Continuous white noise exposure during and after the auditory critical period alters *in vivo* bi-directional auditory cortex plasticity in rats

by

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Abstract

Long-term potentiation (LTP) and long-term depression (LTD) are processes thought to mediate developmental and experience-dependent plasticity in the brain. Both have been demonstrated *in vitro* in the primary auditory cortex (A1) of adult rats, but *in vivo* evidence is lacking. We examined these processes *in vivo* by applying high and low frequency stimulating protocols to the medial geniculate nucleus of urethane anaesthetized rats. Higher frequency theta burst stimulation produced robust LTP (to ~140% of baseline amplitude) of field postsynaptic evoked potentials (fPSP) recorded in the superficial layers of the primary auditory cortex (A1). Low frequency (1 Hz) stimulation resulted in a transient depression (to ~60% of baseline amplitude) of fPSPs recorded in A1. Local, intracortical application of D-(-)-2-amino-5-phosphonopentanoic acid (APV) abolished LTP and reduced synaptic depression, verifying the involvement of cortical N-methyl-D-aspartate receptors in these effects. Thalamocortical plasticity mechanisms were also assessed after continuous white noise exposure, known to arrest auditory cortex maturation, during and after the postnatal critical period of A1 development. Rats housed in continuous white noise during the first 50 days of life exhibited greater LTP (~180%) than controls reared in normal sound environments. More stunningly, the protocol used to elicit depression also resulted in substantial LTP in white noise reared animals (~150%). Adults housed in white noise for the same length of time appeared to exhibit normal LTP, but displayed greater and persistent levels of synaptic depression (to ~30% of baseline amplitude). These results suggest that, in the absence of patterned auditory stimulation during early postnatal life, thalamocortical auditory synapses exhibit a preferential readiness for synaptic potentiation over depression. In adults, however, prolonged deprivation of patterned sound input results in synapses

favouring depression over synaptic enhancement. Together, these data confirm the great potential for plasticity in the mature central auditory system and demonstrate the age-dependent impact of experiential factors on plasticity properties of the thalamocortical auditory system.

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Nomenclature

A1	primary auditory cortex
aCSF	artificial cerebrospinal fluid
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor
BCM	Bienenstock-Cooper-Munro
CF	characteristic frequency
D-APV	DL-2-Amino-5-phosphonopenanoic acid
fPSP	field evoked post-synaptic potential
LFS	low frequency stimulation
LTD	long-term depression
LTP	long-term potentiation
MCPG	(RS)-a-methyl-4-carboxyphenylglycine
MGN	medial geniculate nucleus
mGluR	metabotropic glutamate receptor
NMDAR	N-methyl-D-aspartate receptor
PND	postnatal day
RF	receptive field
TBS	theta burst stimulation
θ_m	synaptic modification threshold

Introduction

During early postnatal development synaptic connections are extremely dynamic, so this period represents a particularly fertile time in which to make inferences with regards to the mechanisms of plasticity. The sensory modalities have provided a rich source of information in this endeavour. However, while the literature on the visual and somatosensory modalities is expansive, until recently, studies assessing the impact of the environment and learning on the development of the auditory cortex have been relatively rare (Zhang, Bao & Merzenich, 2001; Wang, 2004).

Chapter 1: Sensory Cortex Plasticity Across Development

Ontogenesis and Early Development

In all areas of the neocortex ontogenesis involves the migration of new cortical neurons from the columnar epithelium (Mountcastle, 1998). On arrival at their final destination in the cortex, these neurons undergo a process of differentiation, characterized by arrangement into cortical layers with appropriate efferent and afferent connections (Mountcastle, 1998). With respect to layer positioning, the fate of individual neurons is decided simply by the cell's place in the temporal order of production by the columnar epithelium's progenitor cells (Mountcastle, 1998).

Rakic's (1988) 'radial unit hypothesis' provides a framework by which to understand the 'fundamental building block' of the neocortex, the 'ontogenic unit'. This theory states that neurons migrating from the germinal epithelium settle in radial columns in an 'inside-to-outside' pattern, reflective of the order in which they were generated. Cell migration is facilitated by the glial fascicles, which move the new neurons from the ventricular zone to the cortical plate in a precise, topographic manner (Mountcastle,

1998). In addition, Rakic (1988) proposed that the site of neuron generation contains a 'protomap' of the neocortex's ultimate form, defining the gross morphology of cortical anatomic relationships (Krubitzer & Kahn, 2003).

Development of Sensory Maps

The archetypical formulation of a topographical representation is the motor homunculus (Penfield & Rasmussen, 1950, as cited in Carlson), a humanoid figure whose physical proportions illustrate the relative area of the primary motor cortex dedicated to the movement of various body parts. This somatotopic representation was derived from stimulation studies of the primary motor cortex in which activation of particular brain areas resulted in corresponding body movements. Similarly, in the primary auditory cortex (A1), Sally & Kelly (1988) revealed tonotopy using a microelectrode mapping technique whereby tone bursts were used to determine the characteristic frequency (CF) for neurons at each electrode placement. High frequencies were coded rostrally and low frequencies caudally, in A1. In essence, topographic representation is a property of maintenance of spatial relationships throughout the different levels of the central nervous system, with sensory cortices systematically organised in a manner reflective of their sensory epithelia (Weinberger, 2004). For example, in the visual modality, the retinal map is successively preserved in the thalamus and in the visual cortex, in the somatosensory system the body surface is represented from the dorsal column to the somatosensory cortex, and in the auditory system the orderly relationships between the hair cells of the cochlea's basilar membrane are reproduced through successive auditory nuclei up to the auditory cortex (Harrison, Ibrahim & Mount, 1998). In the auditory system this maintains a clear gradient of frequency representation throughout the central nervous system such that the rostrally coded high frequency areas of A1 correspond to the basal portion of the

basilar membrane, which is also reactive to high frequencies, while both the caudal portion of A1 and the apical end of the basilar membrane are responsive to low frequencies (Carlson, 2001). These physical properties of the acoustic environment are primarily channelled through the lemniscal auditory pathway, which projects to the primary auditory cortex. Polysensory integration, and more 'psychological' functions (such as temporal pattern recognition, stimulus significance and certain forms of learning), are managed by the non-lemniscal and diffuse pathways, which terminate in both parts of the primary and secondary auditory cortex (Weinberger & Diamond, 1987).

The study of topographies in the sensory domains has indicated that these representations are more than just anatomical constructs, they are also physiologically modifiable entities (Calford, 2002). Evidence supporting the plastic nature of sensory maps is most easily garnered during the 'sensitive/critical period' of development, a period in which the cortex is believed to be maximally prone to plastic change in an activity-dependent fashion (Mountcastle, 1998; Harrison *et al.*, 1998; Heynen & Bear, 2001). Initially, topographic projections are relatively diffuse, though following experience a large number of these connections are pruned (Pienkowski & Harrison, 2005). This organisational process operates through Hebbian mechanisms, whereby synchronously active connections are strengthened and those that are under utilised, or asynchronously active, are weakened (Katz & Crowley, 2002). Though large-scale reorganisations are effected only within early developmental windows, synaptic plasticity is still evidenced into adulthood, albeit to a lesser degree. In fact, the dynamic properties of the mature cortex are the lynchpin of many modern theories of learning and memory (Mountcastle, 1998), and in the adult sensory cortex can even be considered to bridge the conceptual gap between perception and memory (Weinberger, 1998).

Experiential Manipulations of Developing Sensory Systems

As plasticity functions in an activity-dependent manner, the pattern of synaptic modification is dependent on the environment in which development occurs, such that an impoverished environment may result in distortions in cortical representation (Katz & Crowley, 2002). Wiesel & Hubel's (1963) pioneering study of ocular dominance columns in kittens highlighted the importance of early experience in sculpting cortical circuitry. The two eyes activate different parts of the primary visual cortex, with cells having similar eye preference grouped together into 'ocular dominance' columns. By initiating monocular deprivation during the second postnatal month they found that normal development deviated to such an extent that, in adulthood, input from the once deprived eye no longer activated the primary visual cortex. In essence, the connectivity that had been established during ontogenesis had been manipulated into a new form through the 'competitive imbalance' existing between the two eyes (Tieman, Tumosa & Tieman, 1983; Christen & Mower, 1987). This underscores the interplay of the somewhat autonomous, gene driven, action of early development with the experience-driven mechanisms of the critical period (Krubitzer & Kahn, 2003).

In the auditory modality, techniques comparable to monocular deprivation have been relatively elusive, with attempts to reversibly block the ear canal undermined by issues such as bone conduction (Wang, 2004) and sensorineural loss in the deprived ear (Rauschecker, 1999). However, since synchronous activation of the optic nerve during development has been shown to disrupt normal visual cortex maturation across various species (Zhang, Bao & Merzenich, 2002), Merzenich and his colleagues (Zhang *et al.*, 2001; Zhang *et al.*, 2002; Chang & Merzenich, 2003; Nakahara, Zhang & Merzenich, 2004) have used patterned acoustic inputs to investigate the influence of critical period

auditory environment on subsequent A1 organisation. The critical period for primary auditory cortex development runs from approximately PND12 to PND30 (Chang & Merzenich, 2003). Zhang *et al.* (2001) initially mapped the developing A1 of rats raised in a 'normal' acoustic environment from the onset of the critical period until adulthood. From postnatal day (PND) 13 to 16 the immature A1 showed poor frequency selectivity and broad receptive fields. However, by PND21 a more adult-like degree of refinement and tonotopy was beginning to emerge, illustrating the progressive cortical refinement characteristic of this highly plastic period. When animals were exposed to pulsed tones, at either low (4kHz) or high frequencies (19kHz), for 10 to 16 h a day between PND9 and PND28 neurons rapidly tuned to these frequencies. Notably, representation for these tones expanded at the expense of frequencies higher and lower than the respective pulsed tones. While there was facilitation in the emergence of frequency selectivity, the expanded representation for these frequencies amounted to a distortion. Those areas that did generate frequency selectivity did not sharpen sufficiently, remaining more broadly tuned than evidenced under normal auditory conditions. Confirming that the critical period's particular sensitivity did not extend into maturity, tonotopy did not emerge following introduction of these animals to a normal acoustic environment at PND30. Further, impoverished noise exposure initiated after PND30 failed to cause substantial frequency specific alterations to the tonotopic map (Zhang *et al.*, 2002).

In a later experiment, Zhang *et al.* (2002) examined the developmental impact of early exposure to pulsed white noise (noise composed of all frequencies). As early as PND15 frequency differentiation began to emerge in the cortex of these animals. However, even at the end of the critical period (i.e., ~ PND30), there was no clear, tonotopic gradient of frequency distribution, with a seemingly random arrangement of

tuned areas. There also existed a region of neurons that responded non-selectively to higher frequencies. When re-examined after prolonged exposure to a standard acoustic environment this non-selective region remained, while no neurons exhibited tunings specific to very low frequencies or those above 15 kHz. Clearly this manipulation prevented the normal sharpening of tuning curves during development, while the endurance of these tonotopic deficits when returned to a normally variable auditory environment is again suggestive of the critical period's unique capacity for large-scale cortical reorganisation. The mechanism responsible for these distortions is unclear, though two possibilities present themselves: an overrepresentation of the exposed frequencies, resulting in a competitive loss of less-activated frequencies; or, that the presentation of limited sound patterns simply prevented exposure to more normally distributed environmental inputs. What is clear is that normal auditory development requires complex sound inputs in which temporal patterns are of high significance (Nakahara *et al.*, 2004).

The particular sensitivity of the auditory cortex to environmental inputs during development is not a species-specific phenomenon. For example, the songbird equivalent of the auditory cortex, the field L complex, is also susceptible to early experience (Cousillas *et al.*, 2004; Brainard & Doupe, 2002). Adult birds deprived of early song exposure display broadly responsive neurons lacking refined selectivity, in contrast to the precise, tonotopically organised field L complex of wild birds (Cousillas *et al.*, 2004). In a human context, distortions in the early auditory environment could have ramifications for later speech perception and language learning (Rauschecker, 1999). Beyond cautionary concerns, the effects of early language experience are evidenced by the lack of phonemic preference shown by babies until around the sixth month. After this point

perseveration of native language patterns occurs, making unaccented second language performance, and even language learning, more difficult later in life (Rauschecker, 1999; Cousillas *et al.*, 2004; Kuhl, Williams, Lacerda, Stevens & Lindblom, 1992, Zhang *et al.*, 2001, Nakahara *et al.*, 2004).

Although complete and reversible auditory deprivation has not yet been achieved, continuous white noise presentation has been employed during and beyond the critical period for auditory development in rats as a means by which to block normal auditory inputs (Chang & Merzenich, 2003). While pulsed white noise during A1 development results in broadly tuned CFs that endure even when the animal has been returned to a normal acoustic environment (Zhang *et al.*, 2002), the primitive A1 organisation that results from continuous white noise rearing appears to indicate that the onset of the critical period had been arrested. This conclusion is supported by the susceptibility of adult rats raised in continuous white noise to a subsequent pulsed tone environment, which causes distortions in A1 akin to those generally only inducible during the critical period (Zhang *et al.*, 2002). As well, when continuous white noise reared animals are returned directly to standard housing well after the termination of the critical period for auditory development, they eventually develop CF maps very much like those of control animals. Presumably, continuous white noise constantly excites auditory receptors at all frequencies (Wang, 2004), masking the complex, temporally modulated inputs required for tonotopic organisation and frequency tuning in A1 (Nakahara *et al.*, 2004). Comparable effects are seen in some species of songbirds for whom a lack of normal experience, as in the case of isolation rearing, can allow the brain to remain receptive to song learning for longer durations than typical in standard rearing conditions (Brainard & Doupe, 2002).

Plasticity in the Mature Sensory Cortex

It is well established that early development is a time in which synaptic change is more extensive, and presumably easier to induce. For example, plastic reorganisation in response to a passive acoustic environment is only seen during the critical period, with behaviourally relevant stimuli required to induce cortical reorganisation in the adult auditory cortex (Weinberger, 1995). Presentation of the behaviourally relevant stimuli needed for cortical remodelling in adults coincides with nucleus basalis activation, an area whose cholinergic projections modulate cortical plasticity (Pandya *et al.*, 2005, Kilgard & Merzenich, 1998). Thus, pairing nucleus basalis stimulation with sensory stimuli can circumvent the need for behavioural relevance in anaesthetized animals (McLin, Miasnikov & Weinberger, 2002). For example, Pandya *et al.* (2005) used nucleus basalis stimulation in conjunction with randomly interleaved high and low frequency tones, resulting in tone specific overrepresentations in the adult A1. Attempts have even been made to replace the physical stimuli with intracortical microstimulation at an auditory cortex site responsive to a known frequency, in conjunction with nucleus basalis stimulation (Talwar & Gerstein, 2001). While this resulted in an increase in cortical representation for the frequency it did not translate to an improvement in discrimination for tones at and around this frequency, perhaps due to a lack of RF sharpening or alteration of sub-cortical structures. Cortical plasticity without behavioural expression of learning has also been identified following two-tone conditioning tasks of a difficulty level high enough to elude successful learning (Edeline & Weinberger, 1993).

In adult animals, plasticity has also been observed in lesion, amputation, training and conditioning experiments, though to a less dramatic extent than during the critical period. For example, conditioning procedures, such as frequency discrimination tasks, can

effect increases in cortical and thalamic representation for frequencies of behavioural relevance (Weinberger & Bakin, 1990; Weinberger, 1998), with the magnitude of receptive field (RF) modification reflective of the degree to which the stimulus was behaviourally significant (Weinberger, 2004). RF alterations of this kind can last for as long as eight weeks and potentially beyond (Weinberger, Javid & Lepan, 1993). A human equivalent of use-dependent, functional reorganisation of the auditory cortex can be seen in cochlear implantation studies, in which even post-lingual deaf individuals can regain sufficient hearing ability to converse over a telephone (Pantev *et al.*, 2005). To be an effective mechanism for information storage RF modifiability should operate bi-directionally, also allowing for a decrease in responsivity in appropriate situations. Habituation provides a good example of this, whereby synaptic efficacy has been observed to decrease following a repetitive tone stimulus (Lennartz & Weinberger, 1992). Such decrements are frequency specific, and not representative of a general loss of cortical responsiveness to acoustic stimuli. Online modifications like these are essential when faced with a perpetually varying environment. As Martin and Morris (2002) note, though such physiological forms of memory are conceptually unlike those traditionally studied by cognitive psychologists, receptive field plasticity may provide an understanding of broadly relevant mechanisms of plasticity. It is, therefore, inappropriate to consider the primary auditory cortex as being little more than a sensory detector; it is likely that its cells function as 'adaptive filters', selectively modifying output in response to learning (Weinberger & Diamond, 1987).

Amputation or lesion of sensory organs illustrates the 'filling-in' phenomena (Rauscheker, 1999), another form of adult cortical plasticity. Following digit amputation in monkeys, the adjacent digit and palmar skin region representations expand to fill-in the

denervated area (Merzenich *et al.*, 1983). In the auditory cortex of adult guinea pigs, lesioned areas of the cortex are enveloped by the frequency representations of surrounding cochlear regions (Robertson & Irvine, 1989), while cochlear lesions result in an A1 over representation for frequency representations bordering the damaged area (Harrison *et al.*, 1998). The subjective experience of the filling-in phenomena is well evidenced by amputee patient reports of phantom limb sensations following a touch to another body area, such as the face (Flor *et al.*, 1995). It is possible that these sensations have a correlate in the auditory system in the form of tinnitus. This condition is acquired after exposure to a damagingly loud noise or as a result of age-related loss of hair cells (Rauschecker, 1999), and manifests as the perceptual experience of sound even in the absence of any external or internal sound source (Muhlnickel, Elbert, Taub & Flor, 1998). While the cause of activation in chronic tinnitus is unknown (Flor *et al.*, 2004), in both phantom-limb and tinnitus, it has been noted that the severity of the condition is related to the degree of alteration of the respective cortical topographic map (Muhlnickel *et al.*, 1998).

In summary, the gross morphology of the developing cortex is determined by neocortical 'protomaps', though precise topographic relationships are defined by sensory experience. These organisational processes are facilitated by the highly plastic nature of the cortex during critical periods of development. However, these highly plastic epochs also allow distortions in development if impoverished sensory inputs occur during these developmental windows. In the auditory domain, continuous white noise rearing seems to allow the remarkable plasticity of the critical period for auditory development to be arrested until which time more temporally varied sound inputs occur. It should be noted

that following normal developmental conditions plasticity is still evidenced in the mature cortex, though to a lesser degree than exhibited during critical periods of development.

Chapter 2: Cellular Mechanisms of Plasticity

Long-Term Potentiation (LTP) and Long-Term Depression (LTD)

The cellular processes that facilitate cortical plasticity at different ages are largely unknown (Foeller & Feldman, 2004). The leading candidate mechanisms (Rioult-Perdotti, Friedman & Donoghue, 2000) for these plastic properties are long-term potentiation (LTP) and long-term depression (LTD), which together provide a means by which to explain the activity dependent, bi-directional changes seen throughout the brain (Bear, 1996; Shouval, Bear & Cooper, 2002; Grassi, Dieni, Frondaroli & Pettorossi, 2004) at the level of the synapse (Tsumoto, 1990). Essentially, these mechanisms refer to any enduring up- or down-regulation in synaptic efficacy (Martin & Morris, 2002; Mountcastle, 1998; Malenka & Bear, 2004) of either single neurons or neuronal aggregates (Tsukahara, 1981). As such, the molecular mechanisms of LTP and LTD provide an excellent model by which to understand development, recovery from injury, cortical map plasticity, and learning and memory more generally (Mountcastle, 1998; Malenka & Bear, 2004; Martin & Morris, 2002; Grassi *et al.*, 2004). However, it is important to note that while it is highly likely that together they represent at least one of the mechanisms utilised by the brain to effect long-term synaptic modification, and that the induction paradigms are within the physiological capabilities of the neurons (Andersen & Hvalby, 1986; Shouval, Bear & Cooper, 2002), these are experimentally induced phenomena (Malenka & Bear, 2004; Lamprecht & LeDoux, 2004) for which it has been difficult to conclusively prove functional and naturalistic roles (Malenka &

Bear, 2004). As Martin and Morris (2002, p. 610) point out, "Memory is a property of the entire organism whereas plasticity is a property of synapses," but perhaps for this reason they should not be expected to directly mirror each other in all their properties. Ultimately, there must be a physiological basis for learning. Therefore, it is not so much an issue of whether or not physiological plasticity is involved in information acquisition; rather, it is a case of ascertaining its characteristics (Weinberger & Diamond, 1987). By enhancing understanding of these mechanisms at a cellular level, clarity will be gained with regards to the functioning of the neuronal ensembles and networks that drive the integrative activity of whole organisms (Weinberger & Diamond, 1987).

The predominant forms of LTP and LTD that have been studied are *N*-methyl-D-aspartate receptor (NMDAR) dependent mechanisms (Malenka & Bear, 2004; Mountcastle, 1998). These processes are Hebbian in nature, such that LTP can be thought of as representative of the synaptic strengthening associated with repeated pairing of pre- and post-synaptic firing, and LTD the weakening associated with asynchronous firing. While it is possible to think of LTP and LTD as generalised mechanisms, the same cannot be said with regards to their induction protocols. Just as varied forms of memory can be identified within different brain regions and even different local circuits (Martin & Morris, 2002), there are striking differences in the mechanisms required to induce long-term alterations across the brain (Renger *et al.*, 2002), and even different forms of LTP and LTD expressed at different neurons (Malenka & Bear, 2004; Mountcastle, 1998). A very broad example is the relative resistance of the neocortex to induction of LTP (Trepel & Racine, 1998) compared to the hippocampus, which is modifiable with notably shorter duration stimulation paradigms (Tsumoto, 1990).

The Role of Experience in Bi-Directional Synaptic Plasticity

It is clear that experience across early developmental epochs plays a large role in determining connectivity in the mature cortex, though experiential effects on synaptic responsivity on a temporally shorter scale also need to be considered. With regards to LTP and LTD, Kirkwood, Rioult and Bear (1996) showed that binocular deprivation enhanced LTP (allowing its induction at lower stimulation frequencies) and diminished LTD. Further, it seems that sensory experience may actually be recorded by alterations in the physical properties of NMDARs. Using the same sensory manipulation Philpot, Sekhar, Shouval and Bear (2001) found that after visual deprivation NMDAR-mediated excitatory postsynaptic currents were slowed, impacting excitatory postsynaptic current summation and thus Ca^{2+} entry into the cell such that LTP was favoured over LTD. NMDAR subunit composition has also been implicated, with visual experience resulting in an increase in the NR2A:NR2B ratio, and deprivation causing a decrease (Quinlan, Olstein, & Bear 1999; Quinlan, Philpot, Huganir & Bear, 1999).

Cooper, Lieberman and Oja (1979) proposed a unified synaptic learning rule to account for the influence of environmental experience on synaptic efficacy, stating that both potentiation and depression were borne of presynaptic activity. Their theoretical framework hinged upon the notion of a synaptic modification threshold, denoted by 'θ_m' (not to be confused with the lowest activity threshold, functionally set as 'zero'). In this context, LTP is observed only when the subsequent postsynaptic activity exceeds θ_m, while LTD is the result of a failure to exceed this value. Therefore, the direction of modification is a function of the degree of correlation between pre- and post-synaptic activity (Bear, 2003). In concrete terms, both of these processes involve the flow of Ca^{2+} through postsynaptic NMDARs following depolarisation (Malenka & Bear, 2004). A

cascade of events follow, that, when the Ca^{2+} levels are high, result in an increase of α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPAR) phosphorylation and insertion into the postsynaptic membrane, and thus LTP. When the increase in Ca^{2+} is less pronounced, the occurrence of both of these events is diminished, resulting in LTD. Cooper *et al.'s* notion of a threshold determining the direction of synaptic modification is appealing if Ca^{2+} increase is considered to be the mechanism driving both forms of plasticity. Ultimately, both in terms of function and mechanisms, LTP and LTD can be considered the reciprocal of each other (Mountcastle, 1998).

An important operational consequence of Cooper *et al.'s* theory is that it appeared to allow explanation of how neurons 'decide' the direction of modification in response to different stimuli; stimuli eliciting responses above θ_m potentiate concurrently active synapses, while synapses active during the presentation of stimuli resulting in responsivity below θ_m depress (Bear, 1996). However, the ability of the theory to explain stimulus specific responsivity is limited to situations in which θ_m is fixed, which also means that situations will arise where patterns of stimuli are all above or below θ_m, resulting in the undifferentiated potentiation or depression of all active synapses (Bear, 1996). The Bienenstock-Cooper-Munro (BCM) theory (Bienenstock, Cooper & Munro, 1982) provided an addendum to account for such situations, the 'sliding threshold' hypothesis. To maintain both stability and selectivity in an environment replete with patterned inputs, θ_m is allowed to 'vary as a non-linear function of the average integrated postsynaptic activity' (Bear, 1996, p. 13454). Therefore, it is the history of postsynaptic activity that sets the value of θ_m. Sensory experience should increase θ_m, promoting LTD over LTP, while low activity, or deprivation (e.g., binocular deprivation), should decrease θ_m, increasing the likelihood of LTP over LTD.

Maturational Decline in Synaptic Plasticity

Rather than being a static phenomenon, the molecular mechanisms of LTP appear to change across development (Malenka & Bear, 2004), with a major functional signifier being the relative decline in both naturally occurring plasticity and the experimentally induced phenomena of LTP and LTD (Heynen & Bear, 2001; Crair & Malenka, 1995; Rao & Daw, 2004). Through their LTP induction studies in the adult visual cortex, Heynen and Bear (2001) identify two possibilities for the apparent developmental decline in LTP; a down-regulation of molecules required to support thalamocortical LTP or an up-regulation of inhibitory mechanisms, which would place greater demands on the induction protocol needed to effectively induce LTP. Ultimately, Heynen & Bear (2001) favour the latter due to their ability to overcome difficulties in inducing adult neocortical LTP with thalamic stimulation *in vivo*. However, age related decline in the ability to induce synaptic modification is also correlated with a decline in NMDAR mediated synaptic currents (Crair & Malenka, 1995, Feldman, Nicoll & Malenka, 1999) and alterations in molecular properties of NMDARs, such as changes in their subunit composition (Barth & Malenka, 2001). It is believed that the decrease in the NR2B subunit relative to NR2A may be a factor in the reduction in plasticity seen across development (Philpot *et al.*, 2001; Sun *et al.*, 2005). As well, the disturbance to normal experience-dependent mechanisms caused by ifenprodil blockade of the NR2B subunit throughout the critical period for auditory development results in an enduring impairment in adult synaptic plasticity (Mao, Zang, Zhang & Sun, 2006). There also appear to be layer specific differences in the cortex. For example, the thalamocortical synapses of layer IV are particularly resistant to LTP and LTD induction protocols beyond the termination of the critical period (Crair & Malenka, 1995; Barth & Malenka, 2001; Rao &

Daw, 2004). However, Martin and Morris (2002) point out that failure to induce LTP (or LTD) may simply indicate that the wrong induction parameters are being utilized. Regardless, it is clear that in an experience-dependent manner, plastic processes during development operate to reinforce functionally relevant synaptic connections, with LTP believed to be the cellular mechanism driving developmental plasticity (Kirkwood, Lee & Bear, 1995), while in adulthood it plays an adaptive role, modulating the efficacy of neurons in response to a changing environment (Grassi *et al.*, 2004).

LTP and LTD Induction in the Primary Auditory Cortex (A1)

The paucity of auditory cortex investigation relative to the visual and somatosensory cortices also extends to induction studies of LTP and LTD, which, when studied, have tended towards *in vitro* preparations. In particular, Kudoh, Shibuki and colleagues, have experimentally examined both LTP (Kudoh & Shibuki, 1994) and LTD (Kudoh, Sakai & Shibuki, 2002) in thalamocortical slices, using theta burst stimulation (TBS) and low frequency stimulation (LFS), respectively. The evoked field potentials in A1 are generally bi-phasic, with the late component believed to represent the postsynaptic population spikes in the pyramidal neurons (Kudoh & Shibuki, 1996; Kudoh *et al.*, 2002). While high frequency stimulation was effective in producing long lasting potentiation in A1, low frequency stimulation resulted in transient depression that gradually recovered to baseline over the course of about 20 minutes post induction. The mechanisms for inducing both LTP and LTD may differ across various synapses, with LTD, unlike LTP, seeming to depend on both NMDARs and metabotropic glutamate receptors (mGluRs) (Kudoh *et al.*, 2002). The competitive NMDAR antagonist D-2-amino-5-phosphonopentanoic acid (D-APV) almost completely blocked A1 LTP induction following tetanic stimulation, though LTD was not significantly suppressed by either D-

APV or the competitive mGluR antagonist (RS)-a-methyl-4-carboxyphenylglycine (MCPG), following the requisite LFS (Kudoh & Shibuki, 1994). However, a mixture of the two antagonists was found to block LTD induction at statistically significant levels. It is important to note that these results only held when field postsynaptic potentials (fPSPs) were measured in the supragranular layers following layer IV stimulation. Medial geniculate nucleus (MGN) stimulation achieved comparable levels of depression, though application of MCPG alone was sufficient to significantly block LTD induction. The functional significance of these findings, in the context of the other forms of physiological plasticity already discussed, is exemplified by the fact that enhancements in sound discrimination are also blocked when APV is injected into A1 immediately prior to training (Sakai, Kudoh & Shibuki, 1999).

Together, LTP and LTD provide a unifying, synaptic level mechanism by which to describe the many forms of cortical plasticity outlined in Chapter 1. These mechanisms are experience dependent, such that following a deprivation/reduction of sensory input LTP may be favoured over LTD, while high levels of recent activity may promote LTD over LTP. Further, the well noted decline in plasticity across development is also reflected by the difficulty in inducing both LTP and LTD beyond critical periods of development. This may be the consequence of a reduction in the relative levels of NR2B subunits to NR2A as the cortex matures. With respect to the primary auditory cortex, both LTP and LTD have been successfully induced in adult rats *in vitro*, though the particulars of *in vivo* induction have yet to be elucidated.

Hypotheses

The auditory cortex receives glutamatergic inputs from the MGN (Kudoh *et al.*, 2002), which relays information to the cortex from the inner ear. As such, the *in vitro* work discussed utilised a slicing plane that preserved as many MGN afferents to A1 as possible. However, Kudoh and Shibuki (1997) suggest that *in vivo* work in the auditory thalamocortical system should be more applicable to functioning in the intact organism than that reported by such *in vitro* investigations, owing to the larger number of intact connections facilitating postsynaptic depolarisation. So, while their *in vitro* work utilised stimulation of MGN afferents in the white matter, *in vivo* study can employ direct stimulation of the MGN itself. Under the assumption that their *in vitro* work in the auditory cortex does in fact have an *in vivo* correlate, it seems pertinent to further examine the relationship between the plasticity seen during the critical period of auditory development and that seen in the mature auditory cortex. Evidence clearly suggests a developmental decline in both naturally occurring and artificially induced plasticity. This implies that even if similar levels of synaptic change were possible during these temporally distinct epochs, change would be easier to induce during the critical period. Chang and Merzenich's (2003) use of continuous white noise rearing to indefinitely delay entry into the critical period provides an excellent means by which to examine the experimental induction of LTP and LTD between age matched animals at different stages of A1 development. Further, the experience-dependent synaptic modification threshold proposed by the BCM theory creates a framework by which to examine differences in plasticity between control animals and those experiencing continuous white noise housing for extended periods either during adulthood or development. Together such

investigations should give insight into the differential impact of experiential factors on auditory thalamocortical synaptic plasticity between adulthood and early development

Since there is a lack of *in vivo* data, the parameters for inducing both LTP and LTD in the auditory cortex of adult rats must be determined. It is predicted, as indicated by *in vitro* work, that both synaptic potentiation and depression will be inducible in the adult primary auditory cortex *in vivo*. Recordings will be made in the superficial layers of A1, since it is these layers that are hypothesised to be the location of naturally occurring synaptic modification in the sensory neocortex (Kirkwood *et al.*, 1996). The extent to which these neurons are modifiable will be explored by employing a saturation paradigm whereby stimulation is applied every hour. In A1, LTP is likely to be an NMDAR mediated process, while both NMDAR-dependent and mGluR-dependent forms of LTD are thought to coexist. Once appropriate LTP and LTD induction paradigms are identified, it is predicted that the appropriate antagonists will suppress these plastic events.

To successfully arrest the mechanisms responsible for postnatal A1 development, continuous white noise exposure is essential between PND14 and PND23, since sharpening of receptive fields occurs during this period (Zhang *et al.*, 2002). However, to allow for individual differences in development, white noise will be applied somewhat earlier (PND5), which will also provide an opportunity to gradually increase the intensity of the stimulus to its desired maximal level, minimising maternal distress. With regards to removal from the white noise environment, PND50 is suitably distant from the termination of the critical period to allow electrophysiological comparison to naïve adult animals (Chang & Merzenich, 2003). Further, by PND50 the animal is at an age in which

skull landmarks are relatively stable for the purposes of stereotaxic measurement. For our purposes, PND1 will be considered the day of the pups' birth.

Electrophysiological comparison of adults housed in a standard sound environment with age-matched adults reared and housed in continuous white noise is predicted to reveal differences in the amount of bi-directional plastic change that is inducible, as well as the induction threshold. It is hypothesized that white noise reared animals will show a greater capacity for LTP, owing to lack of refinement in their CFs, and reduced capacity for depression, since these synapses remain in a reasonably weak, immature state. Compared to controls, animals who have experienced continuous white noise housing for extended periods, initiated during adulthood, are expected to show a reduction in LTP and an enhancement in LTD, as predicted by the BCM theory of sliding synaptic modification thresholds in response to recent experience.

Materials and Methods

Experiment 1: Characterisation of bi-directional plasticity of the primary auditory cortex

Subjects

Male Long-Evans rats (250-400 g), obtained from Charles River, Quebec, were housed four per cage in a colony room (12-12 h reverse light cycle, lights on at 0700 h) with food and water *ad libitum*. All procedures were in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

Surgical Preparation

Anaesthesia was induced using urethane administered (*i.p.*) in three doses of 0.5 g/kg, with supplements given as necessary. The suitability of this anaesthetic for non-recovery surgery in electrophysiological investigations lies in its minor influence on cell excitability (Shirasaka & Wasterlain, 1995) and relative lack of impact on neurotransmission in both subcortical areas and the peripheral nervous system (Maggi & Meli, 1986a; 1986b). After insertion into a stereotaxic device (David Kopf, Tujunga, California, USA), the rats' body temperature was maintained at 36-37°C throughout the experiment using a heating pad and cotton wool blanket.

An incision was made to expose the skull surface and a small hole drilled above the medial geniculate body (AP -5.3, L +4.0, V -5.4 to -6.4). Access to the primary auditory cortex (AP -4.5, L +8.0, V -3.4 to -5.4) was gained by parting the muscle from the side of the skull and drilling a hole large enough to allow the entry of both a reverse microdialysis probe and an electrode. Both placements were made ipsilaterally (Figure 1). Holes for ground and recording reference screws were made over the prefrontal cortex and cerebellum, respectively, though on the side contralateral to the electrode placements. All stereotaxic measurements were based on the anatomical work of Paxinos and Watson (1998).

Electrophysiology

A concentric bipolar electrode (Rhodes Medical Instruments Series 100, David Kopf, Tujunga, CA, USA) was used to stimulate the MGN, while A1 fPSP recordings were made against the reference screw, placed in the skull over the cerebellum, using a monopolar recording electrode (125 μm stainless steel, Teflon insulated). The stimulation electrode was connected to a stimulus isolation unit (ML 180 Stimulus Isolator,

ADInstruments, Toronto, ON, Canada, running Chart software, vs. 3.6.5) providing a constant current output. The recording electrode was connected to an amplifier and A-D converter (PowerLab/16s system, ADInstruments, Toronto, ON, Canada) allowing the signal to be amplified, filtered (0.3 Hz to 1 kHz), digitised (10 kHz) and stored for offline analysis. Pharmacological manipulations were made using a reverse microdialysis probe (Mab. 2.14.4; 2mm active PES membrane; 35 kDA cutoff; S.P.E. Limited, North York, ON, Canada) placed immediately adjacent to the recording electrode, with the probe-tip set 1 mm below the electrode.

Once the electrodes had been adjusted ventrally to maximise fPSP amplitude and augmenting responses (100 ms interpulse intervals) in A1 in response to stimulation of the MGN, the animal was left to stabilise for approximately 30 min. An input-output curve was then generated using successive test pulses from 0.1 to 1.0 mA, in 0.1 mA increments, to select the stimulation intensity eliciting a response 50-60% of the maximal fPSP amplitude. Using this test intensity, single pulse stimulation was applied in volleys of 10 at 5, 1, 0.5 and 0.3 s intervals, for a total of 40 pulses. To assess the augmenting response in A1 at varying interpulse intervals, volleys of 10 paired pulses at interpulse intervals of 25, 50, 75, 100, 250, 500 and 1000 ms, were then applied. This process of paired-pulse recording was also repeated at the end of the experiment to examine how each stimulation paradigm affected this response.

Pilot Work

Prior to the main phase of experimentation the appropriate parameters for inducing both LTP and depression, *in vivo*, were determined. It was found that LTP was reliably induced using theta burst stimulation (TBS) consisting of 10 consecutive bursts (five pulses at 100 Hz/burst) at five Hz, followed by a further 10 bursts 10 seconds later.

Depression was induced using low frequency simulation (1800 1 Hz pulses) at twice the test pulse intensity.

Data Collection and Pharmacological Manipulation

Animals were divided into groups receiving either TBS or low frequency stimulation (LFS) of the MGN. Each of these groups was subdivided into two groups, such that one received aCSF (1.0 µl/min) and the other the competitive NMDAR antagonist D-APV (24 mM, 1.0 µl/min) by means of reverse microdialysis application. The relatively high concentration of D-APV was selected in consideration of the ~10% permeability of the microdialysis probe. Twenty minutes prior to baseline fPSP recordings, application of the appropriate solution was initiated using a microdialysis pump (CMA/120, Solna, Sweden) connected to the reverse microdialysis probe by FEP tubing (S.P.E. Limited, North York, ON, Canada). Once started, the solution was applied continuously throughout the experiment.

Before TBS or LFS to induce LTP or LTD, respectively, a baseline measure of fPSP amplitudes was obtained by applying single pulses of 0.2 ms duration every 30 s. Baseline was considered stable once 60 consecutive fPSPs were recorded that were between 95-105% of the average recording during the 30 min baseline period, as assessed by comparing 10 min averages of fPSP amplitudes.

Immediately after obtaining the 30 min baseline fPSP measure, stimulation (TBS or LFS) was applied, followed by a 60 min period of single pulse recording. This pattern of stimulation and recording was repeated three more times, for a total of four induction periods and four hours of recoding (excluding the initial 30 min baseline period). Multiple periods of stimulation were intended to saturate levels of potentiation/depression in the thalamocortical pathway.

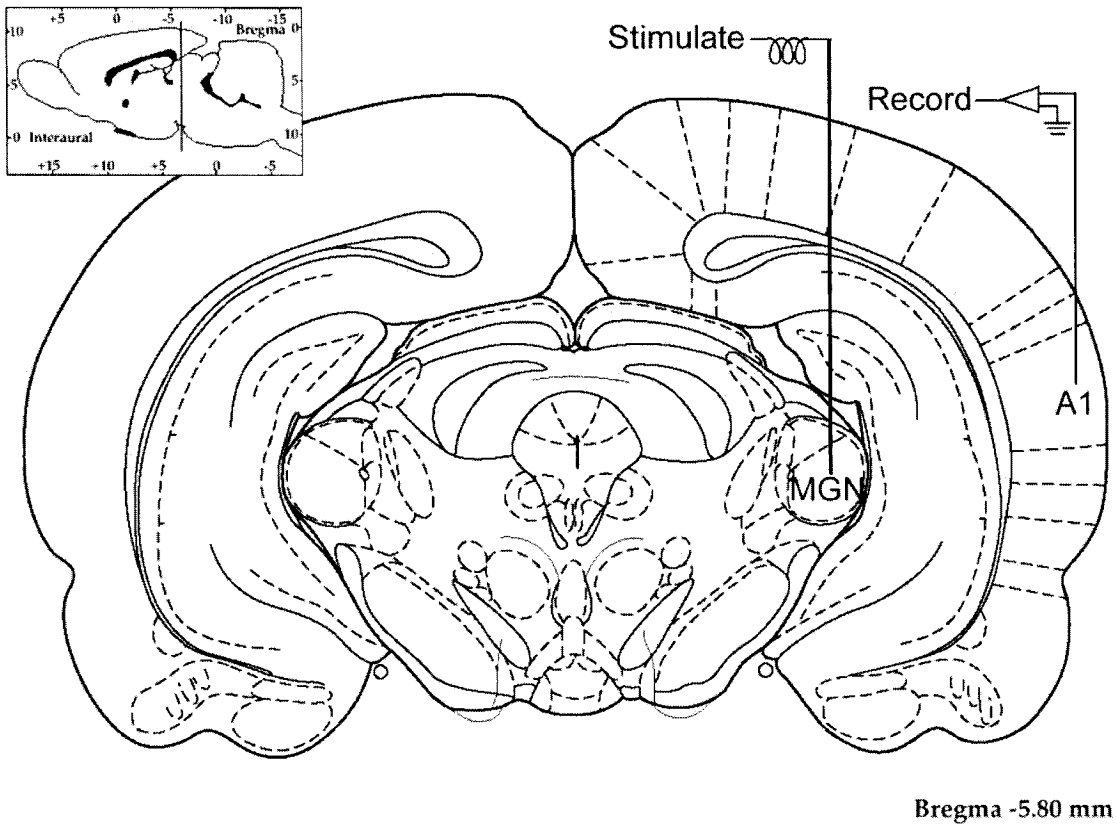

Figure 1. A coronal brain slice schematic of the region 5.80 mm posterior from bregma, indicating the placement of the stimulation electrode in the medial geniculate nucleus (MGN) and the recording electrode in the primary auditory cortex (A1).

Histology

Once all recordings had been completed, the animal was perfused through the heart with 10% formalin and the brain removed and stored in 10% formalin solution for a minimum of 24 hours. A cryostat was then used to section the brains into slices (40 μm), which were then mounted on microscope slides. Standard histological techniques were followed to verify electrode placements (Figure 2), with those showing inaccurate placements excluded from data analysis.

Data Analysis

For each rat, maximum fPSP amplitude measures were calculated using the second peak of each fPSP, which is the portion reflective of synaptic activation of A1 neurons (Kudoh *et al.*, 2002; Kudoh & Shibuki, 1996) (Figure 3). Ten min averages were taken across the four hours of recording, as well as the 30 min of baseline. These averages were then normalized by dividing them by the average baseline amplitude for each animal. All data was expressed as mean ±S.E.M.

For statistical analysis, a two-way analysis of variance (ANOVA) and simple effects were calculated for each stimulation condition group, TBS and LFS, using CLR Anova (v. 1.1, Clear Lake Research Inc., Houston, TX, USA). The between group factor, drug group, had two levels, aCSF and D-APV, with the repeated measures factor being the fPSP amplitude measurements taken every 30 s across the 30 min baseline period and each hour of recording following the four stimulation episodes.

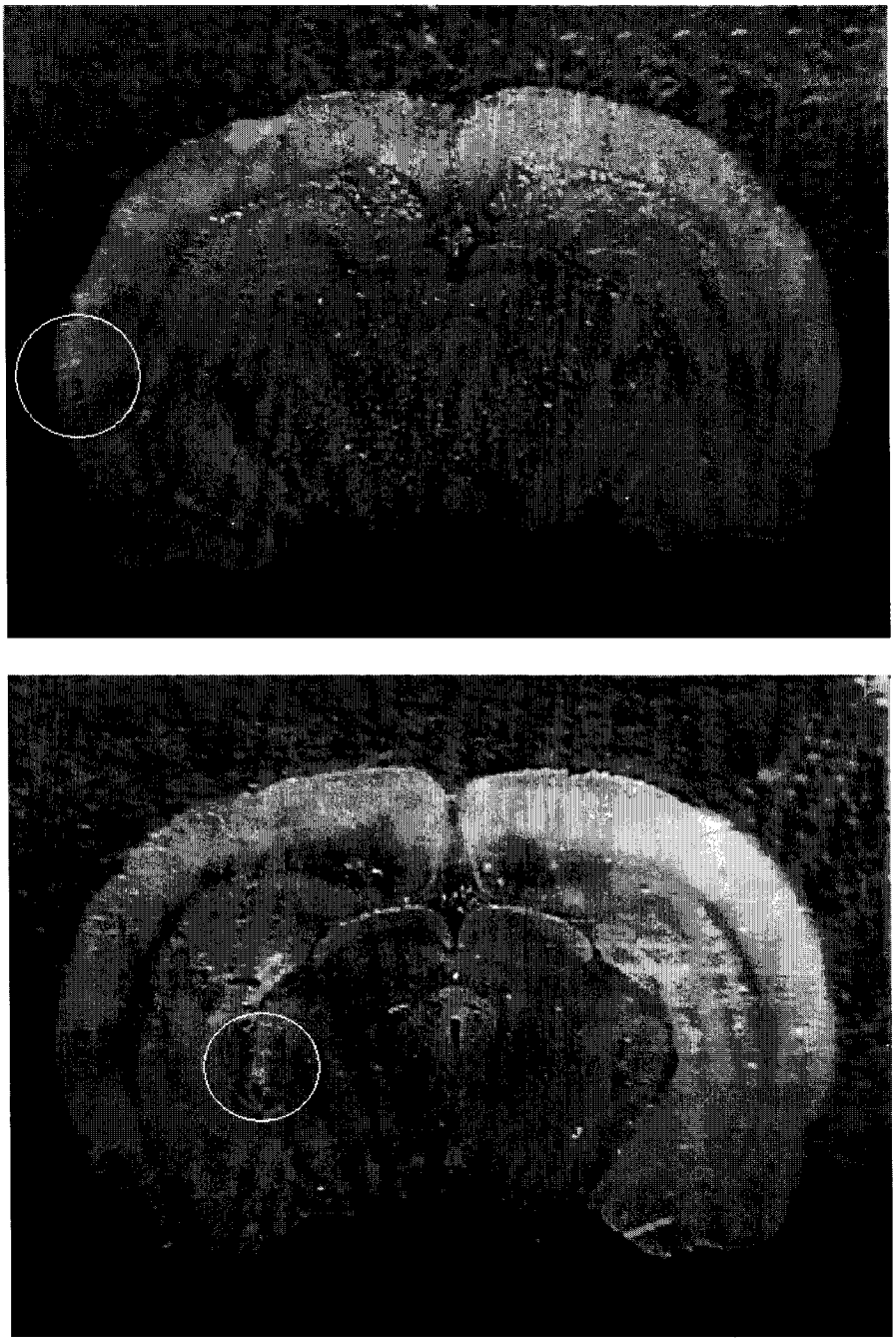

Figure 2. Typical examples of brain slices used in histological analysis to verify electrode and microdialysis probe placement. The white circles highlight the electrode placement. Top: monopolar recording electrode with microdialysis probe in the primary auditory cortex (A1). Bottom: bipolar stimulation electrode in the medial geniculate nucleus (MGN).

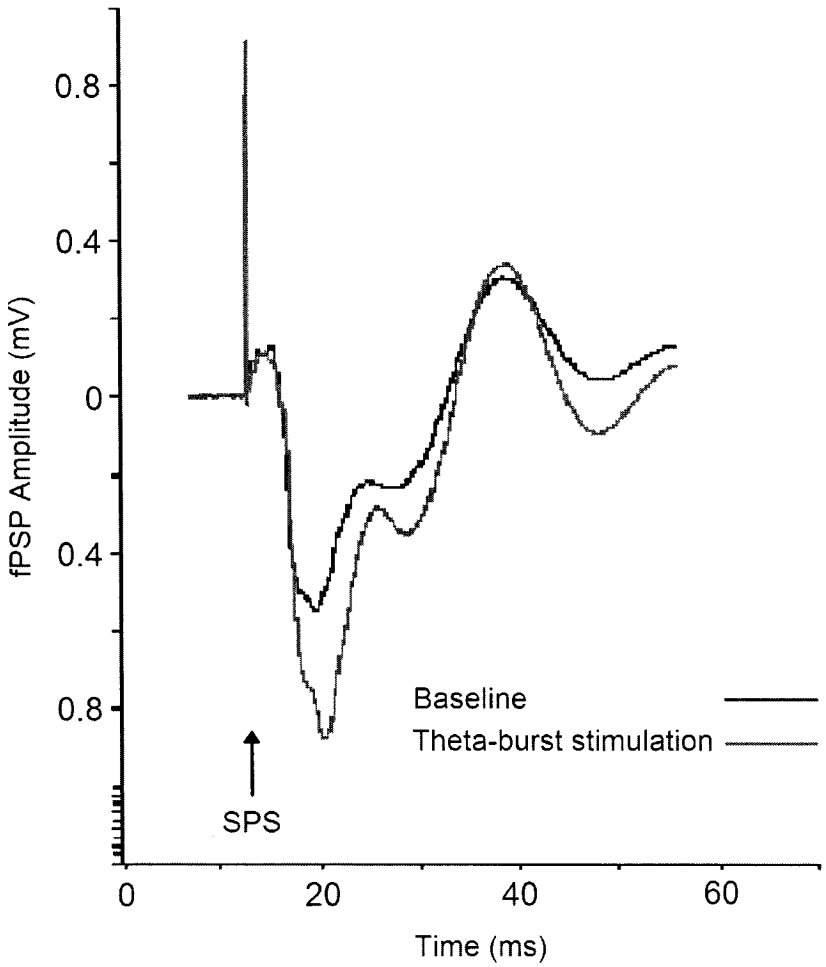

Figure 3. Characteristic auditory cortex evoked potentials, elicited by single pulse stimulation (SPS) of the medial geniculate nucleus (MGN) and recorded in the primary auditory cortex (A1). The darker line indicates the evoked potential before theta-burst stimulation (TBS), while the lighter line shows the potentiated response following TBS.

Experiment 2: Auditory cortex plasticity following continuous white noise rearing

Subjects and Rearing Conditions

Untimed (approx. 14 days) pregnant Long-Evans rats were obtained from Charles River, Quebec. Animals in the control condition (i.e., unaltered sound environment) were housed in a standard colony room, while those in the experimental group were placed in a sound-attenuated chamber (114 x 61 x 66 cm, plywood lined with aluminium, fitted with a time controlled light, a fan, and two equally spaced, centrally mounted ceiling speaker boxes). All animals were maintained on a 12-12 h light cycle, with food and water *ad libitum*.

Starting at PND5, subjects in the sound attenuated chamber were exposed to continuous white noise, incrementally increasing from 68 dB to 82 dB across a five-day period. White noise exposure continued until PND50-60. Chang and Merzenich's (2003) study showed that even at 70dB, white noise was effective in arresting the normal maturation and frequency refinement in A1.

In both conditions, pups were weaned at PND21, with female offspring and mothers removed from the cages. Males were then housed, no more than four per cage (38 x 21 x 49 cm), until PND50-60 (approx. 225-250 g), at which time electrophysiological procedures commenced.

All procedures were in compliance with the guidelines of the Canadian Council for Animal Care and the Queen's University Committee for Animal Care.

Surgical Preparation

All surgical procedures followed the methodology outlined for *Experiment 1*.

Electrophysiology

Electrophysiological methodology mirrored that of *Experiment 1*. However, as microdialysis procedures were not employed in this portion of the study, the recording electrode was not paired with a microdialysis probe prior to insertion into A1.

Data Collection

Though data collection procedures were the same as those employed in *Experiment 1*, no pharmacological manipulations were used in this section of the study. Animals from both the continuous white noise reared group and those reared in a standard noise environment were simply divided into groups receiving either TBS or LFS of the MGN.

Histology

Brain harvesting and histological techniques followed those of *Experiment 1*.

Data Analysis

For each rat, maximum fPSP amplitude measures were recorded and manipulated in the same fashion as in *Experiment 1*.

For statistical analysis, a two-way ANOVA and simple effects were calculated for each stimulation condition group, TBS and LFS, using CLR Anova (v. 1.1, Clear Lake Research Inc., Houston, TX, USA). The between group factor, rearing condition, had two levels, continuous white noise and standard noise environment, with the repeated measures factor being the fPSP amplitude measurements taken every 30 s across the 30 min baseline period and each hour of recording following the four stimulation episodes.

Experiment 3: Auditory cortex plasticity after prolonged continuous white noise exposure during adulthood

Subjects and Rearing Conditions

Male Long-Evans rats were obtained (Charles River, Quebec) at approximately 53 to 59 days of age (226 – 275 g) and placed in a sound-attenuated chamber (construction as in Experiment 2). Continuous white noise was initiated immediately, increasing from 68 dB to 82 dB across a five-day period. White noise exposure continued for 40 days (approximately equal to the time that animals in Experiment 2 experienced noise exposure from onset of hearing to electrophysiology), after which point electrophysiological procedures were carried out.

A 12-12 h light cycle (lights on at 0700 h), with food and water *ad libitum*, was maintained throughout. All procedures were in accordance with the guidelines of the Canadian Council on Animal Care and were approved by the Queen's University Animal Care Committee.

Surgical Preparation

All surgical procedures followed the methodology of the prior experiments.

Electrophysiology

Electrophysiological methodology mirrored that of *Experiment 2*, i.e., did not employ the microdialysis procedures and probe use of *Experiment 1*.

Data Collection

Data collection procedures mirrored those used in *Experiment 2*. Note that this experiment used the control data from Experiment 2 rather than testing another standard noise environment group in TBS and LFS conditions.

Histology

Brain harvesting and histological techniques followed those of the prior experiments.

Data Analysis

For each rat, maximum fPSP amplitude measures were recorded and manipulated in the same fashion as in prior experiments.

For statistical analysis, a two-way ANOVA and simple effects were calculated for each stimulation condition group, TBS and LFS, using CLR Anova (v. 1.1, Clear Lake Research Inc., Houston, TX, USA). The between group factor, housing condition, had two levels, continuous white noise and standard noise environment, with the repeated measures factor being the fPSP amplitude measurements taken every 30 s across the 30 min baseline period and each hour of recording following the four stimulation episodes. Data for the standard noise environment condition was taken from the group used in *Experiment 2*.

Results

Recordings in the superficial layers of A1 showed that single pulse stimulation of the MGN reliably evoked biphasic fPSPs (Figure 3). The first negative peak occurred at a latency of approximately 6.5 ms, while the second negative going potential peaked at about 11.5 ms. In general, the amplitude of the first peak exceeded that of the second. Measurements of fPSP amplitudes were made using the second negative peak, which is thought to reflect synaptic activation of A1 neurons (Kudoh *et al.*, 2002; Kudoh & Shibuki, 1996). Standard histological techniques were used to confirm successful electrode placements (Figure 4), and all subsequent statistical analyses utilised two-way

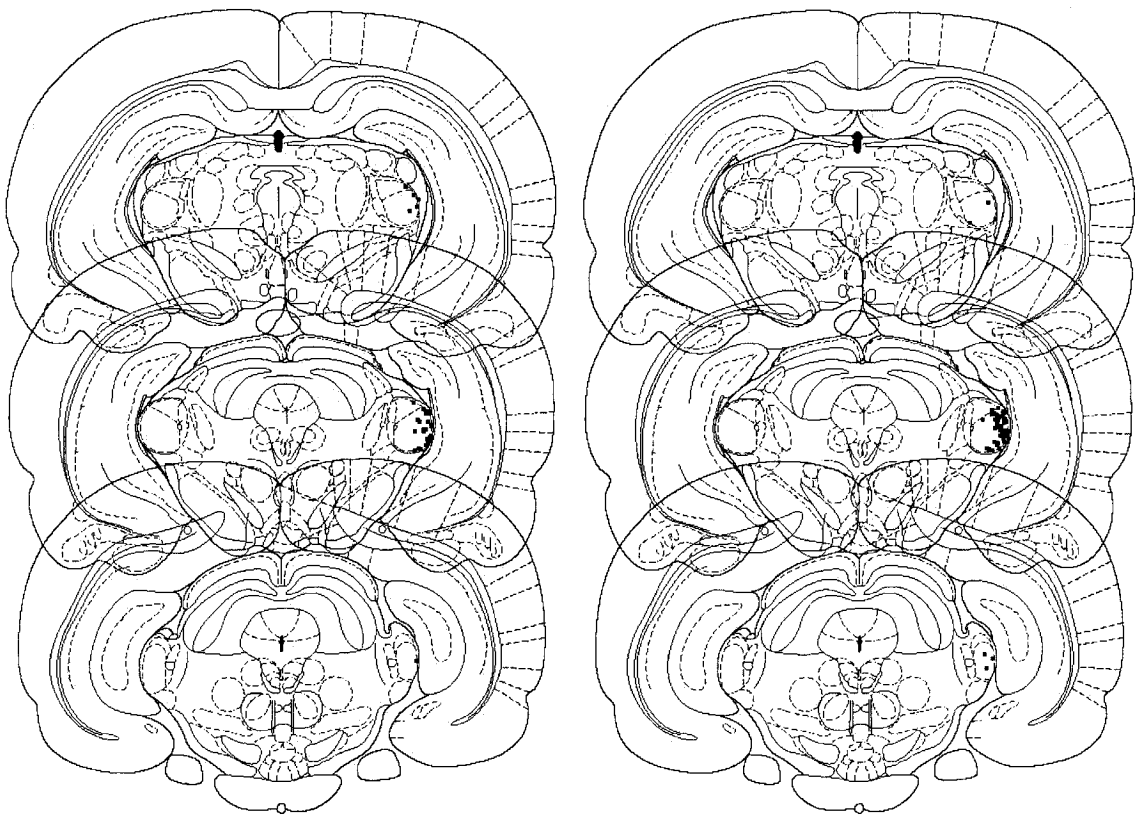

Figure 4. Schematic representing all final bipolar electrode placements that lay within the medial geniculate nucleus (MGN). Subjects who did not meet this criterion were excluded from further data analyses. These three slices collapse across a distance ranging from –4.80 to –6.30 mm posterior from bregma. Left: All placements for Experiment 1. Right: All placements for Experiments 2 and 3.

ANOVA, with post-hoc paired *t*-tests calculated as appropriate to examine simple effects.

Experiment 1: Characterisation of bi-directional plasticity of the primary auditory cortex

Thalamic TBS reliably elicited LTP in animals undergoing auditory cortex aCSF infusion. Following each TBS episode fPSPs increased in amplitude across the subsequent hour-long recording sessions. However, the magnitude of potentiation gradually decreased towards the end of the experiment, suggesting an approach to ceiling levels of enhancement in this pathway. This is reflected by the percentage increase in fPSP amplitudes from baseline levels during the final 10 minutes of each period of recording (i.e., after each of the four TBS sessions): 113%, 128%, 131% and 136% of baseline.

Auditory cortex infusion of APV during recording resulted in a significant suppression of TBS induced potentiation across the four hours of recording. The significant effect of APV (F_{drug} [1, 10] = 5.719, $p < 0.05$) is indicative of its ability to abolish the TBS elicited potentiation seen in the aCSF group. The significant change in fPSP amplitudes across time (F_{time} [26, 260] = 3.849, $p < 0.001$) was due to the change in the fPSP amplitude in the aCSF group, while APV application abolished the enhancement induced by the four TBS sessions ($F_{interaction}$ [26, 260] = 4.593, $p < 0.001$). This observation was confirmed by post-hoc paired *t*-tests, which indicated that it was after the second period of TBS that significant differences between the two groups emerged (Figure 5).

Thalamic LFS, in the presence of aCSF, reliably depressed fPSP amplitudes. However, the phenomenon was transient, returning to baseline levels within 30 min of

each induction episode. Notably, successive periods of LFS resulted in greater levels of depression during the latter half of the experiment (82%, 81%, 71% and 73% of baseline).

While there was a significant change in fPSP amplitudes across time (F_{time} [26, 416] = 4.350, $p < 0.001$), there was no significant effect of APV application (F_{drug} [1, 16] = 0.249, p = 6.109). However, there was a significant interaction of time and drug ($F_{interaction}$ [26, 416] = 1.903, $p < 0.05$). Since depression in this pathway proved to be a transient phenomenon, the number of time points during which the drug could exert any suppressive effect was limited to a period of 20 – 30 min following each LFS. Post-hoc paried *t*-tests (Figure 6) indicated that during the 10 min following the third LFS episode, APV significantly suppressed the LFS induced depression seen under aCSF application ($t(26) = 5.243$, $p < 0.05$). The suppressive effect of APV on depression also approached significance ($t(26) = 3.712$, p = 0.064) in the 10 min following the fourth induction episode. So, it appears that APV can significantly reduce, though not completely abolish, the induction of depression in the auditory thalamocortical pathway.

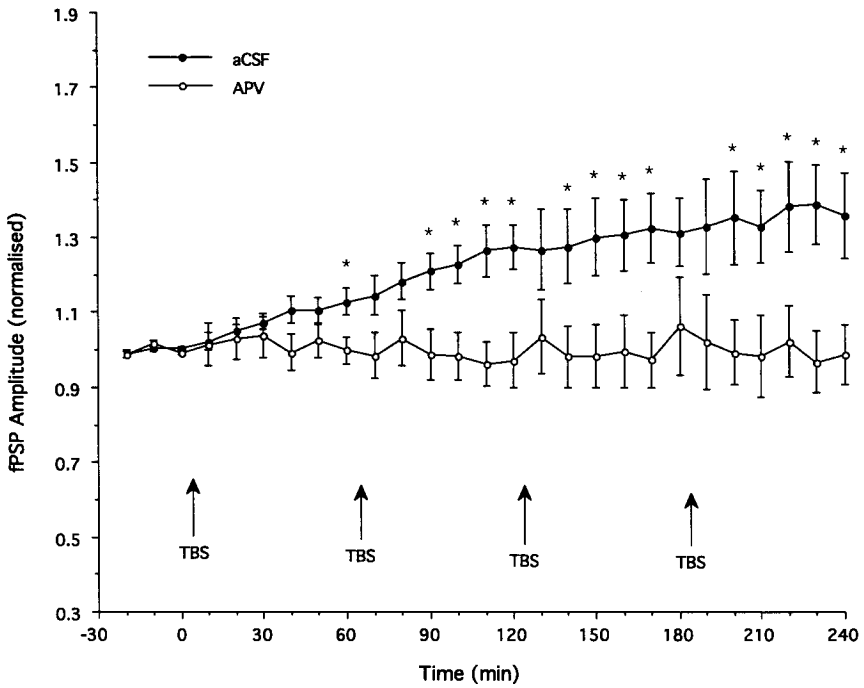

Figure 5. The effects of medial geniculate nucleus theta-burst stimulation (TBS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes. In the presence of aCSF (n = 6), TBS enhanced fPSPs, and this potentiation was abolished by APV application (n = 6).

('*' denotes significance at $p<0.05$, post-hoc paired samples *t*-tests)

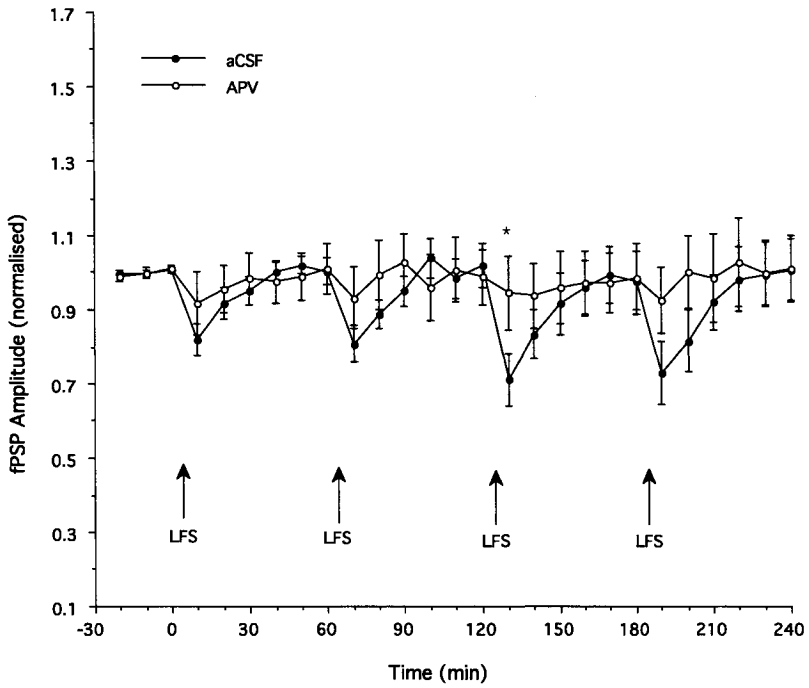

Figure 6. The effects of medial geniculate nucleus low-frequency stimulation (LFS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes. In the presence of aCSF, low-frequency stimulation caused a transient depression in fPSPs (n = 9). APV application (n = 9) resulted in a significant reduction in LFS induced depression immediately following the third period of LFS.

('*' denotes significance at $p<0.05$, post-hoc paired samples *t*-tests)

Experiment 2: Auditory cortex plasticity following continuous white noise rearing

An ANOVA comparing the two rearing conditions (continuous white noise vs. standard noise housing) did not reveal a significant effect of group (F_{noise} [1, 18] = 1.753, p = 0.2020). However, there was a significant effect across time (F_{time} [26, 468] = 13.783, $p < 0.001$). Importantly, the significant interaction of time and noise rearing condition ($F_{interaction}$ [26, 468] = 2.375, $p < 0.001$) indicated that rearing had an effect on change in fPSP over the multiple TBS episodes. This effect is highlighted by the significant simple effects following the final TBS episode (Figure 7), where animals reared in continuous white noise exhibited higher levels of potentiation than animals reared in a normal auditory environment.

The contrast between these two groups was more pronounced following thalamic LFS, in which there were significant effects of noise rearing condition (F_{noise} [1, 22] = 7.853, $p < 0.05$) and time (F_{time} [26, 572] = 6.846, $p < 0.001$), as well as an interaction between the two ($F_{interaction}$ [26, 572] = 4.819, $p < 0.001$). Post-hoc paired *t*-tests show these effects to be more pronounced during the final three hours of recording (Figure 8). Control animals responded to thalamic LFS with a transient depression in auditory fPSPs, returning to baseline levels across a 20-30 min period following each stimulation episode. However, white noise reared animals only showed a 10-20 min period of depression following each induction episode. Notably, this depression was set against a background of substantial potentiation, such that each episode of transient depression was followed by further enhancements of fPSP amplitude.

Experiment 3: Auditory cortex plasticity after prolonged continuous white noise exposure during adulthood

For both adult rats exposed to white noise for 40 days and those experiencing a normal sound environment, there was a significant effect of time (F_{time} [26, 312] = 9.934, $p < 0.001$), indicative of LTP induction with repeated TBS episodes. However, there was no main effect of noise condition (F_{noise} [1, 12] = 0.436, p = 0.5216) and no noise by time interaction ($F_{interaction}$ [26, 312] = 0.320, p = 0.9995), suggesting that sound environment did not alter synaptic potentiation induced by TBS (Figure 9).

Compared to animals housed in a normal sound environment, animals experiencing an extended period of continuous white noise housing during adulthood showed an enhanced response to LFS, with significant main effects of noise condition and time (F_{noise} [1, 14] = 16.437, $p < 0.01$; F_{time} [26, 364] = 10.106, $p < 0.001$), as well as an interaction of the two factors ($F_{interaction}$ [26, 364] = 6.082, $p < 0.001$). Paired *t*-tests indicated that these groups significantly differed from each other at about 50 min following the first LFS episode (Figure 10). Unlike the transient depression from baseline fPSP amplitudes seen in animals that have only experienced a normal sound environment, adults taken from continuous white noise housing show an enduring depression, increasing in magnitude following each LFS episode.

It should be noted (anecdotally) that neither white noise rearing nor exposure during adulthood appeared to cause gross insult to the animals' ability to hear, with startle responses to loud noise evident on their removal from the white noise environment.

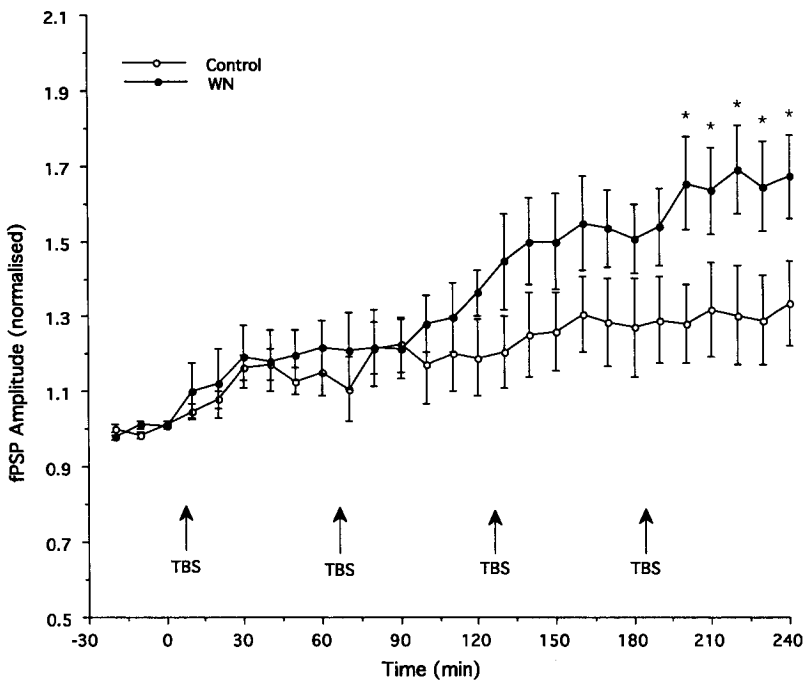

Figure 7. The effects of medial geniculate nucleus theta-burst stimulation (TBS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes. Continuous white noise rearing (WN) (n = 13) significantly enhanced the potentiation elicited by thalamic TBS relative to animals bred and housed in standard noise conditions (n = 7). (* denotes significance at $p<0.05$, post-hoc paired samples *t*-tests). N.B., the same control group (standard noise rearing) is displayed in Figures 6 and 8.

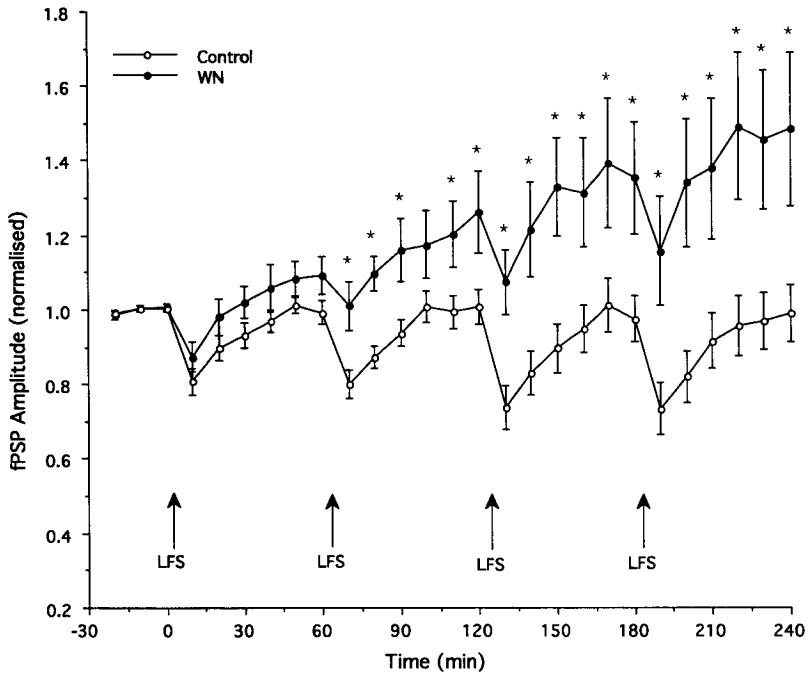

Figure 8. The effects of medial geniculate nucleus low-frequency stimulation (LFS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes. Thalamic LFS induced transient depression (~30 min) in standard noise reared animals (n = 13). However, in continuous white noise reared (WN) animals (n = 11) the same parameters resulted in potentiation, with very brief periods of depression (~10 min) immediately following LFS episodes. (* denotes significance at $p<0.05$, post-hoc paired samples *t*-tests). N.B., the same control group (standard noise rearing) is displayed in Figures 7 and 9.

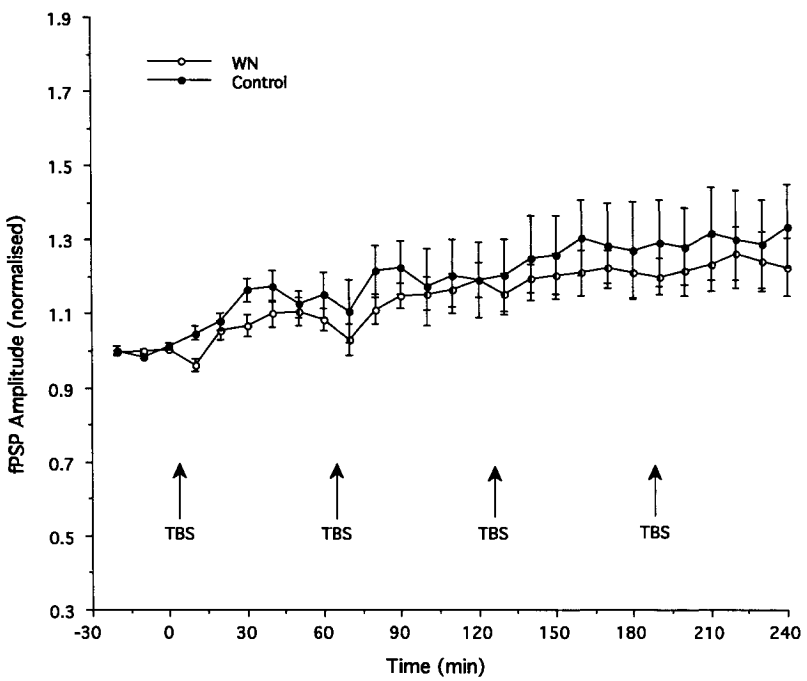

Figure 9. The effects of medial geniculate nucleus theta-burst stimulation (TBS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes. Thalamic TBS resulted in comparable fPSP enhancements in both animals reared and housed in standard noise conditions (n = 7) and those experiencing continuous white noise (WN) housing initiated during adulthood (n = 7). N.B., the same control group (standard noise rearing) is displayed in Figures 6 and 8.

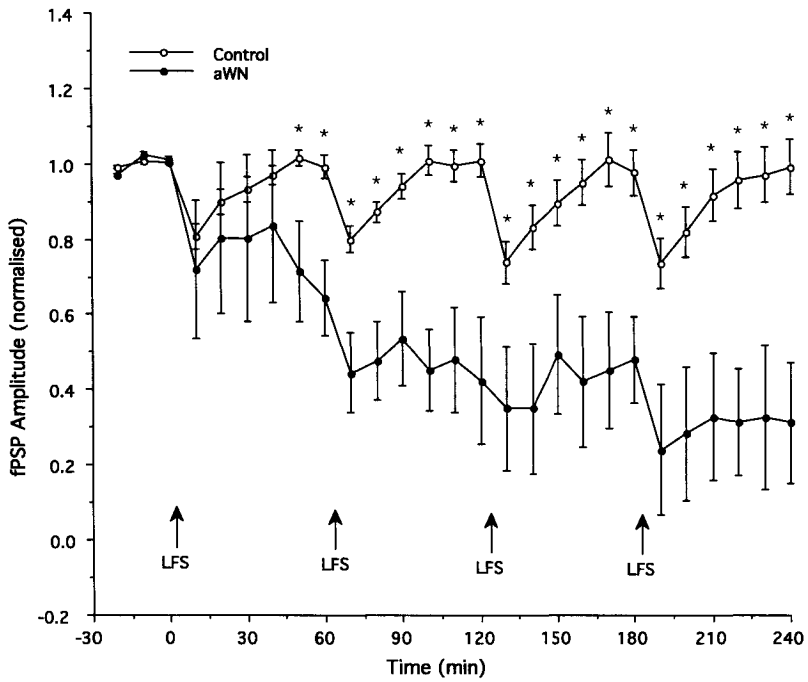

Figure 10. The effects of medial geniculate nucleus low-frequency stimulation (LFS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes. Thalamic LFS induced transient depression (~30 min) in standard noise reared animals (n = 13). However, animals housed in continuous white noise (WN) (n = 3) during adulthood showed an enhanced and enduring depression. (* denotes significance at $p<0.05$, post-hoc paired samples *t*-tests). N.B., the same control group (standard noise rearing) is displayed in Figures 7 and 9.

The age-dependent effects of continuous white noise exposure are summarised in Figures 11 and 12. Figure 11 illustrates the higher levels of potentiation in animals housed in continuous white noise during the critical period for auditory development, compared to animals raised in a normal sound environment and those introduced to a continuous white noise environment during adulthood. These latter two groups showed comparable levels of potentiation under our induction parameters. Figure 12 shows the effect of LFS on fPSP amplitudes, dependent on age of continuous white noise exposure. Adults exposed to continuous white noise during adulthood showed enhanced levels of depression compared to those having experienced a standard sound environment, while those reared in continuous white noise showed potentiation following LFS.

Note Regarding Excluded Animals: Experiments 2 and 3

Due to technical issues with the equipment and examination of electrode placements during histology a large number of animals were excluded from the groups experiencing LFS in Experiments 2 and 3. This left an *n* of 2 for the control group (animals reared in a normal sound environment who were then exposed to episodes of thalamic LFS). The results from these two animals were comparable to those observed in the aCSF-LFS condition of Experiment 1 using animals reared at Charles River (Quebec), i.e., a transient depression, returning to baseline fPSP amplitudes within 20-30 min of each LFS episode. Owing to the period of time between Experiment 1 and the later experiments a further two animals from Charles River were run, confirming that these results were still obtained regardless of any changes in methodology resulting from increased expertise in performing the procedures. As such, the results from these four animals were added to the results from the original aCSF-LFS group of Experiment 1, to

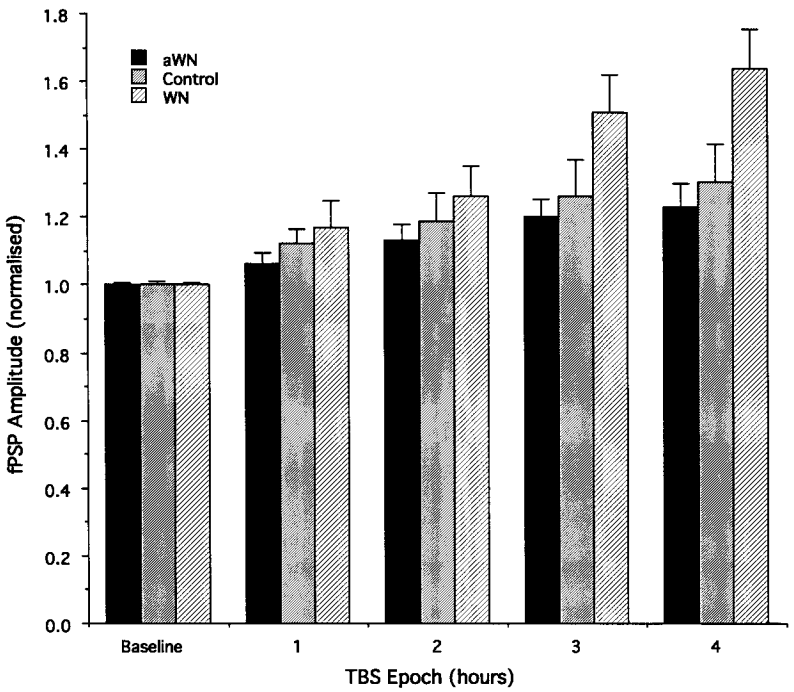

Figure 11. Bar graph showing the effects of medial geniculate nucleus theta-burst stimulation (TBS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes for animals experiencing different sound environments (normal sound environment = control; continuous white noise rearing = WN; adults exposed to white noise housing = aWN). Values shown depict the average fPSP amplitudes across the 30 min baseline period and each hour of recording following TBS episodes. N.B., combines data from Figures 6 & 8.

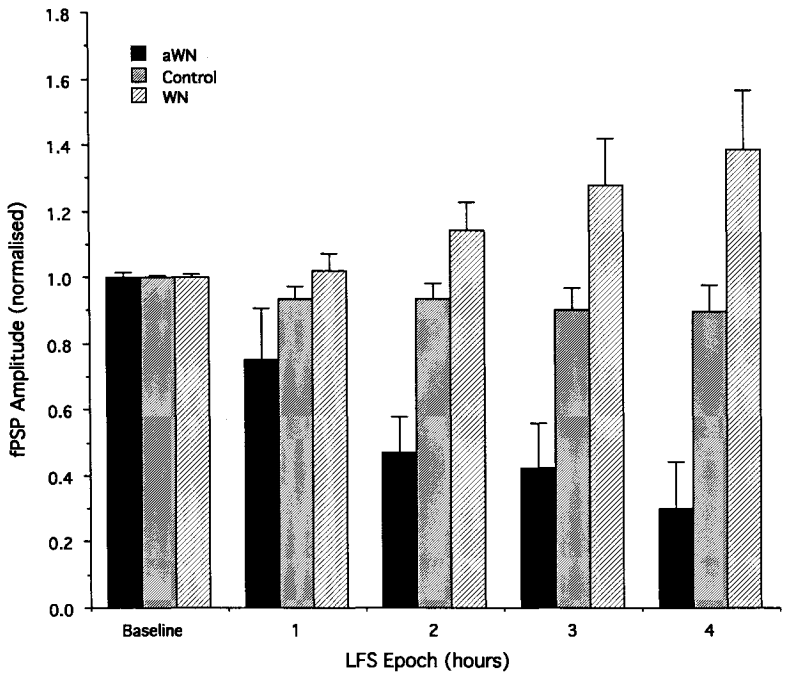

Figure 12. Bar graph showing the effects of medial geniculate nucleus low-frequency stimulation (LFS) episodes on primary auditory cortex field postsynaptic potential (fPSP) amplitudes for animals experiencing different sound environments (normal sound environment = control; continuous white noise rearing = WN; adults exposed to white noise housing = aWN). Values shown depict the average fPSP amplitudes across the 30 min baseline period and each hour of recording following LFS episodes. N.B., combines data from Figures 7& 9.

make the control LFS group for Experiments 2 and 3. Animals are currently being reared at the Department of Psychology, Queen's University, to confirm this decision.

While animals that experienced continuous white noise housing during adulthood showed a significant enhancement in depression compared to animals that only experienced a normal sound environment, the *n* of 3 necessitates the addition of more animals to this group. Continuous white noise exposure of adults has commenced to give further confidence to our findings for this group.

Discussion

Experiment 1: Characterisation of bi-directional plasticity of the primary auditory cortex

This experiment has provided what we believe to be the first *in vivo* example of bi-directional synaptic plasticity in the auditory thalamocortical system. For the most part, our work confirmed *in vitro* findings (Kudoh & Shibuki, 1994; Kudoh, *et al.*, 2002). One minor difference was found in the character of the fPSP. Kudoh's group showed the first two negative peak latencies to occur at about 2 and 3.5 s, while our *in vivo* work found these latencies to be approximately 6 and 11.5 s. Disparity in peak latency measurements is likely the result of basic methodological differences between *in vivo* and *in vitro* investigation, such as the fact that while all recordings were made in the superficial layers of the primary auditory cortex, we stimulated the medial geniculate nucleus in the whole brain, while Kudoh & Shibuki (1994; 1997) stimulated the white matter thalamic afferents in thalamocortical slices. Importantly, the shape of our fPSPs bear a striking resemblance to those seen *in vitro*, and the relative temporal difference between the biphasic peaks is comparable.

While substantial fPSP enhancements were in evidence in the TBS condition, it should be noted that our induction paradigm is different to that commonly used in typical LTP experiments. The rationale for using multiple induction episodes spaced by hour long periods of recording was two fold: by using multiple weaker inductions, we aimed to avoid achieving ceiling levels of potentiation or depression immediately, thus increasing the likelihood of detecting stable differences in LTP with weak induction protocols, while still being able to probe the limits of plasticity in this pathway emerging later in the experiment. Unlike the LTP evidenced *in vitro* (Kudoh & Shibuki, 1994), which shows a rapid increase in fPSP amplitudes following a single strong TBS, our finding of a gentle positive gradient (Figures 4, 6 & 8) is likely a product of the weaker induction episodes used. However, the comparable maximal levels of potentiation achieved by the end of our experiment are likely due to our use of multiple induction episodes. There is need for further exploration of the impact of single episode LTP induction parameters on auditory cortex fPSP amplitudes, with regards to speed of potentiation, duration of the enhancement, and maximal levels of potentiation.

LTD is relatively more difficult to induce than LTP (Massey *et al.*, 2004), with the classic induction parameters being the application of 900 single pulses at 1 Hz (e.g., Kudoh *et al.*, 2002). While these parameters proved sufficient to induce depression *in vitro*, our current study required 1800 pulses at 1 Hz, at twice the test pulse intensity. This is assumed to once again reflect fundamental differences between *in vivo* and *in vitro* work, such as the existence of more intact fibres and connections in whole animals (Kudoh & Shibuki, 1997). Our results are consistent with the pattern of depression noted by Kudoh *et al.* (2002): fPSP amplitudes recovered to pre-induction levels within 30 min. The transient nature of the depression induced in both the current study and in the *in vitro*

work discussed so far precludes it from being accurately described as *long*-term depression. A key result revealed by our multiple induction session approach is that greater levels of depression are achieved following each episode, suggestive of a 'sensitisation' to the induction protocol with multiple induction sessions.

Pre-induction application of the non-competitive NMDAR antagonist APV also confirmed *in vitro* findings (Kudoh & Shibuki, 1994; Kudoh *et al.*, 2002). LTP was abolished, while the apparent reduction in depression did not show a significant effect of drug on fPSP amplitudes. As suggested by the significant time by drug interaction, this may be due to the limited time window in which APV can have its action on the transient depression caused by LFS. Post hoc tests showed that suppression of depression by APV was only significant immediately following the third episode of stimulation, though also approached significance immediately following the fourth induction episode. The limited effect of APV on depression here warrants further pharmacological investigation. Kudoh *et al.*, (2002) note that while mGluR antagonists, such as MCPG, are also not effective in completely suppressing the induction of depression at these synapses, an MCPG-APV mixture is effective. This suggests that depression is mediated by both NMDARs and mGluRs at these synapses. This remains to be confirmed *in vivo*, as does the observation that NMDARs are not responsible for maintenance of potentiation (Kudoh & Shibuki, 1994).

Experiment 2: Auditory cortex plasticity following continuous white noise rearing

Our hypothesis that there would be a greater capacity for LTP in animals housed in continuous white noise from an early age than age matched controls was confirmed by a higher level of potentiation in the noise reared animals during the final two hours of recording. This is congruent with previous findings of heightened plasticity during early

development (e.g., Kato, Artola & Singer, 1991). Stunningly though, parameters that were sufficient to induce transient depression in control animals resulted in robust potentiation in those reared in white noise. One possible explanation is that during this extremely plastic developmental epoch auditory thalamocortical synapses exhibit a preferential readiness for potentiation over depression. However, this is not supported by findings that LTD is easier to induce during development than during adulthood (e.g., Dudek & Bear, 1993; Kikwood *et al.*, 1996). Perhaps varying the induction parameters for both LTP and depression would be informative in this regard. Use of even weaker stimulation parameters may reveal greater differences between the TBS conditions, and though depression was not achieved in these animals, maybe a different pattern of stimulation would be successful. The greater potential for LTP is also highlighted by the observation that induction episodes 'weak' enough to result in depression in adults raised in normal sound conditions are still effective in producing LTP in white noise reared animals.

Continuous white noise presentation throughout auditory cortex development appears to arrest cortical development in this area (Chang & Merzenich, 2003), though the exact mechanism of its action on cortical activity is debatable. It could be masking external patterned input, preventing pattern discrimination by constantly exciting auditory receptors at all frequencies, or even disrupting rhythmic spontaneous activity bursts (Pienkowski & Harrison, 2005). The importance of spontaneous neural activity has been confirmed in the early development of retinotopic maps, whereby ganglion cell activity is correlated with spontaneous waves of action potentials across the retina (McLaughlin, Torborg, Feller & O'Leary, 2003). While this mechanism is also likely to play a role in

early auditory development it is unclear whether our manipulation exerts its effects by disturbing this process.

The BCM theory indicates that high levels of recent cortical activity would result in synapses primed for depression over potentiation. Assuming that continuous white noise exposure increased activity in A1, this hypothesis of the BCM theory was not supported when using young rats exposed to white noise. For these animals, recent experience did not impair LTP and, in fact, caused our LFS paradigm to produce LTP. Perhaps continuous white noise rearing arrests cortical development by rendering the auditory thalamocortical system inactive. Being at or near the floor for synaptic modification would result in a situation where potentiation was favoured, as seen following dark rearing (Kirkwood *et al.*, 1996), and apparently, continuous white noise rearing. The similar impact of these two developmental manipulations on plasticity suggests that white noise may be acting as a form of sensory deprivation, rather than causing a global activation of thalamocortical fibres.

Experiment 3: Auditory cortex plasticity after prolonged continuous white noise exposure during adulthood

Evidence suggests that visually depriving adult rodents reinstates some aspects of juvenile plasticity (Yashiro, Corlew & Philpot, 2005) by lowering the threshold for synaptic potentiation (He, Hodos & Quinlan, 2006). Continuous white noise represents the opposite situation; high levels of activation which should raise the threshold for potentiation. As predicted, in adults, this manipulation enhanced levels of depression. Surprisingly, there was no significant reduction in LTP, though weaker induction parameters may have proved more successful in teasing out a difference. The apparently opposite effects of white noise on the group exposed throughout development to the

group who only experienced it during adulthood indicates that plasticity mechanisms are responding differently between these groups of animals. Unlike continuous white noise rearing (which we suggest acts as a form of sensory deprivation), it seems that in adulthood continuous white noise functions to raise levels of activity, shifting the synaptic modification threshold and enhancing LTD. While dark rearing is a form of deprivation regardless of onset age, continuous white noise may represent a situation where its effects are opposite when initiated during development or adulthood: deprivation versus high levels of activation, respectively.

The effects of light deprivation on the synaptic modification threshold are reversed by two days of light exposure (Kirkwood *et al.*, 1996). The time required for this normalising effect to occur following removal from a continuous white noise environment is not known, though reversibility of the effect is anticipated. With regards to animals reared in these conditions, 'adult levels' of plasticity are expected to emerge as the auditory cortex matures. Chang and Merzenich (2003) found that 10 weeks after rats reared under continuous white noise were returned to a standard sound environment, their cortical mapping profiles were virtually indistinguishable from those of controls. If returned to continuous white noise housing after this complete cortical 'normalisation' has occurred, we would expect patterns of LTP and depression to mirror those of animals exposed to this environment for the first time during adulthood.

Auditory vs Visual Deprivation

Wiesel and Hubel (1963) provided the prototypical example of reversible sensory deprivation during development. However, both their methodology and results lack direct applicability to the manipulation employed by this study. A key difference is that our aim was to examine differences in levels of experience-dependent plasticity between control

adults and those who underwent a developmental manipulation intended to arrest development, while Wiesel and Hubel were illustrating the importance of early experience by employing a methodology that distorted early sensory input and subsequent cortical development. They utilised lid suturing to block visual input, which allows diffuse light to enter through the eyelids (Mower, Berry, Burchfiel & Duffy, 1981). Lid suturing and continuous white noise presentation seem analogous, since both result in abnormal stimulation of the sensory epithelia, while preventing fine pattern information being perceived. However, brightness (Mower, 1981), temporal, and spatial discriminations (Tieman, Tumosa, & Tieman, 1983) are possible through sutured eyelids. At present, it is not known how effective white noise is with respect to interfering with similar abilities in the auditory domain.

In the visual modality, Mower *et al.* (1981) explored the developmental impact of both light diffusion and dark rearing. The diffusion method allows diffuse illumination through the eyelid, so it does not represent the complete absence of visual stimulation, while dark rearing prevents any light stimulation of the retina. Both are forms of visual pattern deprivation. However, analyses show that the complete deprivation caused by dark rearing has similar developmental consequences as continuous white noise rearing, while diffusion has cortical ramifications more akin to that seen in pulsed noise experiments (Zhang *et al.*, 2001; 2002). Unlike the complete deprivation of dark rearing, diffuse stimulation results in permanent manipulations of cortical circuitry (Mower *et al.*, 1981). The effects of these developmental manipulations are restricted to the lateral geniculate nucleus (LGN), causing deficits in X-cell acuity and LGN shrinkage (Christen & Mower, 1987). Further investigation is required to uncover the relative impact of continuous white noise rearing on the MGN and the A1.

Binocular deprivation can extend the visual critical period, resulting in visual cortical cells with non-selectively responsive RFs (Mower *et al.*, 1981), reminiscent of the RFs in the immature auditory cortex (Chang & Merzenich, 2003). Cortical immaturity is also indicated by susceptibility to monocular deprivation following dark rearing, which results in large-scale alterations comparable to those seen when monocular deprivation is initiated early in life (Mower *et al.*, 1981). As is the case in the auditory cortex (Zhang *et al.*, 2002), manipulations initiated in adulthood do not cause these changes (Mower *et al.*, 1981), suggesting that both continuous white noise rearing and dark rearing are able to arrest this plastic developmental period. So, while complete and reversible blockage of auditory input was not achieved (or even attempted) it seems that our method is similar in terms of its impact on cortical development to complete visual deprivation.

Age-Dependent Role of NMDAR Subunits in Experience-Dependent Plasticity

Our results also clearly show that a substantial potential for plasticity is maintained in the mature cortex. However, there are clearly significant differences in terms of plastic response between the immature and mature thalamocortical auditory system. As the brain matures there is a shift in NMDAR subunit composition, with the level of NR2B subunits decreasing relative to NR2A. This may be responsible for the decrease in plasticity in both the visual (Sun *et al.*, 2005; Philpot *et al.*, 2001) and auditory (Mao *et al.*, 2006) cortices beyond their critical periods for development. If our white noise manipulation really has arrested auditory development it may be that the relative NMDAR subunit composition is responsible for the experience dependent effects seen in the current study. In the visual modality, adult ocular deprivation increases NR2B subunit levels, resulting in a more juvenile-like NMDAR composition (He *et al.*, 2006). Interestingly, while this plastic state was similar to cortical immaturity, dark rearing

operated by a different mechanism. It served to delay the increase in NR2A subunits. Therefore, the mechanisms of visual deprivation appear to differ depending on the age at which the manipulation is employed (Yashiro *et al.*, 2005; He *et al.*, 2006). Our data show that continuous white noise during adulthood exerts effects different to those in juvenile animals. Thus, it is useful to consider the likelihood that the age of white noise onset has specific, differential effects on the NR2A:NR2B ratio. In fact, one interpretation of the BCM theory is that the sliding threshold of synaptic responsivity is dependent on the NR2A:NR2B ratio, such that sensory experience results in an increase in the NR2A:NR2B ratio, and vice-versa (Quinlan, Olstein, & Bear 1999; Quinlan *et al.*, 1999). This is a complex story though, with different areas of the brain showing differential effects of NMDAR subunits on plasticity (Mao *et al.*, 2006).

To delineate the relative role of the NR2B subunit in the different groups of our study, an NR2B subunit antagonist, such as Ro 25-6981 hydrochloride, could be applied to the auditory cortex during electrophysiological recording in all conditions. Owing to the relative influence of the NR2A:NR2B ratio on plasticity during different stages of development and in response to the recent history of activation, one would expect a greater impact of this antagonist on plasticity in the animals reared in white noise than either control animals or animals housed in white noise during adulthood.

White Noise as a Stressor

As assessed by levels of neuroendocrine activation, white noise can be considered a mild stressor (Van Raaij, Oortgiesen, Timmerman, Dobbe & Loveren, 1996). As with other stressors, white noise results in activation of the hypothalamic-pituitary-adrenal axis, with associated responses such as increased corticosterone release (Campeau, Akil & Watson, 1997; Windle *et al.*, 1997). However, only higher noise intensities (over 90

dB) significantly increase corticosterone after acute white noise presentation (Campeau & Watson, 1997). This finding confirms the seminal work of Monjan and Collector (1997), who found that three hour, nightly exposure to 120 dB white noise caused short-term increases in corticosterone. Interestingly, the immune response was found to be enhanced four to five weeks following white noise exposure. Similarly, Kulger, Kalvaram and Lange (1990) found that six hours of nightly white noise increased corticosterone release only during the first week of exposure. While none of these techniques are directly comparable to our method of continuous, long-term exposure, they do suggest that it is unlikely that our adult exposed group would be experiencing heightened corticosterone levels at the time electrophysiological recordings were carried out. This possibility is strengthened by both Campeau and Watson's (1997) definition of 70 to 80 dB as 'moderate' noise not resulting in immunoreactive responses, and Van Raaij *et al.*'s (1996) classification of 85 dB as 'low noise' only resulting in subtle physiological changes. It would appear that the exact physiological response to white noise exposure is a function of the noise intensity and exposure time (Van Raaij *et al.*, 1996). Definitive comment on the impact of potential stress resulting from our experimental housing conditions can only be made with further examination, such as searching for the particular pattern of c-*fos* induction that generally follows audiogenic stress (Campeau & Watson, 1997) or measuring corticosterone levels in the blood plasma.

Learned helplessness provides a good adult model of stress and also indicates its detrimental effect on hippocampal dependent forms of learning and memory (Kim, Foy & Thompson, 1996). In young rats, the first three weeks of life appear to be a period of particular sensitivity with regards to dentate gyrus synaptogenesis and receptor site development (Kehoe, Hoffman, Austin-LaFrance & Bronzino, 1995). As such, stress

during early life can impair spatial learning and both hippocampal LTP and synaptogenesis (Huot, Plotsky, Lenox & McNamara, 2002; Champagne, Francis, Mar & Meaney, 2003). Stress results in increased levels of corticosterone in the plasma, which may bear some responsibility for the effects on synaptic plasticity (Xiong *et al.*, 2004; Yang, Huang & Hsu, 2004) and the loss of hippocampal neurons (Woolley, Gould & McEwen, 1990). Interestingly, while hippocampal LTP is impaired, LTD is enhanced following stress (Kim *et al.*, 1996; Xiong *et al.*, 2004). Kim *et al.* (1996) suggest that stress may cause changes in CA1 NMDARs, resulting in an LTP-like mechanism that impairs further LTP, while enhancing LTD. Such a mechanism bears a striking resemblance to the BCM theory of sliding plasticity thresholds, making stress a possible experiential factor that can act to shift the induction threshold (Xiong *et al.*, 2004). This poses an interesting problem for our group that were introduced to continuous white noise during adulthood. While the combination of gradually increased sound intensity and habituation to the environment following prolonged exposure may act to reduce levels of stress in these animals, LTD enhancement conforms to the pattern of activity suggested by Xiong *et al.* (2004) in response to stress. Future work should assess levels of stress hormones during continuous white noise exposure to evaluate the potential role of stress in explaining the changes in synaptic plasticity noted in the present study.

It is clear that the early environment can have a significant impact on development, and thus, the physiology and behaviour of the adult animal (Levine, 2005). While we believe that initiation of white noise prior to the onset of hearing in our rat pups reduces its potential stress producing effects, it is possible that maternal stress could indirectly impact pup development. Variations in mother-pup interactions, such as licking, grooming, and arched back nursing (Stern, 1997), have been shown to affect the

hypothalamic-pituitary-adrenal and behavioural responses to stress in the adult offspring (Weaver *et al.*, 2004). Fortunately, variations between dams in licking and grooming behaviours peak during the first few days postpartum and disappear by the end of the first week, after which point maternal care is not as important a factor in hypothalamic-pituitary-adrenal gene expression (Champagne *et al.*, 2003; Francis, Champagne, Liu & Meaney, 1999). Since our study initiated white noise five days after birth, with sound intensity being slowly increased across the following five days, we do not believe that there would have been significant impact resulting from differences in maternal behaviour between control and white noise reared animals.

Curiously, lactating female rats show reduced, or no, alterations in hypothalamic-pituitary-adrenal activation, such as corticosterone release, to stress (Windle *et al.*, 1997; Torner, Toschi, Nava, Clapp & Neumann, 2002). This has also been confirmed in breast-feeding human mothers (Altemus, Deuster, Galliven, Carter & Gold, 1995). This hyporesponsivity is likely due to increased prolactin in these animals, which is released acutely following suckling (Grosvenor, Shyr, Goodman & Mena, 1986). This enhances lactogenesis and maternal behaviours, as well as the inhibition of the hypothalamic-pituitary-adrenal response (Blake, 1974; Torner *et al.*, 2002; Torner *et al.*, 2004). While white noise exposure does not appear to activate the hypothalamic-pituitary-adrenal axis in lactating dams, it may actually result in more pup directed activity (Windle *et al.*, 1997), which has been shown to reduce later hypothalamic-pituitary-adrenal activity in the developing animals (Francis *et al.*, 1999). Despite the apparent insensitivity of lactating dams to stress, the first week following birth is characterized by a greater hormonal response to stress (Walker, Trottier, Rochford & Lavallee, 1995). This lends further support to our methodological choice to delay white noise exposure until the fifth

day of life, after which point the presence of the pups is likely to buffer the dams' responses to stress.

The Functional Significance of fPSP Modifications

A general point about LTP and LTD is that these are experimental phenomena (Malenka & Bear, 2004; Lamprecht & LeDoux, 2004). That is to say that while these mechanisms may exist in some form in the behaving animal, the particulars of the induction techniques, for example, make them an abstraction from what is likely to occur naturally. Further, neural network models suggest that to attain a high storage capacity, a system like the brain, must have a sparse encoding mechanism (Willshaw & Dayan, 1990). This is not suggested by the large deviations from baseline fPSP levels seen here and in the *in vivo* work of Kudoh and Shibuki (1994). However, similarly large increases in fPSPs have been noted more naturalistically. For example, Rioult-Pedotti *et al.* (1998) trained rats to reach for food through a partition, using only one paw. Following training, slices of primary motor cortex (M1) were taken from both hemispheres. As expected, fPSP amplitudes were enhanced in the trained hemisphere relative to the untrained. Further, LTP was impaired while LTD enhanced in the trained hemisphere, suggestive of recent strengthening of synapses. The 50% fPSP increase from baseline in the trained hemisphere is comparable to the levels of enhancement seen following TBS in our study. Martin and Morris (2002) suggest that enhancements on this scale may only accompany first time instances of learning, and this could genuinely be the case for animals reared in the relatively impoverished conditions of a cage environment. Also, large changes are expected during critical periods. However, if changes of this magnitude were commonplace in adulthood, one would expect the capacity for learning to become saturated very quickly. Unsurprisingly, the role of synaptic plasticity in memory,

particularly with regards to uncovering the engram, remains a hotly debated topic (Martin & Morris, 2002). Regardless of these concerns and questions, our methodology points to age-sensitive differences in the mechanisms of experience-dependent plasticity.

Plasticity and the Tripartate Structure of the MGN

The MGN electrode placements for this study were considered with the knowledge that the average stimulation intensity we used (approx. 0.5 mA) would cause excitation in a tissue area of approximately 1 mm^3 (Ranck, 1975). This is important as the MGN's 'tripartate structure' (Weinberger & Diamond, 1987) has been shown to display varying levels of plasticity across its divisions (Edeline & Weinberger, 1991a; 1991b; 1992). The medial (MGm) division exhibits the greatest degree of RF plasticity, with the dorsal (MGd) and ventral (MGv) divisions showing progressively less plasticity. Further, these areas do not project to the auditory cortex in the same way, with the A1 receiving input from the MGv and MGm, and the secondary auditory cortex receiving input from MGd and MGm (Weinberger & Diamond, 1987). While the large area affected by the stimulation electrode in this study somewhat negates concerns about relative levels of plasticity across the MGN, it is possible that very lateral or medial placements could result in somewhat different levels of responsivity to TBS and LFS. Further, since different subdivisions of the MGN project more heavily to the secondary than the primary auditory cortex, this could also impact optimal placement for the recording electrode, though in the current study, placements in the secondary auditory cortex resulted in exclusion of the subject from the data set. However, the impact of electrode placement, both within the subdivisions of the MGN, and AI and AII, warrant systematic investigation with regards to levels of *in vivo* LTP and LTD, to fully appreciate whether or not these issues could have resulted in any variability in our data.

'Critical' vs 'Sensitive' Periods

As noted by Mower *et al.* (1981), the critical period is not simply an age-dependent event, but one that is also dependent on sensory experience. Our results, in conjunction with those of Chang and Merzenich (2003) provide support for this notion in the auditory modality. Therefore, the use of the phrase 'critical' seems somewhat outmoded, such that 'sensitive period' may be more appropriate. The environmental enrichment work of Engineer *et al.* (2004) has interesting implications in this regard. They found that animals kept in enriched conditions (cage constructions providing a variety of noise stimuli in response to various actions and movements) from an early age (PND30) exhibited a doubling of tone-evoked potentials, an increased selectivity to quieter sounds and an enhancement of tone frequency selectivity, compared to standard housed animals. However, within two weeks of subsequent standard housing evoked potential amplitudes decreased to typical standard housed levels. Interestingly, adults moved from a standard to an enriched environment showed the same doubling in responsivity seen in animals that have experienced this environment since adolescence, highlighting a form of plasticity that does not appear to diminish in adulthood. This is reminiscent of He *et al.*'s (2006) finding of a return to juvenile-like plasticity mechanisms following adult visual deprivation.

Munakata and Pfaffly (2004) provide an interesting challenge to the notion of time locked 'critical' periods by applying Hebbian learning techniques to overcome inflexibility in language learning beyond the critical period for phoneme learning. Japanese has a phoneme that combines 'r' and 'l' sounds, making it difficult to distinguish between the separate perceptual representations these have in English. Hence, for a native Japanese speaker, both 'r' and 'l' sounds activate a single perceptual

representation, and training to distinguish the two only serves to maintain this tendency, since both 'r' and 'l' are activating the same area. It is only by initiating training with extremely exaggerated 'r' and 'l' sounds, and then gradually reducing the degree of exaggeration, that successful separation of these two perceptual representations can be achieved in adults. This is akin to Fregnac, Shulz, Thorpe and Bienenstock's (1992) use of Hebbian learning mechanisms in designing an associative learning paradigm to alter the orientation specificity of adult visual cortical cells. Both very young and adult cats were trained with striped environments of specific orientations, artificially reinforcing the neural response to one orientation and suppressing the response to another. Although the largest changes in orientation tuning were found in young animals at the peak of the critical period, changes were also seen in the orientation preference of visual cortical cells in adults. Utilising a 'spike timing' method, similar shifts in *perceived* orientation have been achieved in adult humans (Yao & Dan, 2001). While the difficulty levels in achieving these changes in adulthood speaks to the particular sensitivity of critical periods, it also points to the plastic nature of the mature brain, which shows properties one would generally only predict during critical periods.

Applicability to Human Functioning

While there are no *in vivo* demonstrations of human LTP, tissue samples resected from human temporal lobe epilepsy patients have revealed LTP in the dentate gyrus (Beck, Goussakov, Lie, Helmstaedter & Elger, 2000), as well as both LTP and LTD in the inferior and middle temporal cortex (Chen *et al.*, 1996), with all indication being that these forms of synaptic plasticity mirror the properties seen in non-human animals. Non-invasive evidence for an 'LTP-like' mechanism has recently been uncovered in both the human visual (Teyler *et al.*, 2005) and auditory (Clapp *et al.*, 2005) cortices. In Clapp *et*

al.'s (2005) study a tetanic tone pip was presented as a substitute for direct electrical tetanic stimulation while recordings were made using electroencephalogram scalp electrodes. This resulted in an increase in the N1 component of the auditory evoked potential, suggestive of modification in the auditory cortex, which lasted for over an hour. Clearly, basic plasticity mechanisms are broadly conserved across species.

Unlike human foetuses, which show signs of hearing at the beginning of the third trimester (Pienkowski & Harrison, 2005) rats are deaf for the first 12 days after birth (Chang & Merzenich, 2003). This allows for the type of manipulations of the early auditory environment exercised here and in the work of Merzenich and colleagues. Pienkowski and Harrison (2005) performed developmental studies using chinchillas, which, like human beings, begin to hear *in utero*, and are thus born with a more developed tonotopic pathway. Their results with chinchillas led them to suggest that after the initial refinement of connections there are increases in tonal RF complexity, possibly necessary for the emergence of selectivity for species-specific vocalisations. The more contiguous developmental timeline between humans and chinchillas may make them more appropriate for direct comparison to human auditory development (Pienkowski & Harrison, 2005). This research suggests that, in humans, an impoverished early auditory environment may be more likely to lead to deficits in the formation of connections required for the extraction of information needed for complex speech perception rather than distortions in basic tonotopic relationships. Regardless, the validity of our study is not diminished as much can be inferred regarding the mechanisms of plasticity and perhaps even the tonotopic development of an earlier human developmental epoch.

Conclusions

In sum, our results suggest that the absence of patterned auditory stimulation during early postnatal life causes thalamocortical auditory synapses to exhibit a preferential readiness for synaptic potentiation over depression. In adults, prolonged deprivation of patterned sound input with white noise results in synapses favouring depression over synaptic enhancement. Contrary to earlier views that the primary sensory areas are pre-programmed in their patterns of connectivity and are constrained to sensory analysis (Fuster, 1984) this study has highlighted the dynamism of the auditory cortex both during and beyond early development. Plasticity in adults should not be surprising since the auditory thalamocortical system is required to maintain flexibility in the face of constantly changing environmental inputs (Grassi *et al.*, 2004). Not only may plasticity mechanisms differ between adulthood and early development (Harrison *et al.*, 1998), but the history of sensory experience may also exert different effects on synaptic modification (Yashiro *et al.*, 2005). The different responses of our groups to continuous white noise support these notions, though the exact mechanism by which the manipulation exerts its action on the auditory thalamocortical response across development needs to be confirmed. Investigation of potential age-dependent NR2A:NR2B alterations in response to continuous white noise could provide an explanation for the differential expression of synaptic plasticity characterised in the present experiment.

References

Altemus, M., Deuster, P., Galliven, E., Carter, C. & Gold, P. (1995). Suppression of hypothalmic-pituitary-adrenal axis responses to stress in lactating women. *Journal of Clinical Endocrinology and Metabolism, 8*, 2954-2959.

Andersen, P. & Hvalby, O. (1986). Long-term potentiation: Problems and possible mechanisms. In R. L. Isaacson & K. H. Pribram (Eds.), *The hippocampus Vol. 3* (pp. 169-186). New York: Plenum Press.

Bakin, J. & Weinberger, N. (1990). Classical conditioning induces CS-specific receptive field plasticity in the auditory cortex of the guinea pig. *Brain Research, 536*(1-2), 271-286.

Barth, A. & Malenka, R. (2001). NMDAR EPSC kinetics do not regulate the critical period for LTP at thalamocortical synapses. *Nature Neuroscience, 4*(3), 235-236.

Bear, M. (1996). A synaptic basis for memory storage in the cerebral cortex. *Proceedings of the National Academy of Sciences, 93*, 13453-13459.

Bear, M. (2003). Bidirectional synaptic plasticity: From theory to reality. *Philosophical Transactions: Biological Sciences, 358*(1432), 649-655.

Beck, H., Goussakov, I., Lie, A., Helmstaedter, C. & Elger, C. (2000). Synaptic plasticity in the human dentate gyrus. *Journal of Neuroscience, 20*(18), 7080-7086.

Bienenstock, E., Cooper, L. & Munro, P. (1982). Theory for the development of neuron selectivity: Orientation specificity and binocular interaction in visual cortex. *Journal of Neuroscience, 2*, 32-48.

Blake, C. (1974). Stimulation of pituitary prolactin and TSH release in lactating prooestrous rats. *Endocrinology, 94*, 503-508.

Brainard, M. & Doupe, A. (2002). What songbirds teach us about learning. *Nature, 417*, 351-358.

Calford, M. (2002). Dynamic representational plasticity in sensory cortex. *Neuroscience, 111*(4), 709-738.

Carlson, N. (2001). *Physiology of Behaviour* (7th ed.). Massachusetts: Allyn and Bacon.

Campeau, S. & Watson, S. (1997). Neuroendocrine and behavioural responses and brain pattern c-fos induction associated with audiogenic stress. *Journal of Neuroendocrinology, 9*, 577-588.

Campeau, S., Akil, H. & Watson, S. (1997). Lesions of the medial geniculate nuclei specifically block corticosterone release and induction of c-fos mRNA in the forebrain associated with audiogenic stress in rats. *Journal of Neuroscience, 17*(15), 5979-5992.

Champagne, F., Francis, D., Mar, A. & Meaney, J. (2003). Variations in maternal care in the rat as a mediating influence for the effects of environment on development. *Physiology & Behaviour, 79*(3), 359-371.

Chang, E. & Merzenich, M. (2003). Environmental noise retards auditory cortical development. *Science, 300*, 498-502.

Chen, W., Lee, S., Kato, K., Spencer, D., Shepherd, G. & Williamson, A. (1996). Long-term modifications of synaptic efficacy in the human inferior and middle temporal cortex. *Proceedings of the National Academy of Sciences, 93,* 8011-8015.

Christen, W. & Mower, G. (1987). Effects of monocular occlusion and diffusion on visual system development in the cat. *Brain Research, 415*, 233-241.

Clapp, W., Kirk, I., Hamm, J., Shepherd, D. & Teyler, T. (2005). Induction of LTP in the human auditory cortex by sensory stimulation. *European Journal of Neuroscience, 22,* 1135-1140.

Cooper, L., Lieberman, F. & Oja, E. (1979). A theory for the acquisition and loss of neuron specificity in visual cortex. *Biological Cybernetics, 33*, 9-28.

Cousillas, H., Richard, J., Mathelier, M., Henry, L., George, I. & Hausberger, M. (2004). Experience-dependent neuronal specialization and functional organization in the central auditory area of a songbird. *European Journal of Neuroscience, 19*, 3343-3352.

Crair, M. & Malenka, R. (1995). A critical period for long-term potentiation at thalamocortical synapses. *Nature, 375*, 325-328.

Dudek, S. & Bear, M. (1993). Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *Journal of Neuroscience, 13*, 2910 –2918.

Edeline, J. & Weinberger, N. (1991a). Subcortical adaptive filtering in the auditory system: Associative receptive field plasticity in the dorsal medial geniculate body. *Behavioural Neuroscience, 105*, 154-175.

Edeline, J. & Weinberger, N. (1991b). Thalamic short-term plasticity in the auditory system: Associative retuning of receptive fields in the ventral medial geniculate body. *Behavioural Neuroscience, 105*, 618-639.

Edeline, J. & Weinberger, N. (1992). Associative retuning in the thalamic source of input to the amygdala and auditory cortex: Receptive field plasticity in the medial division of the medial geniculate body. *Behavioural Neuroscience, 106*, 81-105.

Edeline, J. & Weinberger, N. (1993). Receptive field plasticity in the auditory cortex during frequency discrimination training: Selective retuning independent of task difficulty. *Behavioural Neuroscience. 107*, 82-103.

Engineer, N., Percaccio, C., Pandya, P., Moucha, R., Rathbun, D. & Kilgard, M. (2004). Environmental enrichment improves response strength, threshold, selectivity, and latency of auditory cortex neurons. *Journal of Neurophysiology, 92*, 73-82.

Feldman, D., Nicoll, R. & Malenka, R. (1999). Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. *Journal of Neurobiology, 41*(1), 92-101.

Flor, H., Elbert, T., Knecht, S., Wienbruch, C., Pantev, C., Birbaumer, N., Larbig, W. & Taub, E. (1995). Phantom-limb pain as a perceptual correlate of cortical reorganization following arm amputation. *Nature, 375*(6531), 482-484.

Flor, H., Hoffmann, D., Struve, M. & Diesch, E. (2004). Auditory discrimination training for the treatment of tinnitus. *Applied Psychophysiology and Biofeedback, 29*(2), 113-120.

Foeller, E. & Feldman, D. (2004). Synaptic basis for developmental plasticity in somatosensory cortex. *Current Opinion in Neurobiology, 14*, 89-95.

Francis, D., Champagne, F., Liu, D. & Meaney, M. (1999). Maternal care, gene expression, and the development of individual differences in stress reactivity. *Annals of the New York Academy of Sciences, 896*, 66–84.

Fregnac, Y., Shulz, D., Thorpe, S. & Bienenstock, E. (1992). Cellular analogs of visual cortical epigenesis. I. Plasticity of orientation selectivity. *Journal of Neuroscience, 12*(4), 1280-1300.

Fuster, J. (1984). The cortical substrate of memory. In L. Squire (Ed.) & N. Butters (Ed.), Neuropsychology of memory (pp. 279-286). The Guilford Press: New York.

Grassi, S., Dieni, C., Frondaroli, A. & Pettorossi, V. (2004). Influence of visual experience on developmental shift from long-term depression to long-term potentiation in the rat medial vestibular nuclei. *Journal of Physiology, 560*(3), 767-777.

Grosvenor, C., Shyr, S., Goodman, G., Mena, F. (1986). Comparison of plasma profiles of oxytocin and prolactin following suckling in the rat. *Neuroendocrinology, 46*(6), 679-685.

Harrison, R., Ibrahim, D. & Mount, R. (1998). Plasticity of tonotopic maps in auditory midbrain following partial cochlear damage in the developing chinchilla. *Experimental Brain Research, 123*, 449-460.

He, H-Y., Hodos, W. & Quinlan, E. (2006). Visual deprivation reactivates rapid ocular dominance plasticity in adult visual cortex. *Journal of Neuroscience, 26*(11), 2951-2955.

Heynen, A. & Bear, M. (2001). Long-term potentiation of thalamocortical transmission in the adult visual cortex *in vivo*. *Journal of Neuroscience, 21*(24), 9801-9813.

Hubel, D. & Wiesel, T. (1963). Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. *Journal of Neurophysiology, 26*, 994-1002.

Huot, R., Plotsky, P., Lenox, R. & McNamara, R. (2002). Neonatal maternal separation reduces hippocampal mossy fibre density in adult Long Evans rats. *Brain Research, 950*, 52–63.

Imbert, M. & Buisseret, P. (1975). Receptive field characteristics and plastic properties of visual cortical cells in kittens reared with or without visual experience. *Experimental Brain Research, 22*, 25-36.

Kato, N., Artola, A. & Singer, W. (1991). Developmental changes in the susceptibility to long-term potentiation of neurones in rat visual cortex slices. *Developmental Brain Research, 60*, 43-50.

Katz, L. & Crowley, J. (2002). Development of cortical circuits: Lessons from ocular dominance columns. *Nature Reviews: Neuroscience, 3*, 34-42.

Kehoe, P., Hoffman, J., Austin-LaFrance, R. & Bronzino, J. (1995). Neonatal isolation enhances hippocampal dentate response to tetanization in freely moving juvenile male rats. *Experimental Neurology, 136*(2), 89-97.

Kilgard, M. & Merzenich, M. (1998). Plasticity of temporal information processing in the primary auditory cortex. *Nature Neuroscience, 1*(8), 727-731.

Kim, J., Foy, M. & Thompson, R. (1996). Behavioural stress modifies hippocampal plasticity through *N*-methyl-D-aspartate receptor activation. *Proceedings of the National Academy of Sciences*, 93, 4750-4753.

Kirkwood, A., Lee, H-K. & Bear, M. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature, 375*, 328-331.

Kirkwood, A., Rioult, M. & Bear, M. (1996). Experience-dependent modification of synaptic plasticity in visual cortex. *Nature, 381*, 526-528.

Krubitzer, L. & Kahn, D. (2003). Nature versus nurture revisited: An old idea with a new twist. *Progress in Neurobiology, 70,* 33-52.

Kudoh, M. & Shibuki, K. (1994). Long-term potentiation in the auditory cortex of adult rats. *Neuroscience Letters, 171*, 21-23.

Kudoh, M. & Shibuki, K. (1996). Long-term potentiation of supragranular pyramidal outputs in the rat auditory cortex. *Experimental Brain Research, 110*, 21-27.

Kudoh, M. & Shibuki, K. (1997). Importance of polysynaptic inputs and horizontal connectivity in the generation of tetanus-induced long-term potentiation in the rat auditory cortex. *Journal of Neuroscience, 17*(24), 9458-9465.

Kudoh, M., Sakai, M. & Shibuki, K. (2002). Differential dependence of LTD on glutamate receptors in the auditory cortical synapses of cortical and thalamic inputs. *Journal of Neurophysiology, 88*, 3167-3174.

Kuhl, P., Williams, K., Lacerda, F., Stevens, K. & Lindblom, B. (1992). Linguistic experience alters phonetic perception in infants by 6 months of age. *Science, 271*(5245), 27-8.

Kulger, J., Kalvaram, K. & Lange, K. (1990). Acute, not chronic, exposure to unpredictable noise periods affects splenic lymphocytes and plasma corticosterone in the mouse. *International Journal of Neuroscience, 51*, 233-234.

Lamprecht, R. & LeDoux, J. (2004). Structural plasticity and memory. *Nature Reviews: Neuroscience, 5*, 45-54.

Lennartz, R. & Weinberger, N. (1992). Frequency-specific receptive field plasticity in the medial geniculate body induced by Pavlovian fear conditioning is expressed in the anesthetized brain.

Levine, S. (2005). Developmental determinants of sensitivity and resistance to stress. *Psychoneuroendocrinology, 30*, 939-946.

Maggi, C. & Meli, A. (1986a). Suitability of urethane anaesthesia for physiopharmacological investigations in various systems. Part 1: General considerations. *Experientia, 42*(2), 109-114.

Maggi, C. & Meli, A. (1986b). Suitability of urethane anaesthesia for physiopharmacological investigations in various systems. Part 2: Cardiovascular system. *Experientia, 42*(3), 292-297.

Malenka, R. & Bear, M. (2004). LTP and LTD: An embarrassment of riches. *Neuron, 44*, 5-21.

Mao, Y., Zang, S., Zhang, J. & Sun, X. (2006). Early chronic blockade of NR2B subunits and transient activation of NMDA receptors modulate LTP in mouse auditory cortex. *Brain Research, 1073-1074*, 131-138.

Martin, S. & Morris, R. (2002). New life in an old idea: The synaptic plasticity and memory hypothesis revisited. *Hippocampus, 12*, 609-636.

Massey, P., Johnson, B., Moult, P., Auberson, Y., Brown, M., Molnar, E., Collingridge, G. & Bashir, Z. (2004). Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *Journal of Neuroscience, 24*(36), 7821-7828.

McLaughlin, T., Torborg, C., Feller, M. & O'Leary, D. (2003). Retinotopic map refinement requires spontaneous retinal waves during a brief critical period of development. *Neuron, 40*(6), 1147-1160.

McLin, D., Miasnikov, A. & Weinberger, N. (2002). Induction of behavioural associative memory by stimulation of the nucleus basalis. *Proceedings of the National Academy of Sciences, 99,* 4002-4007.

Merzenich, M., Kaas, J., Wall, J., Nelson, R., Sur, M. & Felleman, D. (1983). Topographic reorganization of somatosensory cortical areas 3b and 1 in adult monkeys following restricted deafferentation. *Neuroscience, 8*(1), 33-55.

Mirescu, C., Peters, J. & Gould, E. (2004). Early life experience alters response of adult neurogenesis to stress. *Nature Neuroscience, 7*(8), 841-846.

Monjan, A. & Collector, M. (1997). Stress-induced modulation of the immune response. *Science, 196*, 307-308.

Mountcastle, V. (1998). Perceptual Neuroscience: The Cerebral Cortex. Harvard University Press.

Mower, G., Berry, D., Burchfiel, J. & Duffy, F. (1981). Comparison of the effects of dark rearing and binocular suture on development and plasticity of cat visual cortex. *Brain Research, 220*, 255-267.

Muhlnickel, W., Elbert, T., Taub, E. & Flor, H. (1998). Reorganization of auditory cortex in tinnitus. *Proceedings of the National Academy of Sciences, 95,* 10340-10343.

Munkata, Y. & Pfaffly, J. (2004). Hebbian learning and development. *Developmental Science, 7*(2), 141-148.

Nakahara, H., Zhang, L. & Merzenich, M. (2004). Specialisation of primary auditory cortex processing by sound exposure in the "critical period". *Proceedings of the National Academy of Sciences, 101*(18), 7170-7174.

Pandya, P., Moucha, R., Engineer, N., Rathbun, D., Vazquez, J. & Kilgard, M. (2005). Asynchronous inputs alter excitability, spike timing, and topography in primary auditory cortex. *Hearing Research, 203*, 10-20.

Pantev, C., Dinnesen, A., Ross, B., Wollbrink, A. & Knief, A. (2005). Dynamics of auditory plasticity after cochlear implantation: A longitudinal study. *Cerebral Cortex Advance Access, [epub ahead of print].*

Paxinos, G. & Watson, C. (1998). *The Rat Brain in Stereotaxic Coordinates* (4th Ed.). Academic Press.

Philpot, B., Sekhar, A, Shouval, H. & Bear, M. (2001). Visual experience and deprivation bidirectionally modify the composition and function of NMDA receptors in visual cortex. *Neuron, 29*, 157-169.

Pienkowski, M. & Harrison, R. (2005). Tone frequency maps and receptive fields in the developing chinchilla auditory cortex. *Journal of Neurophysiology, 93*, 454-466.

Quinlan, E., Olstein, D. & Bear, M. (1999). Bidirectional, experience-dependent regulation of N-methyl-D-aspartate receptor subunit composition in the rat visual cortex during postnatal development. *Proceedings of the National Academy of Sciences, 96*, 12 876-12 880.

Quinlan, E., Philpot, B., Huganir, R. & Bear, M. (1999). Rapid, experience-dependent expression of synaptic NMDA receptors in visual cortex *in vivo*. *Nature Neuroscience, 2*, 352-357.

Rakic, P. (1988). Specification of cerebral cortical areas. *Science, 241*(4862), 170-176.

Ranck, J. (1975). Which elements are excited in electrical stimulation of mammalian central nervous system: A review. *Brain Research, 98*, 417-440.

Rao, Y. & Daw, N. (2004). Layer variations of long-term depression in rat visual cortex. *Journal of Neurophysiology, 92*, 2652-2658.

Rauschecker, J. (1999). Auditory cortical plasticity: A comparison with other sensory systems. *Trends in Neuroscience, 22*(2), 74-80.

Renger, J., Hartman, K., Tsuchimoto, Y., Yokoi, M., Nakanishi, S. & Hensch, T. (2002). Experience-dependent plasticity without long-term depression type 2 metabotropic glutamate receptors in developing visual cortex. *Proceedings of the National Academy of Sciences, 99*(2), 1041-1046.

Rioult-Pedotti, M-S., Friedman, D., Hess, G. & Donoghue, J. (1998). Strengthening of horizontal cortical connections following skill learning. *Nature Neuroscience, 2,* 898-905.

Rioult-Pedotti, M., Friedman, D. & Donoghue, J. (2000). Learning-induced LTP in the neocortex. *Science, 290,* 533-536.

Robertson, D. & Irvine, D. (1989). Plasticity of frequency organization in auditory cortex of guinea pigs with partial unilateral deafness. *Journal of Comparative Neuroscience, 282*(3), 456-471.

Sakai, M., Kudoh, M. & Shibuki, K. (1999). Long-lasting enhancement of sound discrimination ability after sound exposure in rats. *Neuroscience Research, 33*(2), 87-97.

Sally, S. & Kelly, J. (1988). Organization of auditory cortex in the albino rat: Sound frequency. *Journal of Neurophysiology, 59*(5), 1627-1638.

Shirasaka, Y. & Wasterlain, C. (1995). The effect of urethane anaesthesia on evoked potentials in dentate gyrus. *European Journal of Pharmacology, 282*(1-3), 11-17.

Shouval, H., Bear, M. & Cooper, L. (2002). A unified model of NMDA receptor-dependent bidirectional synaptic plasticity. *Proceedings of the National Academy of Sciences, 99*(16), 10831-10836.

Stern, J. (1997). Offspring-induced nurturance: Animal-human parallels. *Developmental Psychobiology, 31,* 19-37.

Sun, W., Mercado, E., Wang, P., Shan, X., Lee, T-C. & Salvi, R. (2005). Changes in NMDA receptor expression in auditory cortex after learning. *Neuroscience Letters, 374*, 63-68.

Talwar, S. & Gerstein, G. (2001). Reorganization in awake rat auditory cortex by local microstimulation and its effect on frequency-discrimination behaviour. *Journal of Neurophysiology, 86*, 1555-1572.

Teyler, T., Hamm, J., Clapp, W., Johnson, B., Corballis, M. & Kirk, I. (2005). Long-term potentiation of human visual evoked responses. *European Journal of Neuroscience, 21,* 2045-2050.

Tieman, D., Tumosa, N. & Tieman, S. (1983). Behavioural and physiological effects of monocular deprivation: A comparison of rearing with diffusion and occlusion. *Brain Research, 280*, 41-50.

Torner, L., Toschi, N., Nava, G., Clapp, C. & Neumann, I. (2002). Increased hypothalamic expression of prolactin in lactation: involvement in behavioural and neuroendocrine stress responses. *European Journal of Neuroscience, 15*, 1381-1389.

Torner, L., Maloumby, R., Nava, G., Aranda, J., Clapp, C. & Neuman, I. (2004). In vivo release and gene upregulation of brain prolactin in response to physiological stimuli. *European Journal of Neuroscience, 19*, 1601-1608.

Trepel, C. & Racine, R. (1998). Long-term potentiation in the neocortex of the adult, freely moving rat. *Cerebral Cortex, 8*(8), 719-729.

Tsukahara, N. (1981). Synaptic plasticity in the mammalian central nervous system. *Annual Review of Neuroscience, 4*, 351-379.

Tsumoto, T. (1990). Long-term potentiation and depression in the cerebral neocortex. *Japanese Journal of Physiology, 20*, 573-593.

Van Raaij, M. Oortgiesen, M., Timmerman, H., Dobbe, C. & Loveren, H. (1996). Time-dependent differential changes of immune function in rats exposed to chronic intermittent noise. *Physiology & Behaviour, 60*(6), 1527-1533.

Walker, C., Trottier, G., Rochford, J. & Lavallee, D. (1995). Dissociation between behavioural and hormonal responses to the forced swim stress in lactating rats. *Journal of Neuroendocrinology, 7*, 615-622.

Wang, X. (2004). The unexpected consequences of a noisy environment. *Trends in Neurosciences, 27*(7), 364-366.

Weaver, I., Cervoni, N., Champagne, F., D'Alessio, A., Sharma, S., Seckl, J., Dymov, S., Szyf, M. & Meaney, M. (2004). Epigenetic programming by maternal behaviour. *Nature Neuroscience, 7*(8), 847-854.

Weinberger, N. & Diamond, D. (1987). Physiological plasticity in auditory cortex: Rapid induction by learning. *Progress in Neurobiology, 29*, 1-55.

Weinberger, N., Javid, R. & Lepan, B. (1993). Long-term retention of learning-induced receptive-field plasticity in the auditory cortex. *Proceedings of the National Academy of Sciences, 99,* 2394-2398.

Weinberger, N. (1995). Dynamic regulation of receptive fields and maps in the adult sensory cortex. *Annual Review of Neuroscience, 18*, 129-158.

Weinberger, N. (1998). Physiological memory in primary auditory cortex: Characteristics and mechanisms. *Neurobiology of Learning and Memory, 70*(1-2), 226-251.

Weinberger, N. (2004). Specific long-term memory traces in primary auditory cortex. *Nature Reviews: Neuroscience, 5,* 279-288.

Wiesel, T. & Hubel, D. (1963). Single-cell responses in striate cortex of kittens deprived of vision in one eye. *Journal of Neurophysiology*, 26, 1003-1017.

Willshaw, D. & Dayan, P. (1990). Optimal plasticity from matrix memories: What goes up must come down. *Neural Computation, 2,* 85-93.

Windle, R., Wood, S., Shanks, N., Perks, P., Conde, G., da Costa, A., Ingram, C. & Lightman, S. (1997). Endocrine and behavioural responses to noise stress: Comparison of virgin and lactating female rats during non-disrupted maternal activity. *Journal of Neuroendocrinology, 9*, 407-414.

Woolley, C., Gould, E. & McEwen, B. (1990). Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Research, 531*(1-2), 225-231.

Xiong, W., Wei, H., Xiang, X., Cao, J., Dong, Z., Wang, Y., Xu, T. & Xu, L. (2004). The effect of acute stress on LTP and LTD induction in the hippocampal CA1 region of anaesthetized rats at three different ages. *Brain Research, 1005*, 187-192.

Yang, C-H., Huang, C-C. & Hsu, K-S. (2004). Behavioural stress modifies hippocampal synaptic plasticity through corticosterone-induced sustained extracellular signal-regulated kinase/mitogen-activated protein kinase activation. *Journal of Neuroscience, 24*(49), 11029-11034.

Yashiro, K., Corlew, R. & Philpot, B. (2005). Visual deprivation modifies both presynaptic glutamate release and the composition of perisynaptic/extrasynaptic NMDA receptors in adult visual cortex. *Journal of Neuroscience, 25*(50), 11684-11692.

Yao, H. & Dan, Y. (2001). Stimulus timing-dependent plasticity in cortical processing of orientation. *Neuron, 32*(2), 315-323.

Zhang, L., Bao, S. & Merzenich, M. (2001). Persistent and specific influences of early acoustic environments on primary auditory cortex. *Nature Neuroscience, 4*(11), 1123-1130.

Zhang, L., Bao, S. & Merzenich, M. (2002). Disruption of primary auditory cortex by synchronous auditory inputs during a critical period. *Proceedings of the National Academy of Sciences, 99*(4), 2309-2314.