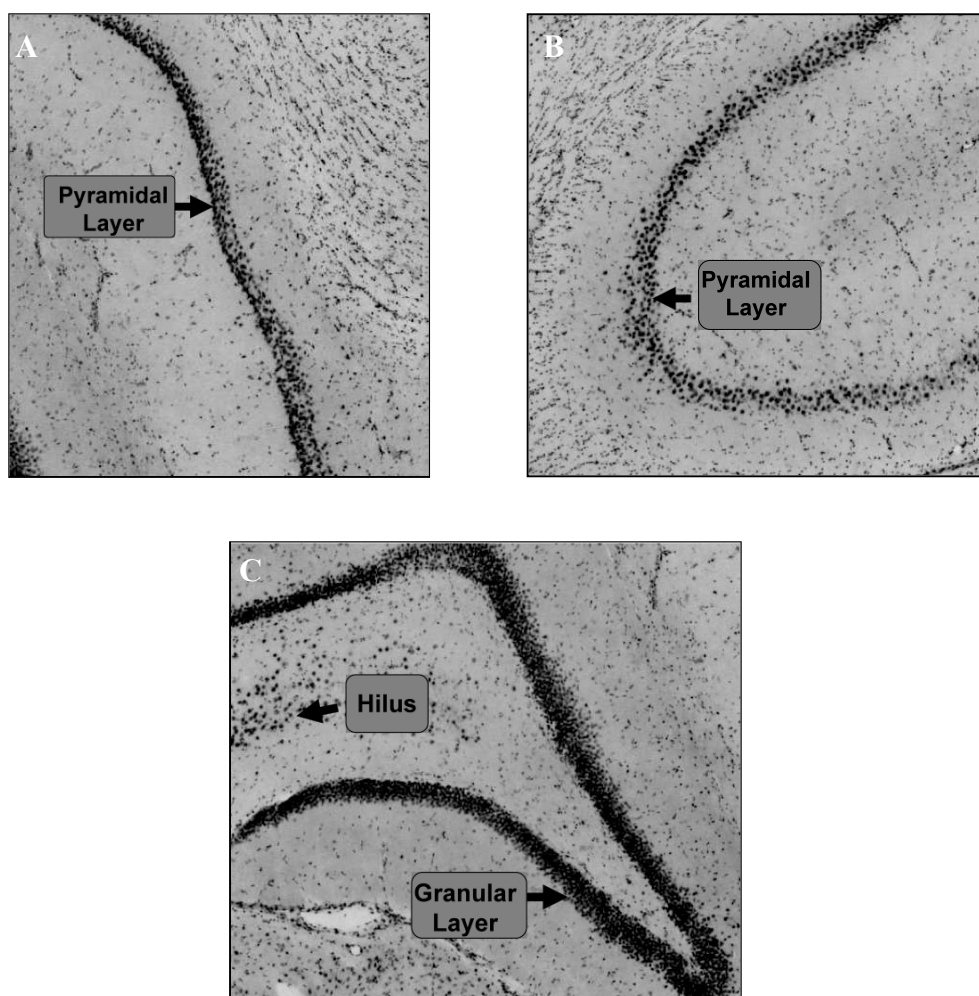


Figure 6. Illustration of pCREB Immunoreactivity Representative photomicrographs of hippocampal pCREB immunopositive cells within the pyramidal cell layer in CA1 (A), CA3 (B), hilus and the dentate gyrus (C).

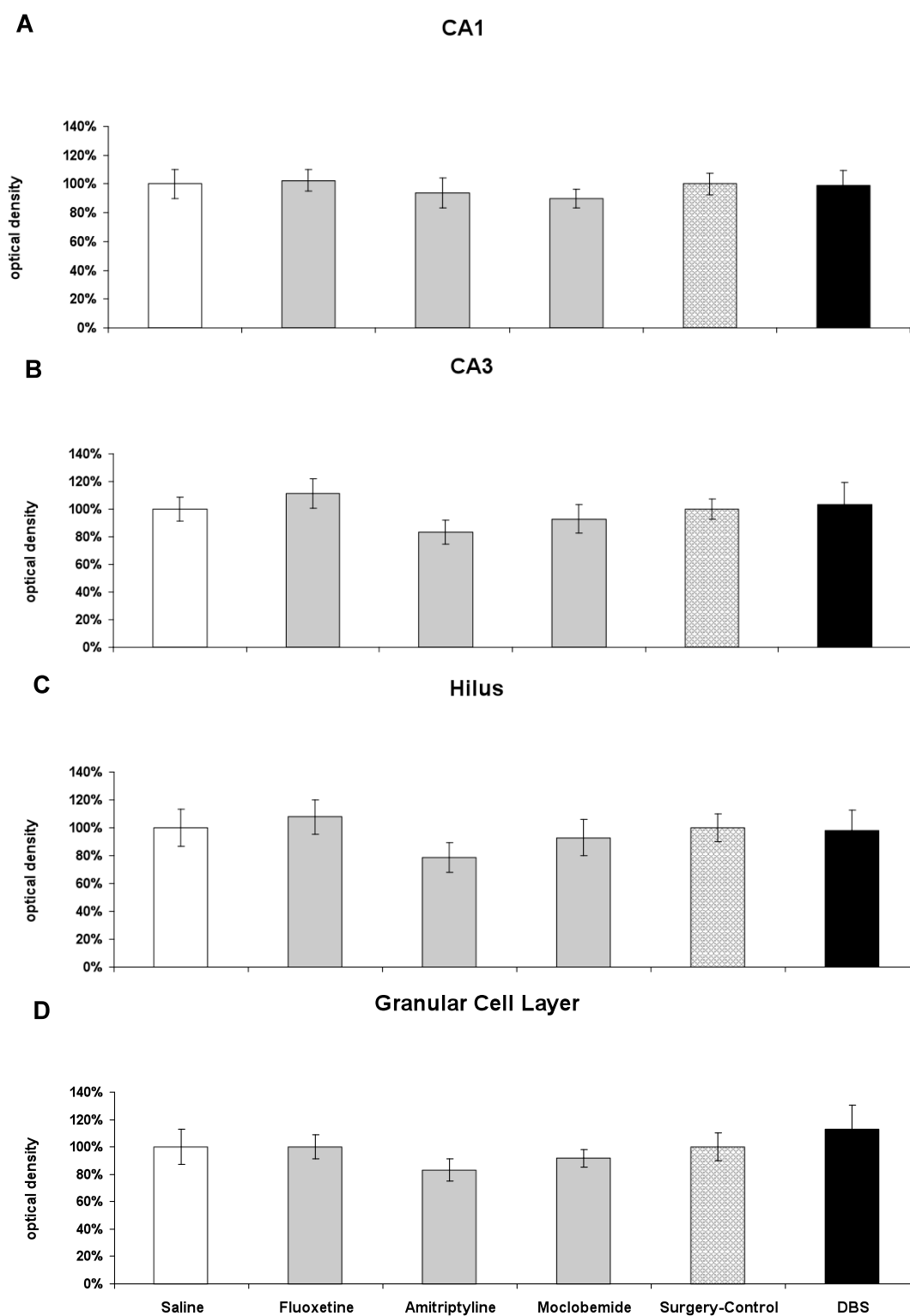


Figure 7. pCREB Effects. Values are ratios of treatment to appropriate controls (saline vehicle for drug treatments, sham surgery for DBS). Error bars indicate standard error. No significant effect of chronic antidepressant treatments or DBS was observed in any of the hippocampal subregions.

3.4 Cytogenesis: Ki-67

Figure 8 illustrates the typical appearance of Ki-67 immunopositive cells in the subgranular zone (SGZ) and the insets illustrate the appearance of cell clusters in each case. Results are shown in Figure 9 and are summarized in Table 4. Chronic treatment with moclobemide caused a 39% increase in cell counts in the SGZ which approached significance ($P = 0.059$), and a 20% increase in the number of cell clusters ($P = 0.097$). Both amitriptyline and DBS treatment induced non-significant decreases in proliferated cell and cluster expression, while chronic fluoxetine treatment did not induce any changes for either measure.

3.5 Cell survival and neuronal differentiation: BrdU and DCX

Figure 10 illustrates the typical appearance of BrdU immunopositive cells in the SGZ and the insets illustrate the appearance of cell clusters in each case. The results are shown in Figure 11 and are summarized in Table 4. All treatments tended to increase the number of BrdU-positive cells, but only DBS increases (81%) were significant ($P = 0.02$). Fluoxetine increased labeling by 96%, amitriptyline by 46%, and moclobemide by 48%.

Figure 12 illustrates the typical appearance of DCX immunopositive cells in the SGZ and the insets illustrate the appearance of cell clusters in each case. DCX results are shown in Figure 13 and are summarized in Table 4. All treatments tended to increase the number of DCX-immunopositive cells, but only in the case of amitriptyline (61%) was this effect significant ($P = 0.009$). Fluoxetine increased labeling by 13%, moclobemide by 16%, and DBS by 8%.

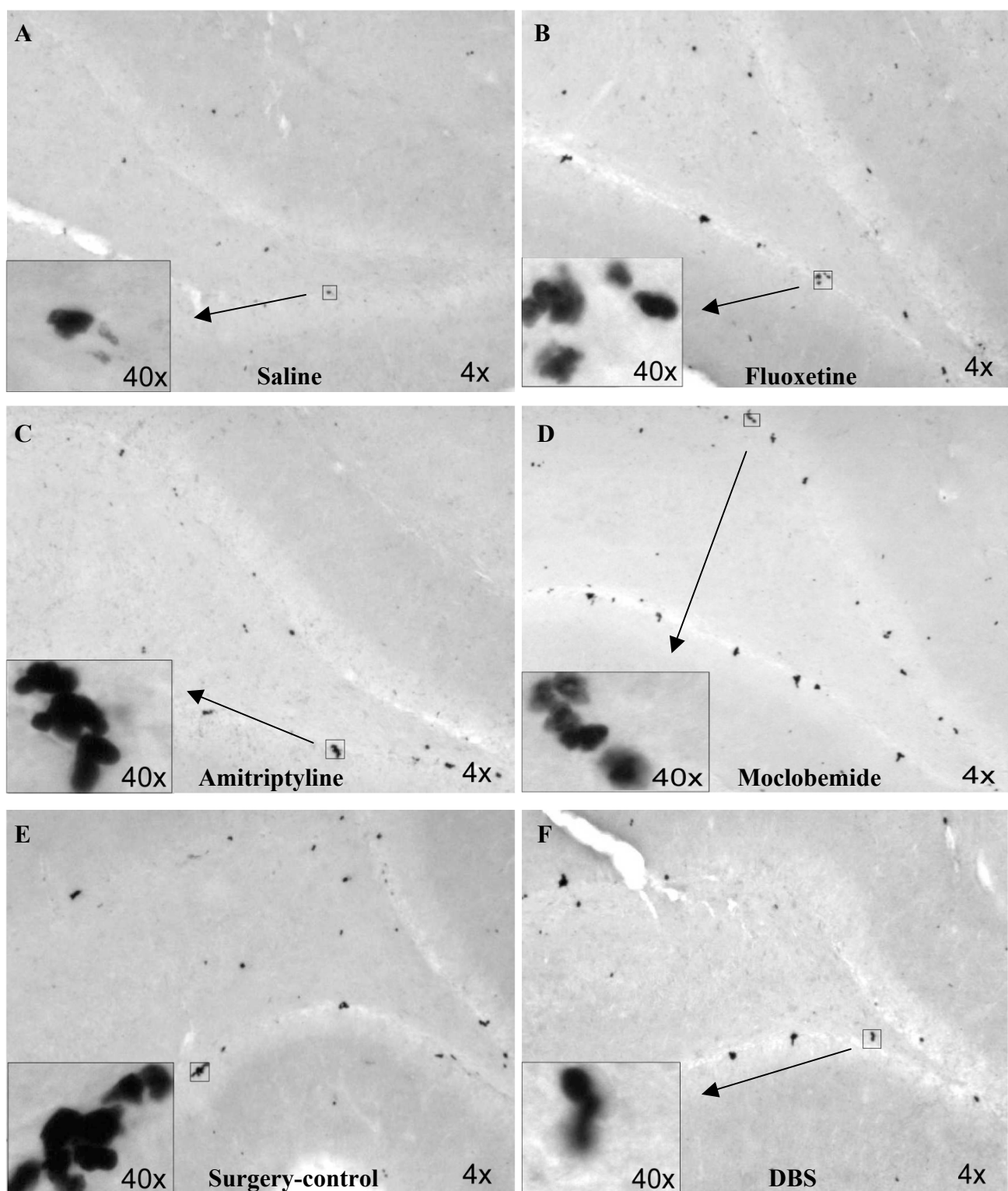


Figure 8. Ki-67 Immunoreactive Cells in Subgranular Zone. Rats were treated for 21 days with saline (A), fluoxetine (B), amitriptyline (C), moclobemide (D), surgery-control (E), or DBS (F). For each treatment, the insets show a typical cluster of cells within the subgranular zone of the hippocampus.

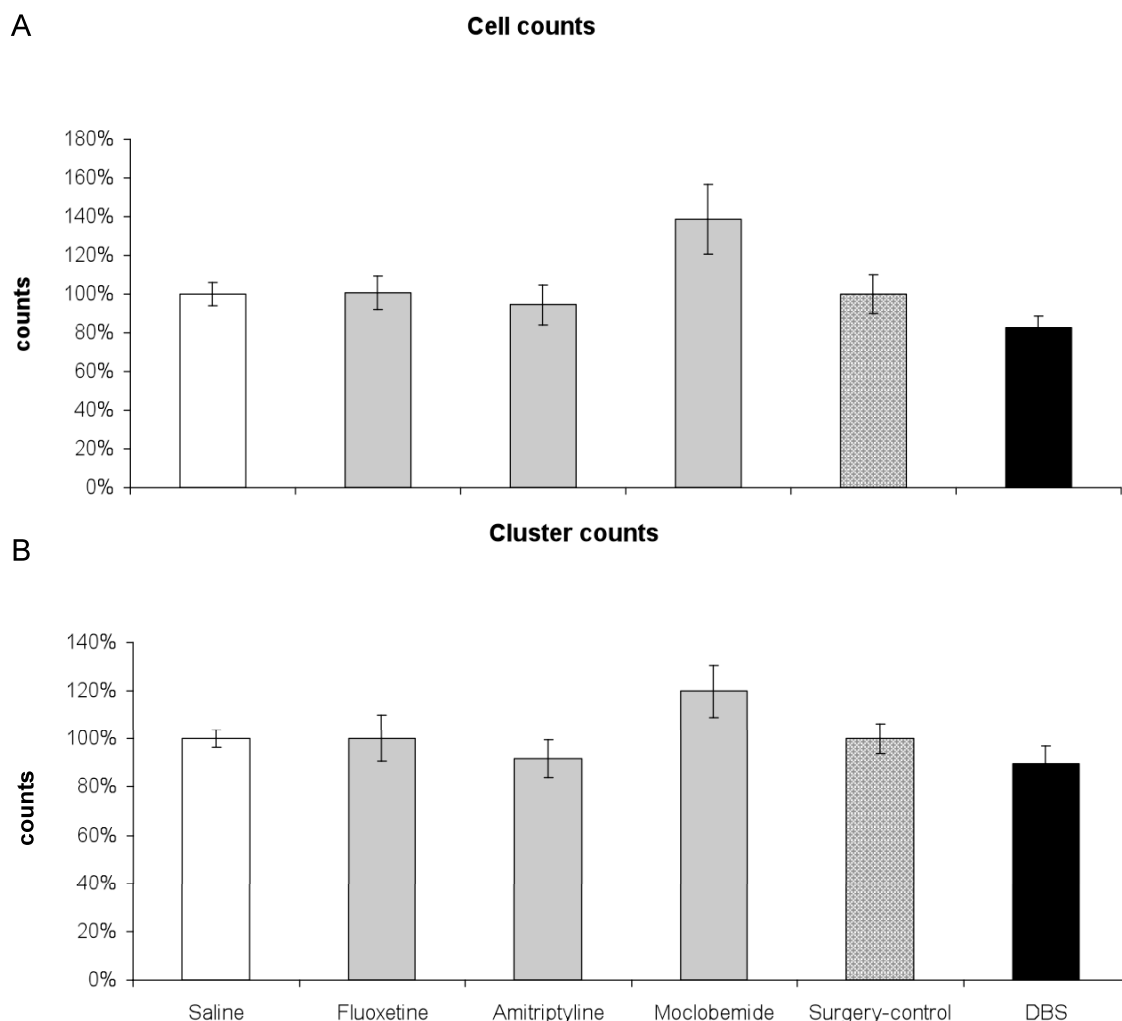


Figure 9. Ki-67 Effects. Values are ratios of treatment to appropriate control (saline vehicle for drug treatment and sham surgery for DBS). Error bars indicate standard errors. (A) An increase in the number of Ki-67-positive cells (39%) was observed following chronic treatment with moclobemide, which just missed statistical significance ($P = 0.059$). (B) A non-significant increase in Ki-67-positive clusters of cells (20%) was observed following chronic treatment with moclobemide ($P = 0.097$). Chronic treatment with fluoxetine, amitriptyline, or DBS did not produce significant changes in numbers of Ki-67 immunoreactive cells or clusters of cells.

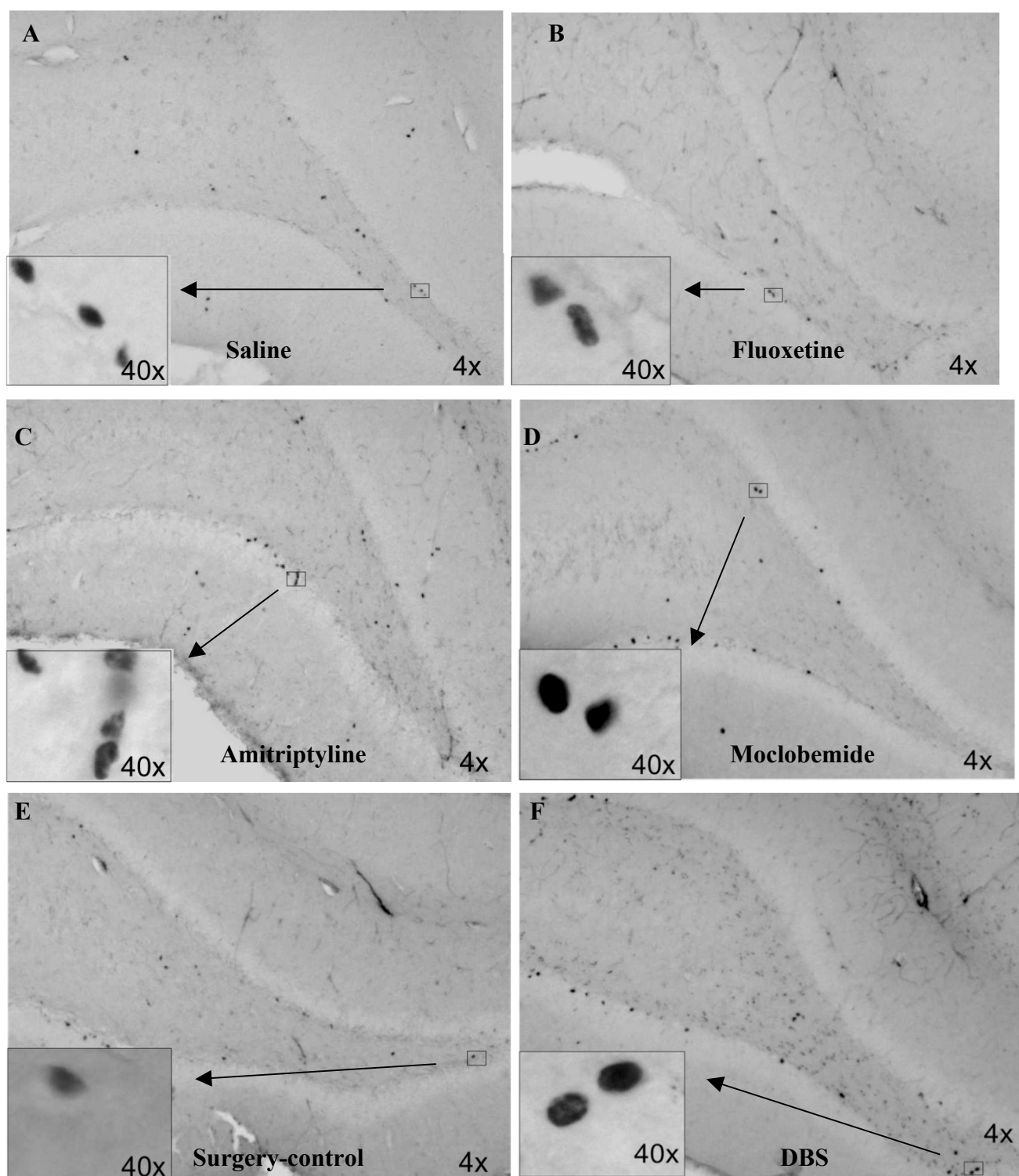


Figure 10. BrdU Immunoreactive Cells in the Subgranular Zone. Rats were treated for 21 days with saline (A), fluoxetine (B), amitriptyline (C), moclobemide (D), surgery-control (E), or DBS (F). BrdU was injected on the 14th day of drug treatment. For each treatment, the inset shows a typical cluster of cells within the subgranular zone of the hippocampus.

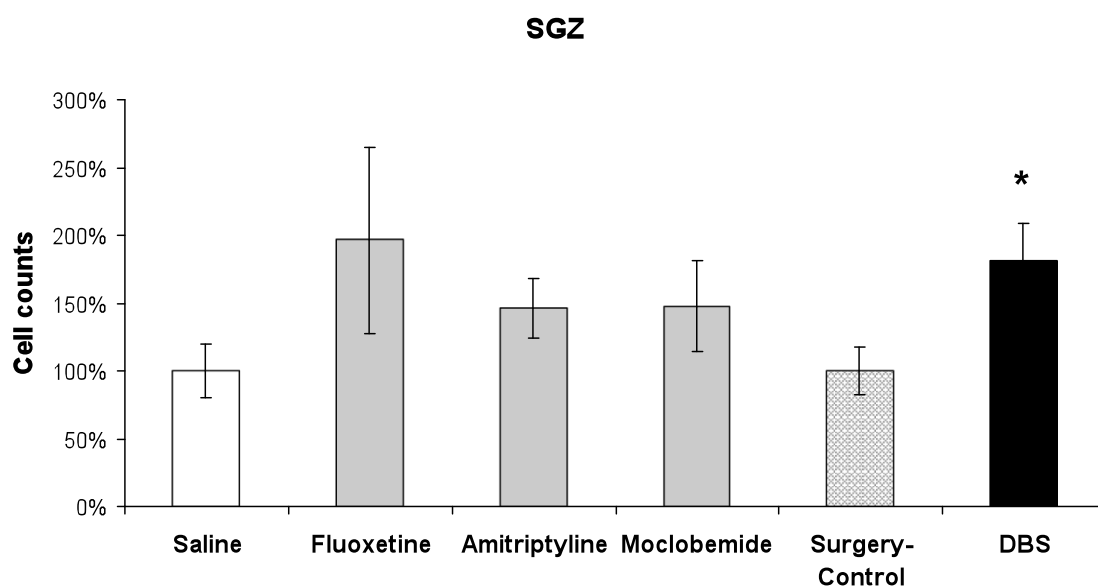


Figure 11. BrdU Effects. Values are ratios of treatment to appropriate control values (saline vehicle for drugs and sham surgery for DBS). Error bars indicate standard errors. A tendency towards increased numbers of BrdU-positive cells was observed following all chronic treatments, but only the DBS effect reached significance (81%, $P = 0.02$).

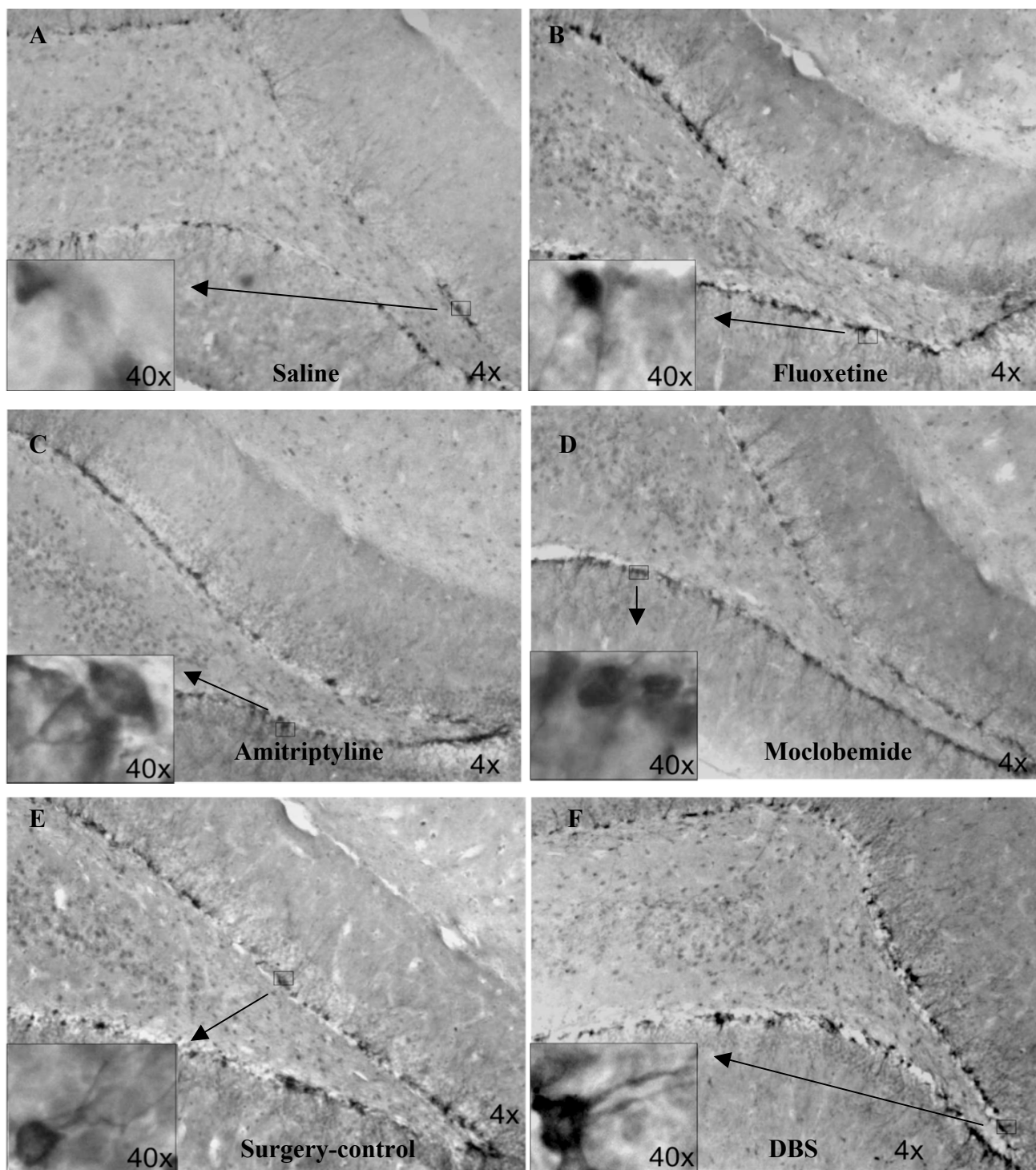


Figure 12. DCX Immunoreactive Cells in the Subgranular Zone. Rats were treated for 21 days with saline (A), fluoxetine (B), amitriptyline (C), moclobemide (D), surgery-control (E), or DBS (F). For each treatment, the inset shows a typical cluster of cells within the subgranular zone of the hippocampus.

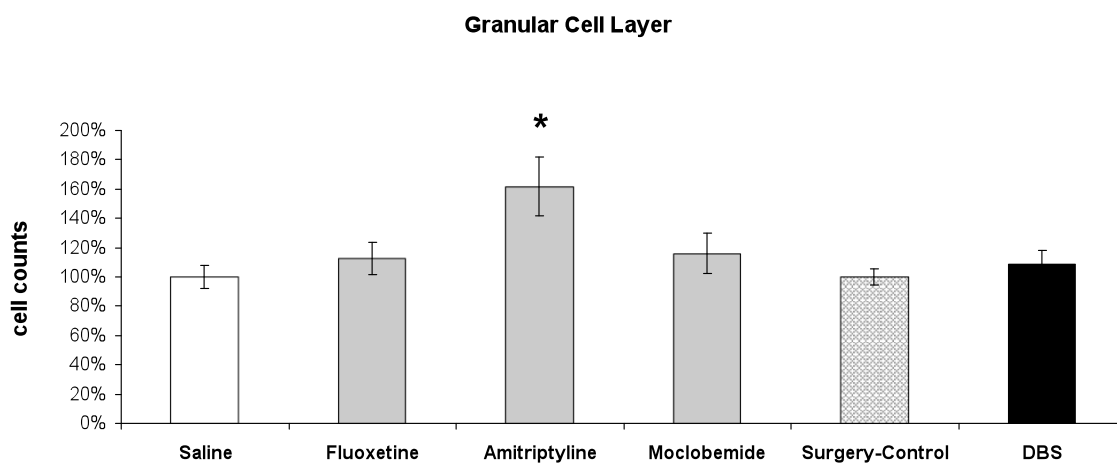


Figure 13. Doublecortin Effects. Values are ratios of treatment over appropriate controls (saline vehicle for drugs and sham surgery for DBS). Error bars indicate standard errors. An increase in DCX-positive cells (61%) was observed following chronic treatment with amitriptyline ($P = 0.009$). No other significant effects were observed.

Table 4. Summary of Effects

Marker:	BDNF mRNA			pCREB				Ki-67		BrdU	DCX
Hippocampal Region:	CA1	CA3	DG	CA1	CA3	Hilus	GCL	SGZ cells	SGZ clusters	SGZ	SGZ
Fluoxetine	-7%	-5%	+3%	+3%	+11%	+8%	n/c	n/c	n/c	+96%	+13%
Amitriptyline	+16%	+9%	+28% (*)	-6%	-17%	-21%	-17%	-6%	-8%	+46%	+61% (**)
Moclobemide	-4%	-4%	-4%	-10%	-7%	-7%	-8%	+39%	+20%	+48%	+16%
DBS	-21% (*)	-9%	-13%	n/c	+3%	-2%	+13%	-17%	-10%	+81% (*)	+8%

Values are ratios of treatment over respective control values (saline treatment for drugs, sham surgery for DBS). ** = $P < 0.01$; * = $P < 0.05$.

Chapter 4

Discussion and Conclusions

4.1 Discussion

Twenty-one days of treatment with various antidepressant drugs or DBS resulted in inconsistent effects, or no effects on BDNF mRNA, pCREB and Ki-67 levels. In contrast, all treatments resulted in substantial increases in BrdU (range of increase: 46% to 96%) and DCX levels (8% to 61%), although only in the cases of DBS and amitriptyline, respectively, did such increases reach statistical significance. This overall pattern of results suggests that antidepressants may increase neurogenesis via increased cell survival up to post-mitotic day 7, and via increased neuronal differentiation, as indexed by BrdU and doublecortin levels in the hippocampus.

While many studies have sought to compare several classes of antidepressant treatments, often on more than one parameter, to the best of the author's knowledge this is the first study to investigate a significant number of parameters (BDNF mRNA expression, pCREB expression, cytogenesis, proliferated cell survival and neuronal differentiation) in a single study, using three different classes of pharmacological antidepressant treatments and one surgical intervention: deep brain stimulation.

DBS has recently been applied to treatment-resistant depression patients and continues to show promising results. In order to investigate the mechanisms of SCG DBS, Hamani et al. (Hamani, Diwan et al. 2010b) developed a rat model where the ventral-medial prefrontal cortex was stimulated in a similar manner as in depressed patients.

In order to further pursue the mechanisms of DBS effect, this study sought to determine whether DBS shares any of the well-known effects of pharmacological antidepressants, in an attempt to determine whether there are common antidepressant mechanisms to these treatments, or whether DBS functions through completely separate mechanisms. Contrary to several current theories (Duman and Monteggia 2006; Krishnan and Nestler 2008; Pittenger

and Duman 2008), the current results indicate that increased BDNF mRNA, CREB phosphorylation and increased cytochrome c may not in fact be common effects of all antidepressant treatments. The results of this study also suggest that increased proliferation, cell survival and neuronal differentiation could be common to all of the tested treatments. This shall be discussed in further detail below.

BDNF mRNA

The results of this study indicate that among the antidepressants investigated, only amitriptyline significantly increased BDNF mRNA expression in the dentate gyrus of the hippocampus, while DBS decreased its expression in the CA1. Fluoxetine and moclobemide had no significant effect on the expression of this mRNA.

This finding is contrary to those of several earlier studies (Nibuya, Morinobu et al. 1995; Nibuya, Nestler et al. 1996; Rogoz, Skuza et al. 2008) that demonstrate increases in BDNF mRNA in unstressed rats after fluoxetine, tranylcypromine, mianserin, desipramine, sertraline, or electroconvulsive seizure (ECS) treatments. The two studies by Nibuya et al. and further work on BDNF protein expression in humans (Chen, Dowlathshahi et al. 2001) and in rats (De Foubert, Carney et al. 2004), or following chronic unpredictable stress treatment (Li, Zhang et al. 2004; Zhang, Gu et al. (in press)), have provided the basis for the conclusion that increases in BDNF expression is a common feature of many antidepressant treatments. However, many other studies have found either no change in BDNF mRNA expression or even a decrease within the hippocampus (Russo-Neustadt, Beard et al. 1999; Fujimaki, Morinobu et al. 2000; Miro, Perez-Torres et al. 2002; Altar, Whitehead et al. 2003; Coppel, Pei et al. 2003; Dias, Banerjee et al. 2003; Saarelainen, Hendolin et al. 2003; Jacobsen and Mork 2004; Bravo, Diaz-Veliz et al. 2009). This inconsistency appears for most antidepressants studied, across all classes.

There is as of yet no clear reason for this variability in results. It has been proposed however, that the cause of these inconsistencies may be post-transcriptional regulation of this protein. Immature pro-BDNF and mature BDNF, are translated from the same mRNA, from which the pro- domain is cleaved to form the mature protein (Castren and Rantamaki 2010). Proneurotrophins bind the pan-neurotrophin receptor p75^{NTR}, as opposed to Trks, and have

the effect of inducing apoptosis rather than cell survival (Volosin, Song et al. 2006). This has led to the “yin-yang hypothesis”, where pro- and mature BDNF (as well as other neurotrophins) induce opposing biological effects by binding to p75^{NTR} and Trk receptors, respectively (Martinowich, Manji et al. 2007). Therefore, measurement of BDNF mRNA does not distinguish between the pro- and mature protein types, whereas antidepressants may be eliciting an effect at several levels to promote mature BDNF protein expression and cell survival, over the immature protein and apoptosis. There is evidence for post-transcriptional regulation of BDNF by antidepressants, since the time course for upregulation of BDNF protein following antidepressant treatment does not match that of its mRNA (Musazzi, Cattaneo et al. 2009).

It is notable that transgenic mice which over express BDNF in the forebrain exhibit *both* anxiogenic- and antidepressant-like effects (Govindarajan, Rao et al. 2006), which could point to the different behavioural effects simultaneously elicited by the pro- and mature-BDNF proteins.

A recent finding is that amitriptyline, but not other antidepressants, is a TrkA and TrkB receptor agonist (Jang, Liu et al. 2009). This is relevant in that TrkB stimulation induces BDNF transcription (Yasuda, Fukuchi et al. 2007). These findings are supported by consistent observations of amitriptyline increasing BDNF mRNA and protein expression (Okamoto, Shino et al. 2003; Xu, Steven Richardson et al. 2003; Hellweg, Ziegenhorn et al. 2008; Hu, Yang et al. 2010; Lee, Myint et al. 2010). In the current study, the increase in BDNF mRNA observed in the dentate gyrus of the hippocampus following chronic treatment with amitriptyline, but not following any other treatment, is consistent with the observation that amitriptyline, but not other antidepressants, is a direct TrkA and TrkB receptor agonist, which upregulates BDNF transcription.

Although BDNF is considered to be important for proliferated cell survival (Sairanen, Lucas et al. 2005), DBS decreased BDNF mRNA expression in the CA1, but yet increased proliferated cell survival up to post-mitotic day 7 as measured by BrdU, more so than any other antidepressant treatment. Given the yin-yang hypothesis of BDNF action, it is possible that DBS regulates BDNF and cell survival through different mechanisms than the other antidepressants studied. DBS may, instead, decrease overall BDNF transcription to reduce

levels of pro-BDNF protein expression, and therefore decrease apoptosis. There may also be post-transcriptional mechanisms involved. Further studies would be required in order to clarify the role of DBS-induced down-regulation of BDNF mRNA in the hippocampus.

pCREB

After 21 days of antidepressant treatment, no significant effects on expression of phosphorylated CREB were detectable. However, it is notable that in all layers of the hippocampus that were measured, amitriptyline and moclobemide treatment induced a non-significant trend towards decreased pCREB, while fluoxetine treatment caused a trend towards increased pCREB levels. DBS treatment caused a non-significant increase in pCREB in the granular cell layer only.

Similar to BDNF, increased CREB function is widely associated with antidepressant treatment (Nibuya, Nestler et al. 1996; Frechilla, Otano et al. 1998; Thome, Sakai et al. 2000; Chen, Shirayama et al. 2001; Rantamaki, Hendolin et al. 2007). However, several of these studies as well as other studies demonstrate that effects on CREB activity are both drug- and region-specific. Specifically, pro-serotonergic antidepressants are much more effective at increasing CREB activity within the hippocampus than pro-noradrenergic antidepressants, but both classes induce similar effects in the frontal cortex (Nibuya, Nestler et al. 1996; Frechilla, Otano et al. 1998; Thome, Sakai et al. 2000; Chen, Shirayama et al. 2001; Tiraboschi, Tardito et al. 2004). Further, several studies demonstrate either a lack of effect or even a decrease in CREB function by pro-noradrenergic antidepressants (Manier, Shelton et al. 2002; Bravo, Diaz-Veliz et al. 2009; Herrold, Shen et al. 2009). It is still unknown why these differences exist between antidepressant classes and between brain regions affected.

Although apparent effects on pCREB in this study were not found to be statistically significant, it is interesting to note that the direction of the observed trends in each case were all consistent with the above noted differences between pro-serotonergic and pro-noradrenergic antidepressants. Amitriptyline, which binds both the 5-HT and NE transporters, caused a slight reduction in pCREB levels. Moclobemide, which also increases 5-HT and NE along with dopamine, also caused a small reduction in pCREB levels. Fluoxetine increased pCREB, and interestingly, although DBS is a non-pharmacological

treatment, it has been demonstrated to be pro-serotonergic (Hamani, Diwan et al. 2010b), and in this study also increased pCREB, although in a more subtle manner.

Cell proliferation, survival and neuronal differentiation

Except for a non significant 40% increase in Ki-67 levels following chronic moclobemide, no other notable effects were observed on cytogenesis by any of the antidepressant treatments studied. There was no effect observed following fluoxetine treatment, a small decrease by amitriptyline treatment, and a more pronounced but still non-significant effect by DBS. Similar findings were found with cluster counts.

BrdU was injected in rats on treatment day 14 to label cells proliferating on that day, enabling measurement of their survival and that of their daughter cells over the final 7 days of treatment. All treatments appeared to increase BrdU labeling, signifying increased survival and proliferation of daughter cells over the last 7 days of treatment. Fluoxetine, for example, induced a 96% increase in BrdU signal. However, because of high variability in BrdU counts, only the effects of DBS achieved significance.

Similar to BrdU, all treatments tended to increase DCX immunoreactivity, signifying increased neuronal differentiation. Despite the general tendency towards increased levels, most treatments induced a modest effect, except for amitriptyline, which caused a pronounced and highly significant increase in neuronal differentiation.

Increased cytogenesis following antidepressant administration of various classes has been reported repeatedly (Malberg, Eisch et al. 2000; Czeh, Michaelis et al. 2001; Malberg and Duman 2003; Santarelli, Saxe et al. 2003; Jayatissa, Bisgaard et al. 2006; Perera, Coplan et al. 2007; Ibi, Takuma et al. 2008). However, it is notable that the observed effects of antidepressants on cytogenesis following various stress paradigms is more pronounced than in the resting state, where a change in proliferation is sometimes absent (Czeh, Welt et al. 2002; Choi, Cho et al. 2007; Holick, Lee et al. 2008; Huang, Bannerman et al. 2008; David, Samuels et al. 2009; Su, Li et al. 2009; Marlatt, Lucassen et al. 2010; Schiavon, Milani et al. 2010). Therefore antidepressants appear to have the effect of normalizing cytogenesis rates.

It is possible that the actions of antidepressants are often normative with respect to rates of cell birth, as opposed to propagative, which may explain the lack of increased cytogenesis observed following fluoxetine, amitriptyline and DBS treatments in this study.

It is possible that the non-significant decrease in DBS-induced cytogenesis is reflective of different mechanisms for neuroplasticity, with respect to resources allocated instead towards increased cell survival, more so than other antidepressants. This is discussed further below.

Increased cell survival following antidepressant administration of various classes, across time points ranging from 7 to 28 days is often observed (Chiou, Ku et al. 2006; David, Samuels et al. 2009; Sui, Zhang et al. 2009; Egeland, Warner-Schmidt et al. 2010; Klempin, Babu et al. 2010). However, several studies failed to observe a significant effect by antidepressants (Wang, David et al. 2008; Su, Li et al. 2009; Schiavon, Milani et al. 2010).

In this study, all treatments caused large increases in BrdU cell counts, although only in the case of DBS did the effects achieve statistical significance. Given that none of fluoxetine, amitriptyline or DBS increased cell proliferation as measured by Ki-67 signaling, it may be concluded that the increased BrdU cell counts induced by these treatments is the result of increased proliferated cell *survival* across the 7 days, given that these treatments do not appear to increase the number of cells undergoing mitosis (see Figure 14 for illustration, page 59). Therefore these results support the current knowledge base that antidepressants increase proliferated cell survival up to 7 days, including and especially DBS. With respect to increased BrdU levels by moclobemide treatment, it is likely reflective of either increased cytogenesis across the 7 days, or both increased cytogenesis and increased survival, which may be a contributing factor.

Similarly, all treatments increased DCX cell counts, although to varying degrees. Given the extent to which DBS and fluoxetine increased cell survival as measured by BrdU immunoreactivity, it is likely that a greater number of cells reached the stage of neuronal differentiation simply due to increased survival of proliferated cells up to that time point, without an increased total number of proliferated cells, and without increasing the *ratio* of cells achieving neuronal differentiation over those that do not (see Figure 14 for illustration, page 59). With respect to increased DCX levels by moclobemide treatment, it is possible

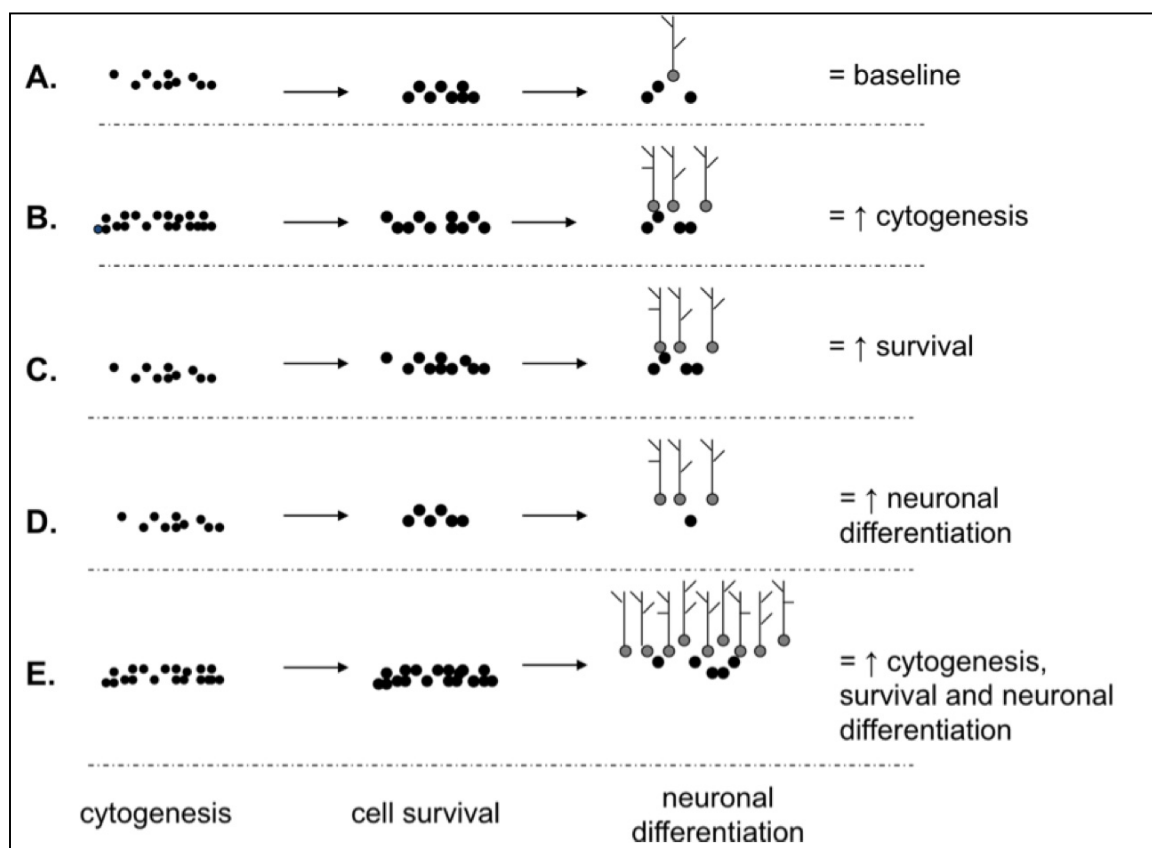


Figure 14. Three Suggested Mechanisms for Increasing Neurogenesis. From left to right, black circles denote newborn, growing and surviving, and undifferentiated cells and/or immature glia, respectively, in the subgranular zone. Grey circles denote immature neuronal cell bodies. (A) In a resting state, cytogenesis occurs within the subgranular zone of the dentate gyrus. Some cells grow and survive, and some do not but undergo apoptosis instead. At the time of doublecortin staining, of the surviving cells, some have differentiated to become neurons, some have become glia, and others have not yet undergone differentiation. (B) Some treatments, including several antidepressants, increase cytogenesis. Although rates of cell survival and neuronal differentiation do not change, increased neurogenesis still occurs due to a larger pool of newborn cells. (C) Treatments may increase neurogenesis without increasing cytogenesis, as the results of this study have demonstrated. Without increasing the pool of newborn neurons, more neurons survive from birth to the time point when neuronal differentiation occurs, and therefore neurogenesis is increased. (D) Neurogenesis may be increased at resting levels of cytogenesis and survival, if neuronal differentiation is increased. Although the pool of newborn and surviving neurons is the same as baseline and less than in scenarios B or C, a greater proportion of these cells become neurons instead of glia or remaining undifferentiated, at this particular time point. (E) Any combination of these three mechanisms for increasing neurogenesis is possible. Also, other mechanisms not mentioned here may be involved.

that this was caused by increased cytogenesis, resulting in a larger pool of both neuronal and glial cells, in all stages of development, as opposed to an increase specifically in the ratio of cells achieving neuronal differentiation. Amitriptyline, however, significantly increased DCX immunoreactivity, which cannot be explained by increased cytogenesis or solely by increased survival. Given that amitriptyline increased survival to a lesser extent than fluoxetine or DBS, but increased DCX signals to a greater extent than these two treatments, there must be another factor involved. Given the current knowledge base, it is likely that amitriptyline caused one or both of the following: it may have accelerated the rate of maturation of proliferated cells to the stage of neuronal differentiation, and/or increased the percentage of cells pursuing neuronal fate over non-neuronal cell types, over the other treatments.

Indeed, it has been postulated that antidepressants increase not only the survival of proliferated cells, but also their rate of maturation (Klempin, Babu et al. 2010). It has also been demonstrated that antidepressants can increase the ratio of proliferated cells that pursue a neuronal fate, as opposed to a glial fate (Egeland, Warner-Schmidt et al. 2010).

Other possibilities for the observed increase in DCX signaling include dematuration of existing mature granule cells back to the immature state. Dematuration of mature granule cells has been demonstrated in one study (Kobayashi, Ikeda et al. 2010) following chronic fluoxetine treatment that induced dematuration to a state similar to that of 3-4 week old neurons. However, this is unlikely to be the case in this study, given that DCX is involved specifically in and expressed during neuronal migration, therefore presumably dematuration would not involve further migration *back* to the subgranular zone, and forth again to the granular cell zone. Therefore DCX would not be expressed in neurons that have already completed the migration phase of development. However this would have to be confirmed through appropriate testing. Lastly, dematuration of neurons has only been demonstrated for SSRIs, and therefore it is unknown as to whether any TCAs are capable of producing this same effect.

The distinction between these various processes is an important one. Although many studies may use the umbrella term “neurogenesis” to describe specific processes such as cytogenesis, the various processes involved in neurogenesis are differentially regulated within the

hippocampus (Sairanen, Lucas et al. 2005; Airan, Meltzer et al. 2007; Bessa, Ferreira et al. 2009; David, Samuels et al. 2009; Lee, Kim et al. 2009; Klempin, Babu et al. 2010), and as has been demonstrated in this study, are effected in a different manner by various antidepressant types. These processes are also differentially effected by stress, as has been recently demonstrated after chronic mild stress, where reductions in total numbers of granule cells precede reduced proliferation rates (Jayatissa, Henningsen et al. 2010).

Proliferation, but not survival or differentiation, is reduced by blocking 5-HT or NE signaling (Kulkarni, Jha et al. 2002; Santarelli, Saxe et al. 2003). In contrast, animals with reduced BDNF do not demonstrate reduced cell proliferation, but robust decreases in cell survival following antidepressant treatment (Sairanen, Lucas et al. 2005). It is yet unknown whether BDNF participates in increasing the rate of maturation of proliferated cells.

Functional relevance of neurogenesis

Young neurons migrate from the subgranular zone into the granular cell layer, where they integrate into existing hippocampal circuitry. As mentioned above, these young neurons possess membrane properties distinct from those of mature neurons and similar to those of neurons in the developing nervous system; these properties include increased excitability and facilitated long-term potentiation. It has been demonstrated that these new neurons are preferentially incorporated over mature neurons into hippocampal spatial memory networks.

Several studies have also demonstrated that antidepressant treatment leads to increased synaptic plasticity of DCX-positive cells (Wang, David et al. 2008; David, Samuels et al. 2009). This is measured as increased dendritic complexity such as increased tertiary dendrites, dendritic length, and number of intersections.

The importance of cyto genesis can be determined by blocking cell proliferation either by focal X-irradiation to the temporal lobe, or by administering methylazoxymethanol (MAM), a cytostatic agent used to arrest neurogenesis. Cyto genesis–dependent and -independent effects of antidepressants have been discovered.

Blocking cyto genesis can prevent antidepressant-induced improvements in performance on several tasks. These tasks include the novelty-suppressed feeding test, which characterizes

anxiolytic properties displayed by the animal, and grooming and coat scores following the chronic unpredictable stress paradigm, which models depression (Santarelli, Saxe et al. 2003; David, Samuels et al. 2009). These studies demonstrate a role for adult-born cells in certain features of anxiety and depression.

It is notable that several hippocampus-dependent memory tasks are also affected by blocking cell proliferation. These tasks include delayed contextual fear conditioning (Saxe, Battaglia et al. 2006; Winocur, Wojtowicz et al. 2006), formation of trace memories (Shors, Miesegaes et al. 2001), and long-term spatial memory (Madsen, Kristjansen et al. 2003; Raber, Rola et al. 2004; Snyder, Hong et al. 2005). Therefore blocking cytogenesis induces cognitive defects involved in hippocampus-dependent memory, which is impaired in many depressed individuals.

There are several behaviours that are cytogenesis-independent, that are not affected by blocking cell generation. These behaviours include learned helplessness, which models depression as measured by the forced-swim test, they include anxiety, as measured by the open-field test (Airan, Meltzer et al. 2007; Bessa, Ferreira et al. 2009; David, Samuels et al. 2009), the elevated plus maze test, and the light-dark choice task, and finally emotional learning, as measured by the passive avoidance test (Raber, Rola et al. 2004; Saxe, Battaglia et al. 2006). These studies demonstrate that adult-born cells do not play a role in antidepressant action on learned helplessness, on several measures of anxiety, and on emotional learning.

It is important to note that blocking cell proliferation does not induce a depressive-like state. When cytogenesis is blocked and vehicle treatment is administered (as opposed to antidepressant treatment), rodents show no significant differences from sham-treated rodents in various depression models (Santarelli, Saxe et al. 2003; Raber, Rola et al. 2004; Airan, Meltzer et al. 2007; Surget, Saxe et al. 2008; Bessa, Ferreira et al. 2009; David, Samuels et al. 2009). Therefore, reduced cytogenesis cannot be a cause of depression, but rather its increase can be an effect of antidepressants *through* which they exert antidepressant-like effects in rodents. The observation that a CRH antagonist and a vasopressin receptor antagonist induce antidepressant-like effects that remain unaffected by X-irradiation,

provides further evidence that adult-born cells are not required for antidepressant effect by all antidepressant treatments (Surget, Saxe et al. 2008).

It is hypothesized that the role of increased cytotogenesis and neuron production is to affect local network dynamics within the hippocampus, that causes increased hippocampal activity, which in turn reduces HPA axis activity (Surget, Saxe et al. 2008). This could explain the efficacy of the CRH antagonist and vasopressin receptor antagonist, in spite of blocked cytotogenesis, in that they by-passed hippocampal activation and directly blocked HPA axis activity. In support of this possibility, it has been found that the ratio of electrical activity within the dentate gyrus relative to CA1 during forced swim testing is specifically reduced by chronic mild stress (CMS) treatment, and increased by both fluoxetine and imipramine treatment (Airan, Meltzer et al. 2007). Therefore the behavioural effects of CMS and antidepressant treatments are directly reflected in hippocampal network activity, irrespective of antidepressant class.

DBS mechanisms

A major purpose of this study was to ascertain whether there are common core antidepressant mechanisms for effective antidepressant drug medications and surgical interventions. DBS may be efficacious for treatment-resistant depression patients because it is more potent at activating the same antidepressant mechanisms, or it could function by engaging unique brain mechanisms not shared by conventional antidepressant interventions. It was hypothesized in this study that common mechanisms exist and that they involve changes in neuroplasticity.

However, it is also possible that there are various mechanisms through which antidepressants could exert their effects. Thus antidepressants may not necessarily overlap in their antidepressant mechanisms, since several are possible. In this case, DBS could conceivably be efficacious for treatment-resistant individuals because it employs at least one mechanism that is entirely different from the one or more mechanisms that antidepressants may activate.

Certainly both scenarios may be concurrently true for DBS. This treatment may be more effective when employing the same mechanisms as other antidepressants, and may also

utilize one or more different mechanisms not shared by any other treatment, that underlie its efficacy in treatment-resistant patients.

The results of this study suggest that there are indeed mechanisms common to effective antidepressant treatments, which include increased proliferated cell survival and differentiation. These results also suggest that DBS is more effective than other antidepressant treatments specifically at promoting proliferated cell survival, which could underlie its efficacy with treatment-resistant depression patients. This study does not, however, rule out the possibility that there are other antidepressant mechanisms that are common to all treatments, and yet still other mechanisms that are unique to DBS.

Summary

The findings of this study suggest that antidepressants do not have common effects on several markers of neuroplasticity, nor on cytochrome. However, the overall pattern suggested by the direction of observed trends could denote that a common mechanism of diverse antidepressant treatments could be increased neurogenesis via increased cell survival and increased neuronal differentiation.

BDNF mRNA expression was increased following amitriptyline treatment, likely due to it being a direct agonist of TrkA and TrkB. However in this study, increased BDNF mRNA is an effect that is unique to this particular treatment. This may be due to the fact that BDNF mRNA is destined to become either the mature form of BDNF protein which binds TrkB, or pro-BDNF, which binds the p75^{NTR} receptor that induces opposing biological effects to Trks, in increasing apoptosis rather than cell survival. Therefore the other antidepressants studied may regulate BDNF at a different level, such as post-transcriptionally via protein cleavage, to increase mature BDNF protein levels over pro-BDNF.

No antidepressant treatment had a significant effect on pCREB expression in this study, however it is interesting to note that the directions of non-significant changes follow the current observations that pro-serotonergic agents increase pCREB expression, whereas pro-noradrenergic agents decrease its expression. It is intriguing that, although DBS is a surgical treatment, it has previously been demonstrated to utilize the 5-HT system (Hamani, Diwan et al. 2010b), which is further reflected in its effect on pCREB expression.

Contrary to current opinion, cytogenesis was not a common effect of the antidepressant agents tested. Only moclobemide treatment induced a trend towards increased cytogenesis after 21 days of treatment. It is postulated here that cytogenic effects of antidepressants are likely more of a restorative effect of antidepressants following reductions in cytogenesis caused by depression or prolonged stress, and not a baseline function of all antidepressants.

Common to all antidepressant treatments were trends towards increases in BrdU and DCX expression, which therefore result in increased neurogenesis, although arising from different underlying mechanisms. DBS and fluoxetine treatments resulted in increases in cell survival up to 7 days post-mitosis, as measured by BrdU, when compared with cytogenesis rates, as measured by Ki-67. Amitriptyline treatment significantly increased DCX expression, which is likely attributable to either an accelerated rate of maturation, or an increased ratio of progenitor cells assuming a neuronal fate over a glial fate.

The current results suggest that despite the fact that antidepressants of different types create their own unique profiles of neuroplastic changes, increased cell survival and neuronal differentiation could possibly be common to all antidepressant interventions.

4.2 Study limitations

Statistical considerations

The suggestions and conclusions presented here are primarily based on the direction and magnitude of observed trends. It would be important to confirm the statistical significance of these trends, since a number of apparently strong effects seem to have been obscured by within-group variation – a good example being the BrdU effects. A larger number of observations per group combined with technical refinements in methodology could help bring the statistical significance of these effects to the fore.

Dose considerations

One limitation of this study is that dose-response curves were not generated with respect to the individual antidepressant treatments and their possible effects on neuroplasticity and

neurogenesis. While the target doses were carefully chosen on the basis of literature (Jain and Subhedar 1993; Vilpoux, Carpentier et al. 2002; Qi, Lin et al. 2008; Reines, Cereseto et al. 2008; Hamani, Diwan et al. 2010a), it is possible that different doses of any of the antidepressant treatments investigated could have yielded different results on any of the parameters studied.

Survival time frame

Newborn cells generally require 3-4 weeks before functionally integrating into the granular cell layer network as immature neurons (Perera, Park et al. 2008). Therefore a limitation of the present study is that proliferated cell survival was only measured at one time point (of 7 days), and did not include measurements of any time points closer to when the proliferated cells become functionally integrated into the hippocampal network.

Functional role of observed changes

The present study was descriptive in nature, and as such does not allow statements about the functional role of the observed effects induced by the various interventions. While all of the parameters studied (BDNF mRNA expression, pCREB expression, cytogenesis, cell survival and differentiation) have been implicated in antidepressant effects, it is not clear whether all of these effects are important for mechanisms of antidepressant effects. In order to determine the mechanistic role of these parameters, they would need to be manipulated (either increased or decreased) and a change in antidepressant effect would need to be observed.

Effects on stressed animals

In designing the present study, a decision was made not to test the effects of treatments in models of depression. There were two reasons for this choice. One was that all of the chosen treatments, including DBS, have previously been well documented to revert depressive-type behaviour in animal models. Second, to the extent that depression models typically involve stress procedures, behavioural testing would almost certainly affect the variables of interest. The decision was therefore made to initially investigate treatment effects in normal, unstressed rats. It remains nonetheless very possible that the markers used

here would show different profiles in stressed animals. This possibility could be considered in future studies.

4.3 Future Directions

In light of the aforementioned yin-yang hypothesis of pro- and mature BDNF functions, and the known importance of BDNF for proliferated cell survival (Sairanen, Lucas et al. 2005; Bergami, Rimondini et al. 2008), it would be informative to determine the effects of DBS on mature BDNF protein expression. As such, a potential study would include immunohistochemical analysis of both mature and pro-BDNF protein expression, to determine whether there is any post-transcriptional regulation by DBS on ratios of these proteins.

Along the same lines, it has been demonstrated that mice with reduced BDNF levels (BDNF^{+/-}) are incapable of antidepressant-induced increases in proliferated cell survival (Sairanen, Lucas et al. 2005). Given that results of this study suggest that increased proliferated cell survival is a key mechanism of increased neurogenesis induced by DBS treatment, it would be pertinent to determine whether reduced BDNF levels, which in other models have reduced proliferated cell survival, are capable of preventing DBS-induced increases in proliferated cell survival, and whether this is important for its antidepressant effects. This would determine whether proliferated cell survival is required for DBS-induced antidepressant effects.

Similarly, neurogenesis has been demonstrated to be a requirement for antidepressant drug effects, where blocking cytotogenesis and therefore preventing neurogenesis is capable of preventing antidepressant-like effects (Santarelli, Saxe et al. 2003). A future study might involve blocking cytotogenesis to determine if neurogenesis is required for DBS-induced antidepressant effects. This would complement the second study mentioned, given that both cytotogenesis and cell survival are separately regulated.

4.4 Conclusion

In conclusion, three effective antidepressant drugs, with diverse pharmacological properties, and one surgical treatment produced diverse effects on BDNF, pCREB and cytogenesis, which, as other studies have also demonstrated, do not appear to be uniformly involved in antidepressant effects. These same antidepressant treatments produced consistent trends towards increasing neurogenesis via increased cell survival and differentiation, without necessarily increasing cytogenesis. Within the scope of this investigation, the findings suggest that increased cell survival and differentiation could possibly be part of a set of core mechanisms associated with effective antidepressant treatments.

The current findings also suggest that DBS, which seems to be uniquely effective in treatment-resistant depression patients, may also work by increasing the efficacy of proliferated cell survival.

When comparing DBS to the antidepressant drugs studied here, its profile of effects most closely resembled the observed profile of fluoxetine. For pCREB, BrdU and DCX, both DBS and fluoxetine induced changes in the same directions and to a similar extent. This is consistent with data suggesting that DBS effects in animal models of depression require the integrity of the 5-HT system. This may be relevant for treatment-resistant depression patients. Given that DBS appears to target the serotonergic system, it is possible that selection of a pro-noradrenergic antidepressant agent to complement DBS treatment may be most effective to maximize the antidepressant efficacy of both monoaminergic systems.

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