PLASTICITY OF THE RAT THALAMOCORTICAL AUDITORY SYSTEM DURING DEVELOPMENT AND FOLLOWING WHITE NOISE EXPOSURE

by

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Abstract

Synaptic plasticity reflects the capacity of synapses to undergo changes in synaptic strength and connectivity, and is highly regulated by age and sensory experience. This thesis focuses on the characterization of synaptic plasticity in the primary auditory cortex (A1) of rats throughout development and following sensory deprivation. Initial experiments revealed an age-dependent decline in plasticity, as indicated by reductions in long-term potentiation (LTP). The enhanced plasticity of juvenile rats appeared to be mediated by NR2B subunits of the N-methyl-d-aspartate receptor (NMDAR), as NR2B antagonist application reduced LTP to adult-like levels in juveniles, yet had no effect in adults. The importance of sensory experience in mediating plasticity was revealed in experiments using white noise exposure, which is a sensory deprivation technique known to arrest cortical development in A1. Notably, adult rats reared in continuous white noise maintained more juvenile-like levels of LTP, which normalized upon subsequent exposure to an unaltered acoustic environment. The white noise-induced LTP enhancements also appeared to be mediated by NR2B subunits, as NR2B antagonists reversed these LTP enhancements in white noise-reared rats. Given the strong influence that sensory experience exerts on plasticity, additional experiments examined the effect of shorter episodes of white noise exposure on LTP in adult rats. Exposure to white noise during early postnatal life appeared to "prime" A1 for subsequent exposure in adulthood, resulting in enhanced LTP. The necessity of early-life exposure was evident, as repeated episodes of white noise in adulthood did not enhance plasticity. In older rats that typically no longer express LTP in A1, pharmacological methods to enhance plasticity were explored. Moderate LTP was observed in older rats with cortical zinc application, which may act through its antagonism of NR2A subunits of the NMDAR. Additionally, current source density and cortical silencing analyses were conducted to characterize the distinct peaks of field postsynaptic potentials recorded in A1, with the earlier and

later peaks likely representing thalamocortical and intracortical synapses, respectively. Together, this thesis emphasizes the critical role of sensory experience in determining levels of cortical plasticity, and demonstrates strategies to enhance plasticity in the mature auditory cortex.

Co-Authorship

The research in this thesis was conducted by Jennifer Hogsden Robinson under the supervision of Dr. Hans Dringenberg. In all cases, Jennifer Hogsden Robinson participated in the study design, data acquisition, data analysis, and manuscript preparation. Dr. Hans Dringenberg contributed to the study design, data analysis, and manuscript preparation for Chapters 2, 3, and 4, and provided editorial advice for Chapters 1 and 5.

Chapters 2 and 3 have been published in their entirety, and are presented in their published formats.

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List of Abbreviations

θ_m	Synaptic modification threshold
A1	Primary auditory cortex
aCSF	Artificial cerebrospinal fluid
AMPA	Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
ANOVA	Analysis of variance
AP	Anteroposterior
APV	DL-2-amino-5-phosphonopentanoic acid
BCM theory	Bienenstock, Cooper, and Munro theory
Ca^{2+}	Calcium ions
CaMKII	α-calcium-calmodulin-dependent protein kinase II
CNQX	6-Cyano-7-nitroquinoxaline-2,3-dione
CSD	Current source density
dB	Decibel
DNQX	6,7-Dinitroquinoxaline-2,3-dione
EPSC	Excitatory postsynaptic current
ERF	Excitatory receptive field
fPSP	Field postsynaptic potential
GABA	Gamma-aminobutyric acid
i.p.	Intraperitoneal
IRF	Inhibitory receptive field
L	Lateral
LTD	Long-term depression

LTP	Long-term potentiation
MGN	Medial geniculate nucleus
mRNA	Messenger ribonucleic acid
NMDA	N-methyl-d-aspartate
PD	Postnatal day
SEM	Standard error of the mean
SPL	Sound pressure level
TBS	Theta burst stimulation
V	Ventral
V1	Primary visual cortex
WN	White noise
Zn	Zinc
$ZnCl_2$	Zinc chloride

Chapter 1
General Introduction

1.1 Cortical Plasticity and Development

Early postnatal life represents a developmental period during which the cortex is extremely sensitive to its external environment. This capacity of the young cortex to be influenced by experience is incredibly important, as the specific conditions of an environment are largely "unknown" prior to birth and must be learned from experience. For example, developing cortical circuits that code the distinct elements (phonemes) of a language cannot predict the native language of an individual, and are rather guided by directly experiencing the patterns of speech after birth. Establishing networks that respond maximally to the patterns of the native language is essential, as it promotes the perception of phonemes and learning of words (Kuhl, 2004). Clearly, early exposure to sensory input is highly instructive for cortical development, and permits the matching of functional properties of neural circuits to the particular features of an environment.

There can be many unknowns regarding the specific conditions characterizing a sensory environment. While spontaneous activity and molecular guidance cues drive the formation of the basic neuronal circuitry of a sensory system before birth, experience-dependent activity is necessary for the refinement and maturation of connectivity that allows an animal to be well-adapted to its external environment (Katz & Shatz, 1996; Ruthazer & Cline, 2004; Webber & Raz, 2006). For example, ocular dominance columns are present at birth in primary visual cortex (V1; Horton & Hocking, 1996), yet can be later modified by visual experience (Hubel & Wiesel, 1977; Shatz & Stryker, 1978). Similarly, thalamic afferents terminate in primary auditory cortex (A1) in distinct patches that are present several days prior to the onset of hearing, yet are later refined during postnatal life (de Venecia & McMullen, 1994).

An important feature of sensory cortices that is influenced by early sensory experience is topographic map organization. Most sensory inputs to the central nervous system are arranged topographically, wherein adjacent areas on a sensory surface (e.g. retina, cochlea, skin) project to and are represented by adjacent neurons in the thalamus and primary sensory cortex. For example, like the cochlea, A1 is arranged tonotopically, with a gradated change in the specific frequency that produces maximal excitation of a given cell across the cortical surface (Reale & Imig, 1980). Likewise, visual and somatosensory cortices are arranged retinotopically and somatotopically, respectively (Dräger 1974; Udin & Fawcett, 1988). With exposure to sensory input, the correlated activity amongst cells that respond to a particular stimulus results in the strengthening and stabilization of those connections. Conversely, non-correlated activity results in the weakening of connections, and together these activity-dependent changes in synaptic strength promote the refinement of topographic organization (Collingridge & Singer, 1990). Morphological evidence of such changes in synaptic connectivity have been observed at the axonal level, with the selective stabilization or elimination of axon branch tips associated with correlated and non-correlated activity, respectively (Ruthazer et al., 2003). Similarly, dendritic arbor elaboration can also be influenced by correlated activity, with the arborization of some cells restricted to areas innervated by coactive inputs (Katz & Constantine-Paton, 1988; Kossel et al., 1995). Interestingly, sensory deprivation can lead to a reorganization of sensory maps, with the deprived cortical area becoming responsive to inputs that typically only activated adjacent regions (Buonomano & Merzenich, 1998). This may occur via an initial correlation-based weakening and elimination of axonal branches in deprived regions, followed by axon branch addition and the strengthening of connections into these now more sparsely innervated areas by neighboring, non-deprived inputs (Antonini & Stryker, 1996; Ruthazer et al., 2003; Frenkel & Bear, 2004).

1.2 Critical Periods of Cortical Development

The susceptibility of the cortex to be modified by sensory experience is highest during critical periods, which are discrete windows during development characterized by particularly high levels of plasticity. Further, critical periods are distinguished by the relative persistence of synaptic modifications established during these specific time windows. Consequently, exposure to an atypical or deprived sensory environment during the critical period can have lasting consequences on cortical functioning that often cannot be remedied with appropriate experience later in life (Knudsen, 2004). For example, a cataract during infancy degrades normal vision in the affected eye, and results in lasting deficits in spatial and temporal contrast sensitivity even with early diagnosis and removal of the cataract (Ellemberg et al., 2000).

The precise timing of critical periods varies between sensory systems and species. Work examining the susceptibility for alterations in the responsiveness of neurons in the visual cortex was pioneered by Wiesel and Hubel (1963). The critical period for shifts in cortical representation to favour the non-deprived eye following monocular deprivation (ocular dominance plasticity) has been shown to peak by postnatal day (PD) 35-48 in cats (Hubel & Wiesel, 1970), and PD 28-35 in rats (Fagiolini et al., 1994). Critical periods for other features of vision have also been identified, such as directional selectivity, which occurs prior to the critical period for ocular dominance. During this period, the preferred direction of cortical neurons can be modified if the prominent direction of stimuli in the environment is altered (Daw & Wyatt, 1976). In auditory cortex, the critical period during which modifications to tonotopic organization can be made with passive sound exposure closely follows the onset of low-threshold hearing in rodents. Pure-tone exposure results in enlarged cortical representations of that frequency, and a reduction in representations of adjacent frequencies in A1 of rat pups, but only if it was presented during PD 11-13. This effect on A1 tuning was found to persist, as pups exposed to a 7 kHz tone from PD

10-14 displayed a significant expansion of areas tuned to that frequency as compared to controls after six weeks in a normal acoustic environment (de Villers-Sidani et al., 2007). More recent studies have described several sensitive windows during A1 development wherein cortical representations of more complex acoustic features are established, such as characteristic frequency maps (PD 8-15), bandwidth (PD 16-23), and selectivity to sound direction (PD 24-39; Insanally et al., 2009). These findings suggest that the critical period for A1 development is comprised of a series of feature-dependent sensitive periods, with cortical representation of more complex acoustic features occurring later.

Remarkably, the complete absence of appropriate sensory stimulation can delay the closure of the critical period. Kittens reared in darkness during early life retain the ability to express ocular dominance plasticity in layer IV of primary visual cortex later in development, at a time when kittens reared under normal conditions no longer show shifts in cortical representations with monocular deprivation (Cynader & Mitchell, 1980). Continuous white noise exposure is a form of patterned sound deprivation that arrests the development of tonotopicity in A1 when experienced during early postnatal life. Normal adult-like tonotopic organization emerges when rats are later returned to unaltered acoustic environments, suggesting that white noise exposure delays the closure of the critical period in A1 (Chang & Merzenich, 2003). Thus, under extremely deprived conditions, the cortex appears to wait for appropriate sensory stimulation before initiating the stabilization of neuronal circuitry that typically occurs with the closure of the critical period.

1.3 Plasticity in Adulthood

With the stabilization of cortical circuitry following the critical period, the capacity of the cortex to be influenced by sensory experience diminishes into adulthood. Some level of plasticity

remains in the adult cortex, however, changes to functional connectivity typically occur only when environmental stimuli are behaviourally relevant and actively attended to, or are paired with reinforcers or neuromodulators. For example, adult owl monkeys trained to differentiate subtle differences in the frequency of tones for reward showed increased cortical representation and sharpened tuning in A1 for those behaviourally relevant frequencies (Recanzone et al., 1993). Similarly, tone exposure paired with electrical stimulation of the nucleus basalis, which is a structure that releases the neuromodulator acetylcholine in the forebrain during states of arousal and attention, permits the expanded representation of that frequency in A1 of adult rats (Kilgard & Merzenich, 1998). This potential for plastic cortical reorganization in adulthood likely reflects the ability of organisms to acquire new skills and behaviours throughout their lifetime. However, such modifications to responsivity during adulthood are relatively modest, and occur within constraints established during early circuit formation (Recanzone et al., 1993; Knudsen, 2004).

1.4 Long-term Potentiation (LTP)

Changes in synaptic strength are thought to underlie, at least in part, the modifications of cortical responsiveness that occur with sensory experience. Bliss and Lømo (1973) were the first to report persistent, activity-dependent increases in synaptic strength following high-frequency stimulation to excitatory pathways in the hippocampus, which is a region of the brain crucial for some types of learning and memory. This enhancement in synaptic efficacy, referred to as long-term potentiation (LTP), has been proposed as a mechanism by which neuronal activity is translated into functional changes in synaptic connectivity, permitting the refinement of sensory systems and information storage in the nervous system (Collingridge & Singer, 1990; Bliss & Collingridge, 1993; Kirkwood et al., 1996; Bear, 2003).

1.5 Mechanisms of LTP Induction

Several characteristic properties of LTP make it a suitable model for activity-dependent plasticity in the nervous system. One such requirement constitutes the associative nature of LTP, given that induction typically requires the coupling of synchronous presynaptic activation and postsynaptic depolarization. Glutamate released from activated presynaptic terminals can bind to either alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or N-methyl-D-aspartate (NMDA) receptor subunits, the latter of which is important for LTP induction (Collingridge & Bliss, 1987; Malenka & Bear, 2004). In addition to requiring glutamate, voltage-dependent NMDA receptor (NMDAR) channels only permit current flow when the postsynaptic cell is depolarized, which acts to release magnesium ions that are typically bound to the channel and prevent ion flow (Mayer et al., 1984; Mayer & Westbrook, 1987). As such, NMDARs are often referred to as "coincidence detectors," since they are only active when both pre- and postsynaptic cells are activated. Sufficient depolarization is required to relieve the magnesium block, which reflects the cooperativity property of NMDAR-dependent LTP, wherein a threshold number of presynaptic fibres must be activated simultaneously for LTP induction to take place (McNaughton et al., 1978). Notably, threshold may be achieved either with the simultaneous activation of several presynaptic cells that converge cooperatively on a given postsynaptic cell, or with stronger stimulation to single afferents (Barrionuevo & Brown, 1983). Typically, high frequency tetanic stimulation is required, but it is noteworthy that frequency and stimulation strength interact such that lower frequencies may be applied when the stimulation intensity is increased (Bliss & Lømo, 1973; Dunwiddie & Lynch, 1978). Another prominent feature of LTP is input specificity. While postsynaptic cells receive input from a great number of afferent synapses, LTP will only occur in those synapses that received strong tetanic stimulation (Barrionuevo & Brown, 1983; Kelso et a1., 1986). Thus, in a naturalistic setting, the facilitation

of inputs is presumably selective to those that were robustly activated by exposure to a given sensory stimulus. However, LTP may be induced when weaker inputs are paired in close temporal succession with stronger inputs to the same postsynaptic cell, much in the same way that a weak conditioned stimulus can be paired with a strong unconditioned stimulus, thus highlighting the associative nature of LTP (Levy & Steward, 1983).

Following postsynaptic depolarization and relief of the magnesium block, calcium ions (Ca^{2+}) enter the postsynaptic cell, resulting in significant increases in intracellular calcium levels (MacDermott et al., 1986). This elevation of intracellular calcium is thought to be a critical trigger for LTP induction, as the injection of calcium chelators into the postsynaptic cell has been shown to block LTP induction in the hippocampus and neocortex (Lynch et al., 1983; Baranyi & Szente, 1987). The sudden influx of calcium through NMDARs that occurs with high levels of synaptic activity triggers a cascade of intracellular events that are thought to result in the expression of LTP. Activation of the protein kinase α-calcium-calmodulin-dependent protein kinase II (CaMKII) is believed to be a crucial component of the molecular mechanisms underlying LTP induction. It is thought that the downstream effects of CaMKII activation lead to the phosphorylation of existing, and insertion of additional AMPA receptors (AMPARs) in the synaptic membrane, which would act to facilitate postsynaptic responding (for review see: Malenka & Nicoll, 1999; Manilow & Malenka, 2002). Depending on the preparation and stimulation protocol, LTP can last for several hours to even weeks. The maintenance of LTP is thought to occur via changes to protein synthesis and gene transcription, as well as morphological changes, such as increases in dendritic spine number or the enlargement of existing spines (for review see Malenka & Bear, 2004).

1.6 Subunits of the NMDA Receptor

It is clear that NMDARs are important for the induction of LTP in the central nervous system. Interestingly, different subunit configurations of these receptors influence the ability of synapses to undergo plastic change. NMDARs are tetrameric ion channel complexes comprised of two obligatory NR1 subunits, and two regulatory NR2 (NR2A→D) or NR3 (NR3A→B) subunits (Monyer et al., 1994; Cull-Candy et al., 2001). NR2A and NR2B subunits have been the focus of research on developmental plasticity, as they are the predominant subtypes found in the forebrain, and are thought to confer distinct functional properties to the receptor (Yashiro & Philpot, 2008). Notably, these subunits show a distinct developmental alteration, with a clear shift from primarily NR1/NR2B receptors to NR1/NR2A in thalamic and cortical neurons during early postnatal life (Monyer et al., 1994; Liu et al., 2004b). Such changes are thought to contribute to the shortening of NMDAR-mediated excitatory postsynaptic currents (EPSCs) that occurs with maturation, given the different current kinetic properties associated with each subunit. For example, compared to NR2A-containing receptors, NMDAR-mediated EPSCs of NR2B-containing receptors are longer in duration and permit enhanced Ca^{2+} entry into postsynaptic cells (Monyer et al., 1994; Flint et al., 1997). Given the importance of intracellular Ca^{2+} for the induction of LTP, greater relative levels of NR2B are thought to facilitate plasticity by lowering LTP induction thresholds, and may act as a synaptic mechanism underlying enhanced critical period plasticity (Bear, 2003).

Studies in visual cortex have suggested that the shift in relative levels of NR2A and NR2B occurs with sensory experience (Quinlan et al., 1999; Philpot et al., 2001). Notably, NR2A-deficient mice display deficits in receptive field properties in visual cortex, suggesting the involvement of NR2A-mediated increases in plasticity thresholds for the development of receptive field organization (Fagiolini et al., 2003). Thus, these developmental alterations to

NMDAR subunit composition likely promote experience-driven cortical organization, with the heightened plasticity associated with NR2B-containing receptors important for synapse formation and strengthening in response to sensory input, and the inclusion of NR2A-containing receptors vital for the later refinement and stabilization of functional connectivity. Fittingly, sensory deprivation paradigms have shown a lack of refinement in topographical maps, disruptions in the maturation of feature selectivity, and the maintenance of heightened relative levels of NR2B subunits (Quinlan et al., 1999; White et al., 2001; Chang & Merzenich, 2003).

1.7 Objectives

Much of the literature on developmental plasticity and LTP has been dominated by investigations in the visual and somatosensory cortex, with the examination of these processes in the auditory cortex comparatively rare. The focus of this thesis centres on characterizing plasticity throughout development in the rat A1, and the impact of early sensory experience on the expression of plasticity later in life. Initial experiments characterize age-related changes in cortical LTP in A1 from early life well into adulthood, and examine the role of NR2B subunits in the expression of plasticity at these different developmental stages. Given the importance of sensory experience in cortical development, the impact of patterned sound deprivation on plasticity in A1 was investigated with the use of continuous white noise exposure beginning in early postnatal life. It was hypothesized that sensory experience, at least in part, modulates levels of cortical plasticity, such that exposure to patterned acoustic inputs throughout development results in a gradual decline in plasticity. Accordingly, the absence of typical patterned sound stimulation with white noise exposure would prevent this decline, resulting in the maintenance of more juvenile-like plasticity levels. Additionally, since the ratio of NR2A:NR2B subunits are known to increase with age and sensory experience, we examined the role of the plasticity-

associated NR2B subunit in the alterations in cortical plasticity noted following continuous white noise-rearing, theorizing that a relative upregulation of NR2B underlies the observed plasticity enhancements. In the final chapter, pharmacological and environmental manipulations were utilized in an attempt to restore juvenile-like plasticity (LTP) in the mature rat A1. Cortical zinc application was chosen as the pharmacological agent since it is a potent antagonist of the NR2A subunit, and may be able to modulate the ratio of NR2A:NR2B subunits to promote plasticity. Finally, the impact of prior experience with patterned sound deprivation on the ability of later deprivation episodes to restore LTP in the mature brain was assessed. It was hypothesized that a prior episode of white noise exposure could "prime" the cortex for later exposure to white noise to promote plasticity. The findings of this thesis contribute importantly to the body of research on cortical LTP in the auditory cortex, and provide insight into the factors that govern A1 plasticity throughout the lifespan of the rat.

Chapter 2

Reduction in Auditory Cortex Plasticity and Sensitivity to NR2B Subunit Blockade with Age

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2.1 Abstract

A decline in the ability of synapses to express plasticity and long-term potentiation (LTP) during postnatal maturation has been characterized in primary visual and somatosensory cortices. The present study is the first to document changes in the magnitude of LTP in the primary auditory cortex (A1) of rats at different stages of postnatal life. In urethane-anesthetized rats, field postsynaptic potentials (fPSPs) in A1 were elicited by stimulation of the medial geniculate nucleus, and LTP of cortical fPSPs was induced by application of repeated episodes of theta burst stimulation. Rats tested between postnatal day (PD) 30-35 showed the greatest level of LTP, with fPSP amplitude reaching ~165% of baseline after delivery of TBS. Levels of LTP decreased with increasing age, with ~140% and ~120% potentiation in rats aged PD 40-45 and 100-110, respectively. In rats older than 200 days, LTP could no longer be reliably induced (~105-110% potentiation). Application of an antagonist of NMDA receptor NR2B subunits (Ro 25-6981, 10 mM) in A1 reduced LTP in PD 30-35 rats to levels similar to those in adults, without affecting LTP in adults (PD 100-110). Additional pharmacological experiments demonstrated a critical role of AMPA, but not NMDA, receptors in eliciting baseline, non-potentiated fPSPs in A1, an effect not significantly influenced by age. Together, these experiments demonstrate a pronounced, age-related decline in plasticity of synapses in the rodent A1, with elevated levels of plasticity in

juvenile animals requiring activation of NR2B subunits, known to facilitate plasticity in other cortical and subcortical circuits.

2.2 Introduction

Extensive evidence has shown that younger, immature brains are more plastic than the mature nervous system, due to the fact that the potential for re-organization of synaptic connectivity is at its peak during critical periods of early postnatal development (for reviews see Hensch (2005); Dahmen and King (2007); Daw et al. (2007); Keuroghlian and Knudson (2007)). Reductions in plasticity with maturation are clearly evident in the visual system, as indicated by the decline in ocular dominance plasticity outside the critical period (Hubel and Wiesel, 1970; Daw et al., 1992; Lehmann and Löwel, 2008; Sato and Stryker, 2008) or the greater resistance to the induction of NMDA receptor-dependent long-term potentiation (LTP) in the primary visual cortex (V1) of rodents with increasing age (Kato et al., 1991; Kirkwood et al., 1995; Jiang et al., 2007; Jang et al., 2009). Importantly, such age-related declines of LTP in V1 show marked differences among cortical layers, with thalamorecipient layers (IV and deep III) showing little or no LTP at younger ages (after postnatal day 18 in mice), when supra- and infragranular layers still express substantial LTP in response to afferent stimulation (Wang and Daw, 2003; Jiang et al., 2007).

Results similar to those in V1 have also been obtained for plasticity and LTP measures in the rodent somatosensory cortex. Crair and Malenka (1995) found that LTP at thalamocortical synapses in the rat barrel cortex could only be induced prior to postnatal day (PD) 7. Interestingly, this time period corresponds to the critical period during which alterations in sensory input (e.g., lesioning of whisker follicles) effectively modify barrel cortex synaptic connectivity (Woolsey and Wann, 1976; Schlaggar et al., 1993), consistent with the hypothesis

that LTP-like mechanisms mediate a variety of sensory-induced plasticity phenomena during early postnatal life (Feldman et al., 1999; Malenka and Bear, 2004).

Surprisingly, to the best of our knowledge, age-related changes in the induction and properties of LTP in the rodent primary auditory cortex (A1) have not been characterized. Like other sensory cortices, the synaptic and topographical structure (i.e., tonotopic organization) of A1 is influenced by the specific patterns of sensory input received during early postnatal life. For example, passive exposure to a tone of a specific frequency results in an enlargement of the cortical area in A1 responsive to that frequency if exposure occurs during a relatively tight sensitive period (PD 11-13 in rats; de Villers-Sidani et al., 2007). Further, significant changes in the expression of various NMDA receptor (NMDAR) subunits (e.g., NR2A and NR2B) and GABAergic markers in A1 during postnatal development have been characterized (Hsieh et al., 2002; Bi et al., 2006; Kotak et al., 2008), factors known to impact the ability of synapses in other cortical areas to express LTP (Bear, 2003; Malenka and Bear, 2004). Consequently, the present experiments were designed to characterize LTP in the rat A1 *in vivo* from early adolescence to well into adulthood (>PD 200). In addition, the question of whether the NR2B subunit of the NMDA receptor plays a role in mediating some of the age-related differences in LTP was also addressed by means of local application of an NR2B subunit antagonist in A1.

2.3 Results

2.3.1 Pharmacological characterization of fPSP elicited by MGN stimulation

The location of stimulation electrodes in the MGN and a typical placement of an electrode-dialysis probe assembly in A1 are shown in Fig 2.1. Single pulse stimulation of the medial geniculate nucleus (MGN) in juvenile and adult rats (30-35 and 100-150 days, n = 5 and

7, respectively) under urethane anaesthesia reliably elicited field postsynaptic potentials (fPSPs) recorded in the middle cortical layers (~400-600 μm, layers IV-V) of A1. The fPSPs consisted of two, negative-going peaks, with latencies to peak of approximately 7 and 15 ms, respectively (Fig. 2.2). Previous work suggests that these two peaks reflect sequential current sinks at deeper, thalamorecipient and more superficial, intracortical synapses, respectively (Kaur et al., 2005; J.L. Hogsden and H.C. Dringenberg, unpublished data). It appeared that the first peak of the fPSP had a larger amplitude in adult rats (Fig. 2.2, top), but this effect did not reach statistical significance (effect of age, first peak: $F_{(1,13)} = 2.3$, $p = 0.16$; second peak: $F_{(1,13)} = 0.97$, $p = 0.34$). Surprisingly, application of APV (1 and 5 mM) did not exert significant effects on amplitude of the first or second peak of the fPSP in either 30-35 day old or adult animals (Fig. 2.2). However, significant reductions in fPSP amplitude of both peaks were seen with application of CNQX (1 and 5 mM) in both age groups (Fig. 2.2; effect of drug, first peak: $F_{(4,52)} = 8.8$, $p < 0.01$; second peak: $F_{(4,52)} = 13.0$, $p < 0.01$). Following 1 mM CNQX application, average fPSP amplitudes of the first peak were reduced to 45 and 70% of their initial size during artificial cerebrospinal fluid (aCSF) application for the 30-35 day and adult groups, respectively. Similarly, amplitudes for the second peak decreased to 34 and 59% of their initial size for young and adult rats, respectively. Increasing the concentration of CNQX to 5 mM resulted in a further suppression of fPSP amplitude in adult animals to 51 and 32% of baseline for the first and second peak, respectively. In contrast, a concentration of 1 mM CNQX was maximally effective in suppressing fPSP amplitude in young animals, since increasing its concentration to 5 mM did not exert additional effects (46 and 32% of baseline for first and second peak, respectively; Fig. 2.2). The lack of significant drug by age interactions for either peak (first peak: $F_{(4,52)} = 0.5$, $p = 0.75$; second peak: $F_{(4,52)} = 0.4$, $p = 0.84$) indicates that APV and CNQX exerted similar effects, regardless of the age of the animals.

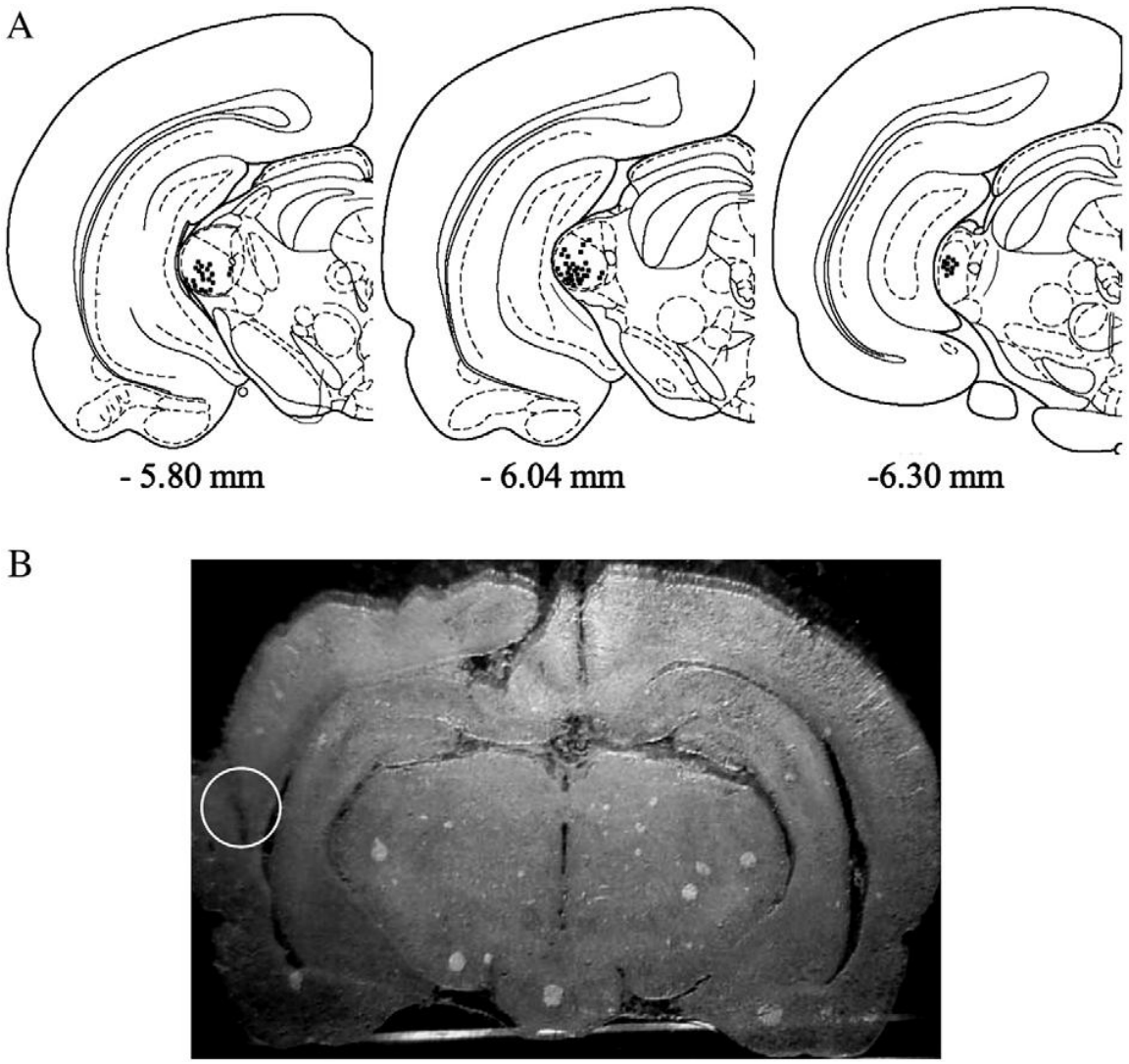

Figure 2.1 Location of stimulation electrodes in the medial geniculate nucleus (A) and a typical placement of an electrode-dialysis probe assembly in the primary auditory cortex (B). Note that the tip of the recording electrode is located about 1 mm above the tip of the dialysis probe. Numbers in A refer to the distance from bregma in mm. Drawings are adapted from Paxinos and Watson (1998).

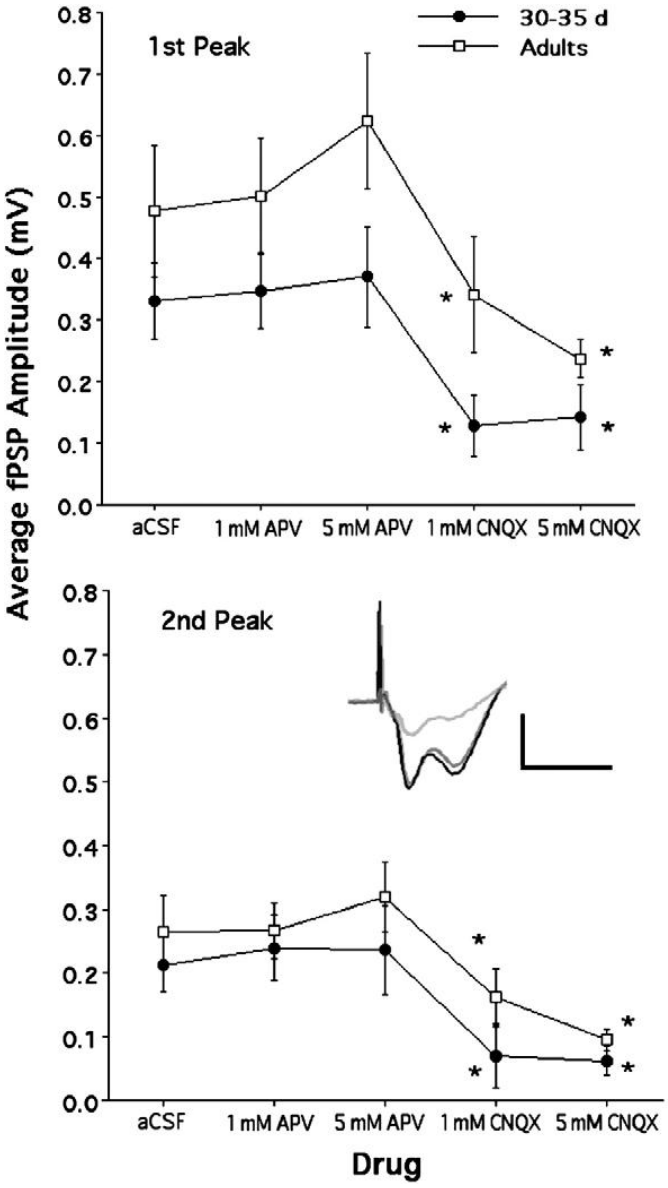

Figure 2.2 Average amplitude of first (top) and second (bottom) peak of field postsynaptic potentials (fPSPs) recorded in the primary auditory cortex (A1) in response to single pulse stimulation of the medial geniculate nucleus. Application of CNQX (1 and 5 mM), but not APV (1 and 5 mM), significantly reduced amplitude of both fPSP peaks in juvenile (30–35 days [d] old, *n*=5) and adult (100–150 days old, *n*=7) rats. The insert depicts typical fPSPs of an adult rat in the presence of aCSF (black), 5 mM APV (dark grey), and additional 5 mM CNQX (light grey; scale bars are 0.25 mV and 20 ms). *indicates significant (*p*<0.05) difference from aCSF level in the respective age group (Newman–Keul's tests).

2.3.2 Characterization of cortical LTP at different ages

A systematic comparison of LTP in animals of four different age groups revealed clear, age-related differences in the magnitude of potentiation elicited in A1 in response to theta burst stimulation (TBS) of the MGN (all experiments conducted with cortical aCSF perfusion). The greatest levels of LTP were elicited in the 30-35 day old age group (n = 6), with potentiation of the first fPSP peak reaching 120, 139, and 153% of baseline for each of the three episodes of TBS (Fig. 2.3, top; values are averages for the last 10 min of each 60 min recording period following a TBS episode). Similarly, amplitude of the second fPSP peak was 127, 157, and 165% of baseline at the corresponding time points (Fig. 2.3, bottom).

For animals in the 40-45 day age group (n = 9), TBS also resulted in robust levels of LTP, even though levels of potentiation at the end of the experiment were lower than those in the youngest rats tested. Amplitude of the first peak reached 130, 136, and 138% of baseline following the three TBS episodes, while the second peak increased to 130, 138, and 144% (Fig. 2.3).

Much lower levels of LTP were observed in the 100-110 day group (n = 9), with first peak amplitude reaching 108, 127, and 116%, while second peak amplitude was at 112, 128, 120% of baseline for each of the three TBS episodes (Fig. 2.3). Finally, in the 200+ day group (n = 10), LTP was not reliably induced, with first peak amplitude averaging 106, 115, and 104%, and second peak amplitude reaching 104, 114, and 107% of baseline for the three TBS episodes (Fig. 2.3).

Together, these data reveal that there is a clear, age-related decline in the ability of TBS of the MGN to elicit LTP in the A1 of rats under the present experimental conditions. These observations were confirmed by statistical analyses, showing significant effects of age for both fPSPs peaks (first peak: $F_{(3,30)} = 6.1$, $p<0.01$; second peak:, $F_{(3,30)} = 5.5$, $p < 0.01$), as well as

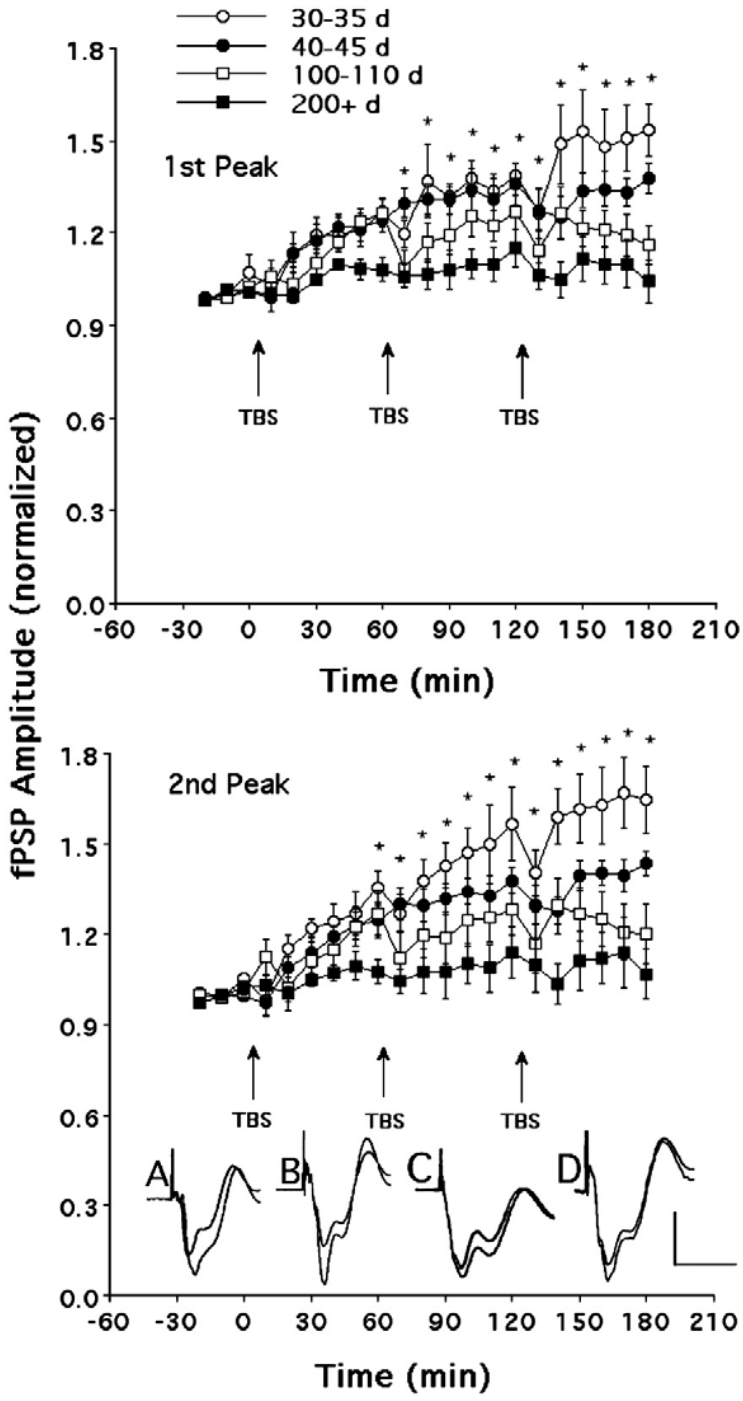

Figure 2.3 The effect of repeated episodes of theta burst stimulation (TBS; at arrows) of the medial geniculate nucleus on amplitude of first (top) and second (bottom) peak of field postsynaptic potentials (fPSPs) recorded in the primary auditory cortex (A1) of rats at different ages (30–35 days [d], *n*=6; 40–45 d, *n*=9; 100–110 d, *n*=9; 200+ d, *n*=10). Levels of

potentiation of both peaks of the fPSP decreased with increasing age. Inserts depict fPSPs recorded before (smaller amplitude) and after the last episode of TBS in rats at the 4 different ages (A, 30–35 d; B, 40–45 d; C, 100–110 d; D, 200+ d; fPSPs shown are averages of 20 individual fPSPs recorded during the last 10 min of baseline and of the experiment, respectively; scale bars are 0.25 mV and 20 ms). *indicates significant (p<0.05) simple effects tests for the comparison between 30–35 d and 200+ d.

significant age by time interactions (first peak: $F_{(60,600)} = 2.9$, $p < 0.01$; second peak: $F_{(60,600)} = 3.9$, $p < 0.01$). Newman-Keul's tests revealed that, for the first fPSP peak, the 30-35 day and 40-45 day groups showed significantly ($p < 0.05$) greater LTP than the 200+ day group. For the second peak, Newman-Keul's tests indicated that the 30-35 day group showed significantly ($p < 0.05$) more LTP than the 100-110 day and 200+ day groups.

A further group of control rats ($n = 4$, ~60-70 days old) was tested to assess the stability of recordings in A1 over the duration of the experiment in the absence of thalamic TBS. In these animals, amplitude of the first peak was 106, 93, and 92%, while the second peak reached 103, 94, and 95% of baseline at time points equivalent to those reported above in rats that did receive TBS. Thus, without TBS, fPSPs remained relatively stable over the course of the experiment.

2.3.3 Effect of NR2B antagonist application on cortical LTP

Next, we applied a selective antagonist of the NR2B subunit of the NMDA receptor locally in A1 to examine a possible role of this subunit in LTP induction in juvenile and adult rats. Cortical application of Ro 25-6981 (10 mM) significantly reduced potentiation of both fPSP peaks in the 30-35 day group ($n = 7$) as compared to controls of the same age receiving aCSF application (Fig. 2.4, i and ii; aCSF animals are the same as above, $n = 6$; first peak: effect of drug, $F_{(1,11)} = 8.4$, $p = 0.01$; drug by time interaction, $F_{(20,220)} = 3.9$, $p < 0.01$; second peak: effect of drug, $F_{(1,11)} = 16.1$, $p < 0.01$; interaction, $F_{(20,220)} = 6.0$, $p < 0.01$). Amplitude of the first peak was reduced to 102, 119, and 103% of baseline during the last 10 min of each 60 min recording period following the three TBS episodes (Fig. 2.4, i). Amplitude for the second fPSP peak was reduced to 102, 124, and 117% of baseline at these time points (Fig. 2.4, ii).

In contrast to the results in young animals, Ro 25-6981 application in A1 did not reduce LTP in rats in the 100-110 day group ($n = 6$) relative to age-matched controls receiving aCSF (Fig. 2.4, iii and iv; aCSF animals same group as above, $n = 9$; first peak: effect of drug, $F_{(1,13)} =$

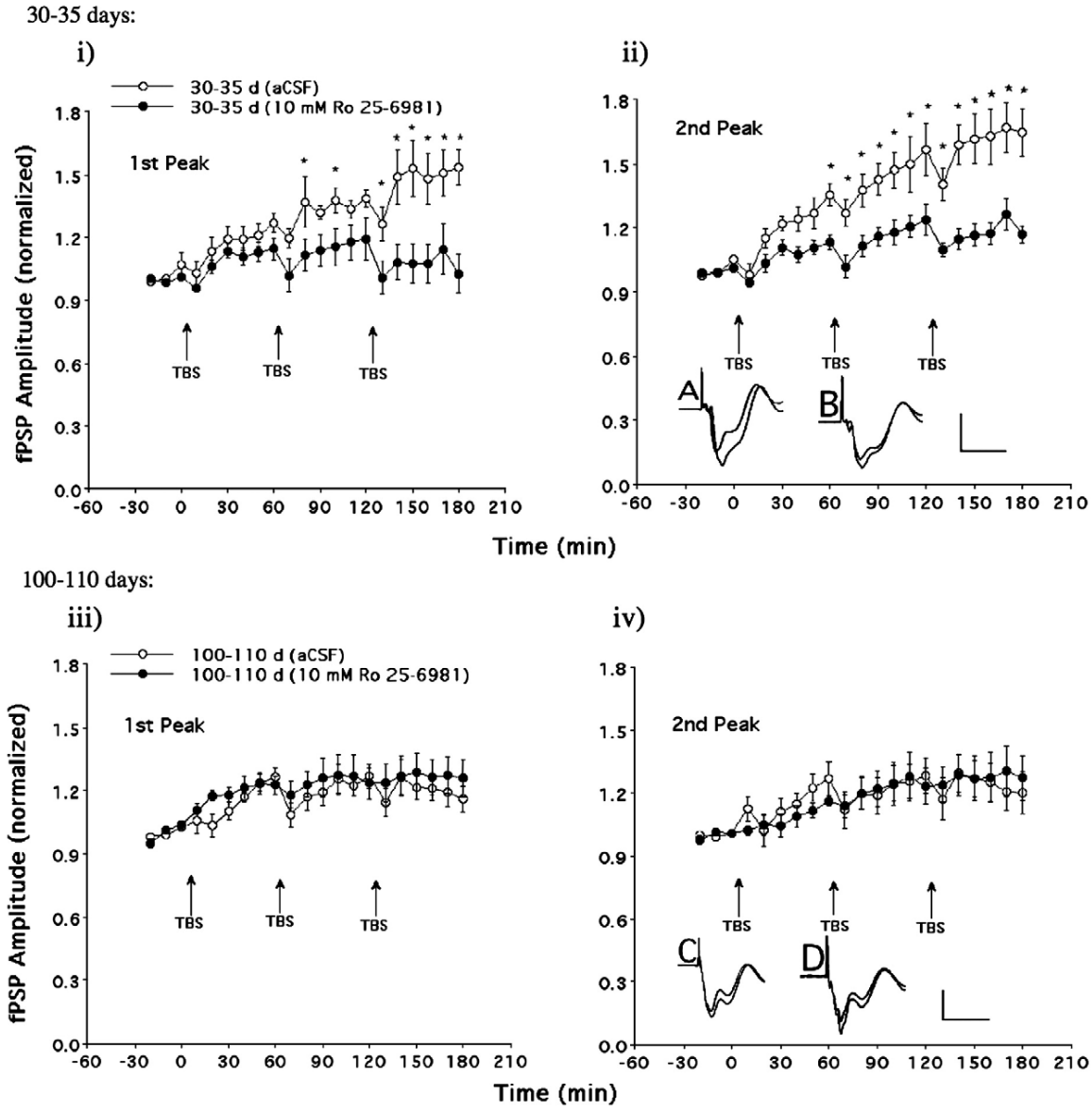

Figure 2.4 The effect of three episodes of theta burst stimulation (TBS; at arrows) of the medial geniculate nucleus on amplitude of the first and second peak of field postsynaptic potentials (fPSPs) recorded in the primary auditory cortex (A1) in 30–35 days (d; top) and 100–110 d (bottom) rats. Local cortical application of Ro 25-6981 (10 mM) significantly reduced LTP of both fPSP peaks in 30–35 d rats (*n*=7; i and ii) relative to age-matched controls receiving aCSF (*n*=6; same animals as above). In contrast, Ro 25-6981 did not

significantly reduce LTP in 100–110 d rats (*n*=6; iii and iv) when compared to age-matched controls receiving aCSF (*n*=9; same animals as above). Inserts depict fPSPs before and after the final episode of TBS in the following conditions: A, 30–35 d, aCSF; B, 30–35 d, Ro 25-6981; C, 100–110 d, aCSF; D, 100–110 d, Ro 25-6981 (fPSPs shown are averages of 20 individual fPSPs recorded during the last 10 min of baseline and of the experiment, respectively; scale bars are 0.25 mV and 20 ms). * indicates significant ($p<0.05$) simple effects tests comparing aCSF and Ro 25-6981 application.

0.4, $p = 0.52$; drug by time interaction, $F_{(20,260)} = 0.7$, $p = 0.82$; second peak: effect of drug, $F_{(1,13)} = 0.01$, $p = 0.92$; interaction, $F_{(20,260)} = 0.6$, $p = 0.89$). Average fPSP amplitudes in 100-110 day animals receiving Ro 25-6981 reached 118, 124, and 126% of baseline for the first peak, and 114, 124, 127% of baseline for the second peak following each of the three TBS episodes (Fig. 2.4, iii and iv, respectively).

2.4 Discussion

Extensive evidence has shown that both age and sensory experience profoundly influence the ability of cortical synapses to undergo activity-dependent alterations in synaptic efficacy. Previous studies have demonstrated clear reductions in the ability of afferents to induce LTP in V1 and somatosensory cortex during early postnatal life, indicative of an increased resistance of cortical circuits to express changes in synaptic connectivity (Kato et al., 1991; Crair and Malenka, 1995; Kirkwood et al., 1995; Jiang et al., 2007; Jang et al., 2009). The current study is the first to characterize age-related changes of LTP in the rodent A1, showing much greater levels of LTP in juvenile animals relative to adults. Further, our findings suggest a critical role of NR2B subunits in the enhanced plasticity seen in younger animals, since blockade of NR2B subunits significantly reduced LTP in young (30-35 d), but not older (100-110 d) rats.

The ages of rats chosen for the current experiment fall within major stages of postnatal development and maturation, as classified in previous work: juvenile/early adolescence (~PD 21-35), middle adolescence (~PD 37-48), and adulthood (>PD 60; Adriani et al., 2002; Macrì et al., 2002; Laviola et al., 2003; but see Spear, 2000). Juvenile rats (30-35 d) clearly showed the greatest levels of potentiation in response to TBS of the MGN, with animals only 10 days older already exhibiting reduced LTP for both peaks of the cortical fPSP. Interestingly, in rats aged 200+ days, TBS of the MGN failed to reliably induce LTP. Overall, this pattern of reduction in

LTP magnitude with increasing age is in line with work in V1 (Kato et al., 1991; Kirkwood et al., 1995), even though we used a wider age range, chronicling plasticity declines into more advanced stages of adulthood than previous work in other sensory modalities.

Our finding that NR2B antagonist application in A1 reduced LTP in juvenile (30-35 d), but not adult (100-110 d) rats is in agreement with the notion that modifications in NMDAR subunit composition are an important contributor to changes in the levels of plasticity during postnatal development (Quinlan et al., 1999; Philpot et al., 2001; Bear, 2003). Extensive work in V1 has shown that, at birth, NR2B levels are high and remain high, whereas NR2A subunits are initially low, but increase with age, resulting in an increase in the ratio of NR2A:NR2B subunits with maturation (Quinlan et al., 1999; Bear, 2003). Relatively higher levels of NR2B subunit expression during early postnatal life are thought to facilitate plasticity and LTP induction, due to longer durations of NMDAR-mediated excitatory postsynaptic currents (EPSCs) in NR2B-containing receptors, resulting in enhanced Ca^{2+} entry into the postsynaptic cell (Carmingnoto and Vicini, 1992; Hestrin, 1992; Monyer et al., 1994; Flint et al., 1997; Philpot et al., 2001; Bear, 2003; Sobczyk et al., 2005).

Similar changes in NMDAR subunit composition have also been reported in the rodent A1, with increases in the NR2A:NR2B ratio evident during postnatal development. However, there appear to be some inconsistencies with regard to the precise pattern and timing of these subunit changes in the rodent A1. Bi et al. (2006) have shown that levels of NR2B subunit mRNA in A1 peak during the first 3 days after birth, followed by a steep decline till about PD 35, after which steady levels appear to be maintained. In contrast, Hsieh et al. (2002) noted more moderate changes in NR2B mRNA expression between PD 4 and adulthood, with a transient peak at PD 10 and apparently adult-like levels at PD 25. Further, these investigators noted much more pronounced changes in NR2A mRNA, with very low levels present at birth, but increasing

rapidly until ~PD 18-25, after which high levels are maintained into adulthood. At present, the reasons of these discrepancies are not known and further work is necessary to resolve this issue. It is worthwhile to note, however, that for both subunits, the majority of changes in expression levels are completed prior to the age of the youngest group of rats tested in the present experiments (PD 30-35), even though Bi et al. (2006) noted a further decline in NR2B mRNA between PD 33 and 37. Consequently, levels of plasticity between MGN and A1 might be significantly higher for rats younger than those examined here, a hypothesis that should be examined in future work.

The observation that NR2B blockade selectively decreased LTP in juvenile, but not adult animals highlights the critical role of this subunit in the expression of heightened levels of synaptic plasticity at younger ages, and is in excellent agreement with previous work. de Marchena et al., (2008) showed that application of the NR2B antagonist ifenprodil reduced LTP in V1 slices of juvenile (PD 21-28), but not adult mice (PD 45-90). Similarly, Massey et al. (2004) found that NR2B subunit blockade by Ro 25-6981 did not affect the induction of LTP in slices of adult (PD 49-84) rat perirhinal cortex. Interestingly, chronic blockade of NR2B subunits with ifenprodil during early postnatal life (PD 13-73) reduces levels of LTP in A1 of adult mice (Mao et al., 2006). Consequently, it appears that the availability of this subunit during development exerts important influences over plasticity mechanisms in adulthood, even though the subunit itself is no longer critical for LTP induction in the mature cortex.

It is important to emphasize that experience and interaction with the sensory environment also play a major role in cortical maturation, in addition to strictly age- and genetically regulated developmental processes (Krubitzer and Kahn, 2003; Hensch 2004). In the visual system, lack of sensory input during early life by means of dark-rearing extends the critical period for ocular dominance plasticity in kittens and rats (Cynader and Mitchell, 1980; Mower, 1991; Fagiolini et

al., 1994), as well as that for LTP induction in slices of rat V1 (Kirkwood et al., 1995; Bear, 2003). Interestingly, dark-rearing also attenuates the increase in the ratio of NR2A:NR2B subunits in V1 normally seen with maturation, resulting in increased relative levels of NR2B (Quinlan et al., 1999; Chen and Bear, 2007). In the auditory system, an effect similar to dark-rearing is induced by rearing rodents under conditions of continuous white noise exposure to mask patterned sound input during early postnatal life. As adults, rats raised under these conditions exhibit an immature tonotopic organization (Chang and Merzenich, 2003), as well high levels of LTP in A1 (Speechley et al., 2007), which are equivalent to those we observed in juvenile rats. These observations strongly suggest that the decline in LTP we noted in older rats is due, at least in part, to experience with the sensory environment, and not merely to the fact that rats were of a more advanced age.

It has been hypothesized that changes in the relative contributions of NMDA and AMPA receptors to baseline (i.e., non-potentiated) synaptic transmission, and the conversion of silent to functional synapses by means of activation of AMPA receptors, also play important roles in the maturation of thalamocortical networks (Feldman et al., 1999). Consequently, we characterized the effects of selective blockade of NMDA and AMPA receptors in rats at two different age ranges (30-35 and 100-150 days). Results of these experiments showed that, regardless of age, neither peak of the fPSP in A1 was significantly altered by local blockade of NMDA receptors with APV. In contrast, both peaks were reduced and, in fact, the second fPSP peak was largely eliminated by local AMPA receptor blockade. Consequently, activation of AMPA, but not NMDA receptors, appears to be necessary for normal synaptic transmission in A1 to occur, at least for the ages tested in this study. These results are consistent with previous *in vitro* work by Kudoh and Shibuki (1994), showing that application of APV did not reduce, while CNQX completely blocked, synaptically evoked fPSP components in slices of rat A1 (28-49 days old)

following white matter stimulation. The fact that Kudoh and Shibuki (1994) noted a complete block of synaptic responses, while we observed some residual, apparently CNQX-resistant responses in A1 likely relates to the different methods of drug application. Kudoh and Shibuki (1994) used bath application, while we used reverse microdialysis, resulting in a more restricted diffusion of drug molecules in an area immediately surrounding the dialysis probe. Our results are also in agreement with similar work in other sensory modalities. Huemmeke et al. (2004) found that DNQX (also an AMPA receptor antagonist) blocked synaptic components of field potentials in V1 slices of juvenile rats (PD 21-25), confirming AMPA receptors as the critical mediators of synaptic transmission in sensory cortices in juvenile and adult animals.

The mechanisms of LTP and long-term depression are thought to mediate aspects of receptive field plasticity seen in cortical sensory fields (Bear, 2003; Malenka and Bear, 2004). The tonotopic organization of the rodent A1 emerges and matures over the first 40-50 days of postnatal life (Zhang et al., 2001; Chang and Merzenich, 2003; Chang et al., 2005). During this development, several processes occur, often with distinct time courses. For example, de Villers-Sidani et al. (2007) have defined a precise temporal window (PD 11-13) that marks the peak of the critical period for receptive field plasticity in A1 induced by pure-tone exposure. On the other hand, the maturation of cortical inhibitory circuits in A1 occurs later during development, with a significant increase in cortical inhibition occurring between of PD 35 and 45 (Chang et al., 2005). Of course, the presence of critical (or "sensitive") periods during early postnatal life does not rule out the occurrence of significant, long-lasting plasticity in later life. Receptive field plasticity in A1 occurs in adulthood, even though the susceptibility to express plasticity clearly decreases with age. Thus, while mere passive exposure to pure tones is sufficient to induce receptive field changes in young animals (Zhang et al., 2001; de Villers-Sidani et al., 2007), adults show equivalent changes only in the context of behavioral learning and/or the release of learning- or

plasticity-related neuromodulators (e.g., acetylcholine; Kilgard and Merzenich, 1998; Weinberger, 2004, 2007). Consequently, the inverse relation between age and LTP noted here is consistent with this model of a gradual increase in resistance against plasticity induction, rather than an absolute loss of plasticity after completion of early, postnatal development. We suspect that even the oldest rats tested (200+ days), which did not reliably show LTP in response to the specific TBS protocol used, can express LTP if stronger induction protocols are delivered, or LTP induction is paired with the release of plasticity-related neuromodulators, as has been shown for thalamocortical LTP in other sensory domains (Dringenberg et al., 2007).

In summary, the findings of the current study are the first to document an age-related decline in NMDAR-dependent LTP in the rat auditory cortex, thus extending previous research in visual and somatosensory cortices by profiling plastic changes in juvenile to adult animals. The enhanced LTP noted in juvenile rats was abolished by cortical NR2B antagonist application, suggesting a critical involvement of this subunit in the heightened level of plasticity observed in the younger cortex. These age-related declines in cortical plasticity can be expected to significantly limit the ability of cortical circuits to respond and adjust to alterations in sensory inputs experienced at different stages of development and adulthood.

2.5 Experimental procedures

2.5.1 Animals

All subjects were treated in accordance with the regulations on animal experimentation established by the Canadian Council on Animal Care and the experimental procedures were approved by the Queen's University Animal Care Committee. Male Long-Evans rats of different ages (25, 35, or 70 days old) were obtained from Charles River (St. Constant, Quebec) and

housed in a colony room until they were of appropriate age for experimentation (tested at PD 30-35, PD 40-45, PD 100-150, or PD >200). All rats were housed as four animals per cage (12-12 h reverse light cycle, lights on at 1900 h) with food and water available *ad libitum*.

2.5.2 Surgical procedure

Rats were placed in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA) under deep urethane anaesthesia (1.5 g/kg, i.p., administered as 3×0.5 g/kg doses every 15 min, with supplements provided as necessary). An incision was made to expose the skull, and small holes drilled over the MGN (AP −5.5, L +4.0, V −5.4 to −6.4) and ipsilateral A1 (AP −4.5, L +8.0, V −3.2 to −5.4). For 30-35 day old rats, lateral coordinates were adjusted to L+3.7 and +7.7 for MGN and A1 placements, respectively. Additional skull holes were drilled for ground and reference electrode screws, located above the contralateral frontal cortex and cerebellum, respectively. All stereotaxic measurements were based on the anatomical rat brain atlas of Paxinos and Watson (1998). Body temperature of the rats was maintained at 36-37 °C throughout the experiment with a heating pad and cotton wool blanket.

2.5.3 Electrophysiology

An electrode-dialysis probe assembly was lowered into A1 for the recording of fPSPs and local drug application. The electrode (125 μm diameter stainless steel wire insulated with Teflon) was situated immediately adjacent to the dialysis probe, with the tip of the electrode positioned approximately 1 mm above the probe tip. This assembly was lowered into A1 so that the recording electrode penetrated the middle cortical layers (depth of about 400–600 μm; layers IV–V).

The dialysis probe (Mab. 2.14.4; 2 mm active polyether sulfone membrane, 35 kDA cut-off; S.P.E Limited, North York, Ontario, Canada) was connected to a 2.5 mL Hamilton airtight

microsyringe driven by a microdialysis pump (CMA/102, CMA Microdialysis, Solna, Sweden). The probe was perfused continuously at a flow rate of 1.0 µl/min with aCSF, which consisted of, in mM: 124.0 NaCl, 4.4 KCl, 1.2 $MgSO_4$, 1.0 NaH_2PO_4, 2.5 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose.

The recording electrode was connected to an amplifier (Model 1800, A-M Systems Inc., Carlsborg,WA; filter settings at 0.3 Hz to 1 kHz) and A-D converter (PowerLab/4s system, running Scope software v. 4.0.2, AD Instruments, Toronto, ON, Canada), which digitized (at 10 kHz) and stored the recorded signal for offline analysis.

Stimulation of the MGN (0.2 ms pulse duration) was provided by a concentric, bipolar stimulating electrode (SNE-100, Rhodes Medical Instruments, David Kopf, Tujunga, CA) connected to a stimulus isolation unit (ML 180 Stimulus Isolator, AD Instruments) providing a constant current output. Final ventral placements for both the recording and stimulating electrodes were adjusted in order to achieve maximum fPSP amplitude in A1 in response to ipsilateral MGN stimulation.

2.5.4 Data collection

Following final electrode adjustments, the brain was allowed to stabilize for 30 min with continuous aCSF application in A1. For all experiments, an initial input-output curve was generated by stimulating the MGN at successively increasing intensities (0.1-1.0 mA, in 0.1 mA increments). The stimulation intensity producing approximately 50-60% of the maximal fPSP amplitude was chosen as the intensity for the subsequent, formal data collection. For LTP experiments, baseline fPSPs were recorded every 30 s until 30 min (i.e., a total of 60 fPSPs) of stable recordings were obtained. Following baseline, TBS of the MGN was applied as trains of 10 bursts (each burst consisting of 5 pulses at 100 Hz, bursts repeated at 5 Hz), with trains repeated every 10 s for a total of 4 trains. Following TBS, single pulse stimulation of the MGN continued

for 60 min. The TBS protocol was repeated twice more for a total of three induction episodes, each followed by a 60 min recording period. Multiple TBS episodes were delivered in order to characterize saturation points of cortical LTP. A control group, consisting of rats that did not receive TBS, was also tested to assess the stability of fPSP recordings over the course of the experiment.

2.5.5 Pharmacological manipulations

Local drug application in A1 was performed by reverse microdialysis, which allows drug molecules to reach neural tissue by means of concentration driven diffusion across the dialysis probe membrane. It is estimated that the drug concentration outside the probe membrane reaches ~10% of the concentration contained in the perfusion medium (i.e., aCSF) with this technique (e.g., Oldford and Castro-Alamancos, 2003). All drugs were dissolved in aCSF, which circulated through the probe at a flow rate of 1.0 μl/min. For LTP experiments, rats received either aCSF alone, or aCSF containing Ro 25-6981 (10 mM), a selective NR2B antagonist, with application beginning immediately prior to the onset of baseline recordings and continuing throughout the entire experiment.

An additional experiment was designed to characterize some pharmacological properties of cortical fPSPs in A1 elicited by single pulse MGN stimulation in rats of different ages (30-35 and ~100-150 days). For these experiments, initial input-output curves were generated (0.1-1.0 mA in 0.1 mA increments, 10 fPSPs/intensity) in the presence of aCSF, after which the following drugs were applied (in the order listed): (a) the NMDA receptor antagonist DL-APV (1 mM); (b) DL-APV (5 mM); (c) the AMPA receptor antagonist CNQX (1 mM); and (d) CNQX (5 mM). Input-output curves were repeated for each new drug and drug concentration at 30 min after the onset of drug delivery. Analyses of the input-output data during aCSF application showed that 0.3 mA constituted both the mean and mode of stimulation intensities to elicit between 50 and 60%

of the maximal fPSP amplitude. Thus, for this subset of experiments, only data obtained with an intensity of 0.3 mA were subjected to formal data analyses and presented in the sections above. All drugs were obtained from Sigma-Aldrich (Oakville, Ontario).

2.5.6 Histology

Following all experiments, rats were perfused through the heart with 0.9% saline, followed by 10% formalin. Brains were extracted, and standard histological techniques were used to examine the placements of electrodes and microdialysis probes. Data from experiments with inaccurate placements were discarded.

2.5.7 Data analysis

For all experiments, fPSP amplitudes were computed offline by calculating the voltage difference between the recording immediately prior to the stimulus artifact and the two negative peaks of the fPSP, occurring at about 7 and 15 ms after the stimulus artifact. The fPSP amplitude measurements for LTP experiments were averaged over 10 min intervals, and normalized by dividing them by the average baseline amplitude recorded for each animal.

All data are expressed as mean ±S.E.M. Statistical comparisons were made using analyses of variance (ANOVA) and, where statistically appropriate, simple effects or Newman-Keul's tests using the CLR ANOVA software package (v.1.1, Clear Lake Research Inc., Houston, Texas).

Chapter 3

Characterization of Auditory Cortex Plasticity Following Early Sensory Deprivation

As published in: "Hogsden JL, Dringenberg HC (2009) NR2B subunit-dependent long-term potentiation enhancement in the rat cortical auditory system in vivo following masking of patterned auditory input by white noise exposure during early postnatal life. Eur J Neurosci, 30, 376-384"

3.1 Abstract

The composition of N-methyl-D-aspartate (NMDA) receptor subunits influences the degree of synaptic plasticity expressed during development and into adulthood. Here, we show that theta-burst stimulation of the medial geniculate nucleus reliably induced NMDA receptor-dependent long-term potentiation (LTP) of field postsynaptic potentials recorded in the primary auditory cortex (A1) of urethane-anesthetized rats. Furthermore, substantially greater levels of LTP were elicited in juvenile animals (30-37 days old, ~ 55% maximal potentiation) than in adult animals (~30% potentiation). Masking patterned sound via continuous white noise exposure during early postnatal life (from postnatal day 5 to postnatal day 50-60) resulted in enhanced, juvenile-like levels of LTP (~70% maximal potentiation) relative to age-matched controls reared in unaltered acoustic environments (~30%). Rats reared in white noise and then placed in unaltered acoustic environments for 40-50 days showed levels of LTP comparable to those of adult controls, indicating that white noise rearing results in a form of developmental arrest that can be overcome by subsequent patterned sound exposure. We explored the mechanisms mediating white noise-induced plasticity enhancements by local NR2B subunit antagonist application in A1. NR2B subunit antagonists (Ro 25-6981 or ifenprodil) completely reversed white noise-induced LTP enhancement at concentrations that did not affect LTP in adult or age-matched controls. We conclude that white noise exposure during early postnatal life results in the maintenance of

juvenile-like, higher levels of plasticity in A1, an effect that appears to be critically dependent on NR2B subunit activation.

3.2 Introduction

Appropriate patterns of sensory input are particularly important during early postnatal development, when cortical maturation is highly dependent on incoming sensory stimuli. This period of heightened, experience-dependent plasticity during early life allows for a precise matching of cortical organization to its particular sensory environment (Hubel & Wiesel, 1970; Knudsen, 2004; Keuroghlian & Knudsen, 2007).

Cortices deprived of sensory input during critical periods do not show their characteristic organizational patterns. For example, monocular deprivation during early postnatal life results in a bidirectional shift in cortical representation of the two eyes; that is, synapses driven by the open eye strengthen and expand, and synapses driven by the deprived eye show depression and atrophy (Wiesel & Hubel, 1963; Shatz & Stryker, 1978). In the rat primary auditory cortex (A1), adult-like tonotopic organization develops over the first 4-6 weeks of postnatal life, characterized by the appearance of narrowly tuned, frequency-selective neurons (Zhang et al., 2001; Chang et al., 2005). Alterations in sensory experience greatly impact this maturational process. Chronic exposure to a repetitive acoustic stimulus, of a specific frequency, results in an overrepresentation of that frequency in A1 (Zhang et al., 2001). Masking patterned sound during early development by continuous white noise or unmodulated tone exposure prevents the emergence of normal tonotopic organization in A1, even though this effect can be reversed with subsequent exposure to an unaltered acoustic environment (Chang & Merzenich, 2003; Chang et al., 2005; Zhou et al., 2008).

Interestingly, rats reared in white noise exhibit greater levels of long-term potentiation (LTP) between the medial geniculate nucleus (MGN) and A1 relative to rats reared in unaltered acoustic environments (Speechley et al., 2007). Given that white noise rearing appears to extend the critical period for postnatal A1 maturation, it is possible that LTP serves as a synaptic mechanism mediating the heightened plasticity present during critical periods and its maintenance following white noise exposure.

Activation of N-methyl-D-aspartate (NMDA) receptors is required for LTP induction in the rat cortical auditory system *in vitro* and *in vivo* (Kudoh & Shibuki, 1994; Speechley et al., 2007). Changes in the expression of different NMDA receptor subunits have been suggested to influence the degree of plasticity at synapses during different developmental stages (Quinlan et al., 1999; Philpot et al., 2001). Over the course of development, NR2A subunit levels in A1 are initially low and increase rapidly, whereas NR2B subunit levels are high and remain relatively stable, or decline slightly (Hsieh et al., 2002; Bi et al., 2006). It appears that the effect of visual deprivation (dark-rearing) in extending ocular dominance plasticity and enhancing LTP in the visual cortex is mediated by a deprivation-induced decrease in the NR2A/NR2B subunit ratio, with relatively higher levels of NR2B subunits (Bear, 2003; Chen & Bear, 2007). Here, we examined the role of NR2B subunits in white noise-induced enhancement of LTP in the cortical auditory system *in vivo* by means of local, cortical NR2B subunit antagonist application following the masking of patterned sound by white noise exposure during the critical period for the emergence of tonotopic organization of A1.

3.3 Materials and methods

3.3.1 Animals

All subjects were obtained from Charles River Laboratories (St. Constant, Quebec, Canada) and treated in accordance with the regulations on animal experimentation established by the Canadian Council on Animal Care. The experimental procedures were approved by the Queen's University Animal Care Committee.

For the initial pharmacological, current source density (CSD) and age comparison experiments, adult (> 100 days old) or juvenile (30-37 days old) male Long-Evans rats were used. Rats were housed four per cage in a colony room (12:12 h reverse light cycle, lights on at 19:00 h) with food and water available *ad libitum*.

For experiments assessing the effects of altered sound environments (white noise and the appropriate control conditions), untimed (~19 days) pregnant female Long-Evans rats were obtained. Upon arrival, pregnant rats were housed singly in cages (food and water available *ad libitum*) that were contained in a sound-attenuated chamber (114 × 61 × 66 cm, plywood lined with aluminum, fitted with a time-controlled light, fan, and two equally spaced speaker boxes mounted on the ceiling of the box; sound attenuation across the chamber wall was about ~27 dB for measurements immediately outside the box containing an ~80 dB white noise signal). Pregnant rats were allowed to give birth, and offspring were housed together with their mothers until weaning [postnatal day (PD) 21], when the mother and female offspring were removed from the cages. Male rats continued to be housed in the chambers in cages containing no more than four individuals. To generate offspring for control (unaltered acoustic) conditions, pregnant females (same as above) were housed and allowed to give birth in cages contained in the same room, but outside of the sound-attenuated chamber (12:12 h light cycle, lights turned on at 07:00 h).

3.3.2 White noise exposure

Each speaker box contained two speakers (one 8 inch woofer and one 3.25 inch tweeter; frequency ranges of 45 Hz to 5 kHz and 2-35 kHz, respectively; American Legacy Series 2 Speakers, Legacy Audio, Brooklyn, NY, USA). Speakers were connected to a custom-made white noise generator (Department of Psychology, Electronics Workshop, Queen's University). Previously published spectral analyses of white noise generated by this set-up and recorded inside the sound-attenuated chamber showed that the signal covered a frequency range up to about 35 kHz, with power gradually declining in the range between 30 and 37.5 kHz (Speechley et al., 2007).

Continuous white noise exposure began at PD 5, which is approximately 5-6 days prior to the onset of low-threshold hearing in rats (de Villers-Sidani et al., 2007). The sound volume was increased incrementally from ~70 to ~80 dB over 5 days, and subsequently maintained at that level until PD 50-60, when electrophysiological procedures were carried out (one group received prolonged white noise exposure until PD 100-110). Continuous white noise rearing of rats using these or similar parameters is known to effectively delay tonotopic refinement of A1 (Chang & Merzenich, 2003) and alter the properties of cortical LTP (Speechley et al., 2007).

3.3.3 Surgical procedure

Rats were placed in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA) under deep urethane anaesthesia (1.5 g/kg, intraperitoneal, administered as 3 × 0.5 g/kg doses every 15 min, with supplements provided as necessary). An incision was made to expose the skull, and small holes were drilled over the MGN (anteroposterior, –5.5; lateral, +4.0; ventral, –5.4 to –6.4) and ipsilateral A1 (anteroposterior, –4.5; lateral, +8.0; ventral, –3.2 to –5.4). Holes were also drilled for ground and reference electrode screws, located above the contralateral frontal cortex and cerebellum, respectively. All stereotaxic measurements were based on the anatomical rat brain

atlas of Paxinos & Watson (1998). The body temperature of rats was maintained at 36-37°C throughout the experiment with a heating pad and cotton wool blanket.

3.3.4 Electrophysiology

Recordings of field postsynaptic potentials (fPSPs) and local drug application in A1 were carried out by means of an electrode-dialysis probe assembly that was lowered into A1. The electrode (125 μm diameter stainless steel wire insulated with Teflon) was positioned immediately adjacent to the dialysis probe, with the electrode tip located approximately 1 mm above the probe tip. This assembly was positioned in A1 so that the probe penetrated throughout the entire cortex (depth of ~1500 μm), placing the electrode tip in the middle cortical layers (depth of ~400-600 μm, layers IV-V).

The dialysis probe (Mab. 2.14.4; 2 mm active polyether sulfone membrane, 35 kDa cut-off; SPE Limited, North York, Ontario, Canada) was connected to a 2.5 mL Hamilton airtight microsyringe driven by a microdialysis pump (CMA/102; CMA Microdialysis, Solna, Sweden) and perfused continuously at a flow rate of 1.0 μL/min with artificial cerebrospinal fluid (aCSF). The aCSF consisted of 124.0 mM NaCl, 4.4 mM KCl, 1.2 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 26.0 mM $NaHCO_3$, and 10.0 mM glucose.

The recording electrode was connected to an amplifier (Model 1800; A-M Systems Inc., Carlsborg, WA, USA; filter settings at 0.3 Hz to 1 kHz) and A–D converter (PowerLab/4s system, running scope software v. 4.0.2; AD Instruments, Toronto, ON, Canada), which digitized (at 10 kHz) and stored the recorded signal for offline analysis.

Stimulation of the MGN (0.2 ms pulse duration) was provided by a concentric, bipolar stimulating electrode (SNE-100; Rhodes Medical Instruments, David Kopf, Tujunga, CA, USA) connected to a stimulus isolation unit (ML 180 Stimulus Isolator; AD Instruments) providing a constant current output. The final ventral placements for both recording and stimulating

electrodes were adjusted in order to achieve maximum fPSP amplitude in A1 in response to ipsilateral MGN stimulation.

3.3.5 Data collection

Following final electrode adjustments, the brain was allowed to stabilize for 30 min with continuous aCSF application in A1. Subsequently, the MGN was stimulated at successively increasing intensities (0.1-1.0 mA, in 0.1 mA increments) to generate an input-output series. The stimulation intensity producing approximately 50-60% of the maximal fPSP amplitude was chosen as the stimulation intensity for the experiment.

Baseline fPSPs were recorded every 30 s until 30 min (i.e. a total of 60 fPSPs) of stable recordings were obtained, defined as fPSP amplitudes within 95-105% of the average of the recordings over the 30 min time period. Following baseline, theta-burst stimulation (TBS) was applied to the MGN in the form of five pulses per burst (pulses repeated at 100 Hz), with bursts repeated ten times at 5 Hz. This stimulation was repeated four times with 10 s intervals. Following TBS, single pulse stimulation of the MGN continued for 60 min. This TBS protocol was repeated for a total of three induction episodes, each followed by a 60 min recording period. Multiple TBS episodes were delivered in order to characterize saturation points of cortical LTP. An additional control group not receiving TBS was also included to verify the stability of fPSPs over the course of the experiment.

3.3.6 Pharmacological manipulations

Local drug administration in A1 was achieved by reverse microdialysis, allowing drug molecules to reach neural tissue by means of concentration-driven diffusion across the dialysis probe membrane. With this technique, the drug concentration outside the probe membrane is estimated to reach approximately 10% of the concentration contained in the perfusion medium

[aCSF; e.g. Oldford & Castro-Alamancos (2003)]. Different groups of rats received one of the following compounds (all dissolved in aCSF, flow rate of 1.0 μL/min): DL-APV (10 mM), a broad-acting NMDA receptor antagonist; Ro 25-6981 (1 or 10 mM), a selective NR2B subunit antagonist; or ifenprodil (1 mM), a selective NR2B subunit antagonist. Drug application was started immediately prior to the onset of baseline recordings and continued throughout the entire experiment. Control animals received continuous aCSF application throughout the experiment. All drugs were obtained from Sigma-Aldrich (Oakville, Ontario).

3.3.7 CSD experiments

The data used for CSD analyses were obtained from depth profiles, carried out in an additional group of animals. For these experiments, a recording electrode (not coupled to a dialysis probe) was placed on the brain surface above A1 (~25° angle from horizontal). The electrode was lowered into the cortex in successive 100 μm steps, and 20 fPSPs were recorded at each depth, to a final depth of ~1500 μm. Subsequently, the electrode was gradually withdrawn (100 μm steps), with another 20 fPSPs being recorded at each depth. The fPSPs collected at each depth during the descent and ascent of the electrode did not show obvious differences and, consequently, were collapsed (i.e. 40 fPSPs/depth) and used to generate average fPSPs for each 100 μm depth increment. At the end of the experiment, the recording electrode was lowered to the deepest level, and small lesions were made to aid with the histological reconstruction of the electrode path.

3.3.8 Histology

At the end of each experiment, while under urethane anaesthesia, the rat was perfused through the heart with 0.9% saline, followed by 10% formalin. Brains were extracted, and standard histological techniques were used to examine the placements of electrodes and

microdialysis probes. Data from experiments with inaccurate placements were discarded. For experiments involving placements of dialysis probe-electrode assemblies, tissue damage created by the probe made accurate histological verification of the precise depth of the electrode tip impossible. Consequently, fPSPs recorded with probe-electrode assemblies were compared with the depth profiles obtained during CSD experiments (without dialysis probes) in order to estimate the depth of recordings obtained in A1.

3.3.9 Data analysis

The cortical fPSPs recorded here consisted of two distinct peaks, with typical latencies of about 7 ms and 14 ms from the stimulus artifact to peak maximum (see Results). The amplitude of both peaks was computed offline by calculating the voltage difference between the recording immediately prior to the stimulus artifact and the two negative peaks of the fPSP elicited by the stimulation. For all LTP experiments, fPSP amplitude measurements were averaged over 5 min intervals and normalized by dividing them by the average baseline amplitude that was recorded for each animal during the first 30 min of the experiment.

In order to characterize local current sinks and sources at different cortical depths, one-dimensional CSD analyses were performed on the data collected during the depth profile experiments according to methods described previously (Nicholson & Freeman, 1975; Mitzdorf, 1985). CSD profiles were calculated by subtracting two times the voltage at a given depth (V_0) from the sum of the voltages recorded 200 μm above (V_a) and below (V_b) this depth, and dividing this value by the squared distance (d^2) between depths (CSD = $[(V_a + V_b) - 2V_0] / d^2$). Using this analysis, current sinks and sources are indicated by positive and negative values, respectively. The CSD profiles obtained in individual animals were collapsed in order to generate an average CSD profile across all experiments.

All data are expressed as mean ± standard error of the mean. Statistical comparisons were made using analyses of variance (ANOVA) and simple effects tests using the CLR ANOVA software package (v.1.1; Clear Lake Research Inc., Houston, TX, USA).

3.4 Results

3.4.1 Characterization of fPSPs elicited by MGN stimulation

Single pulse stimulation of the MGN in urethane-anesthetized rats reliably elicited fPSPs recorded in A1. Typically, stimulation electrodes were located in the ventral division of the MGN and elicited fPSPs that consisted of two distinct, negative-going peaks with latencies to peak of approximately 7 ms and 14 ms, respectively (Fig. 3.1). In order to more fully characterize these fPSPs, we performed CSD analyses of depth profiles collected in a subset of animals (n = 5). These analyses showed that MGN stimulation elicited an initial short-latency, large-amplitude current sink, maximal at depths of about 700-800 μm, followed by a second, longer-latency sink at approximately 500-600 μm (Fig. 3.1). Both current sinks were surrounded by current sources (Fig. 3.1). From the differential distribution of these current sinks and the clear difference in latency, it is likely that the two consecutive, negative-going peaks reflect sequential activation of direct thalamocortical and subsequent intracortical synapses, respectively.

3.4.2 Characterization of cortical LTP

Initially, the stability of fPSP recordings was assessed in a group of adult (> 100 days) rats (n = 4, aCSF applied) not subjected to TBS. In these animals, fPSP amplitude at the end of the experiment was 102% of baseline for both peaks of the fPSP (Fig. 3.2), indicating stable recordings during the course of the experiment. In contrast, delivery of TBS to the MGN of adult rats (n = 8, > 100 days, aCSF applied) produced a reliable potentiation of fPSPs in A1 (Fig. 3.2).

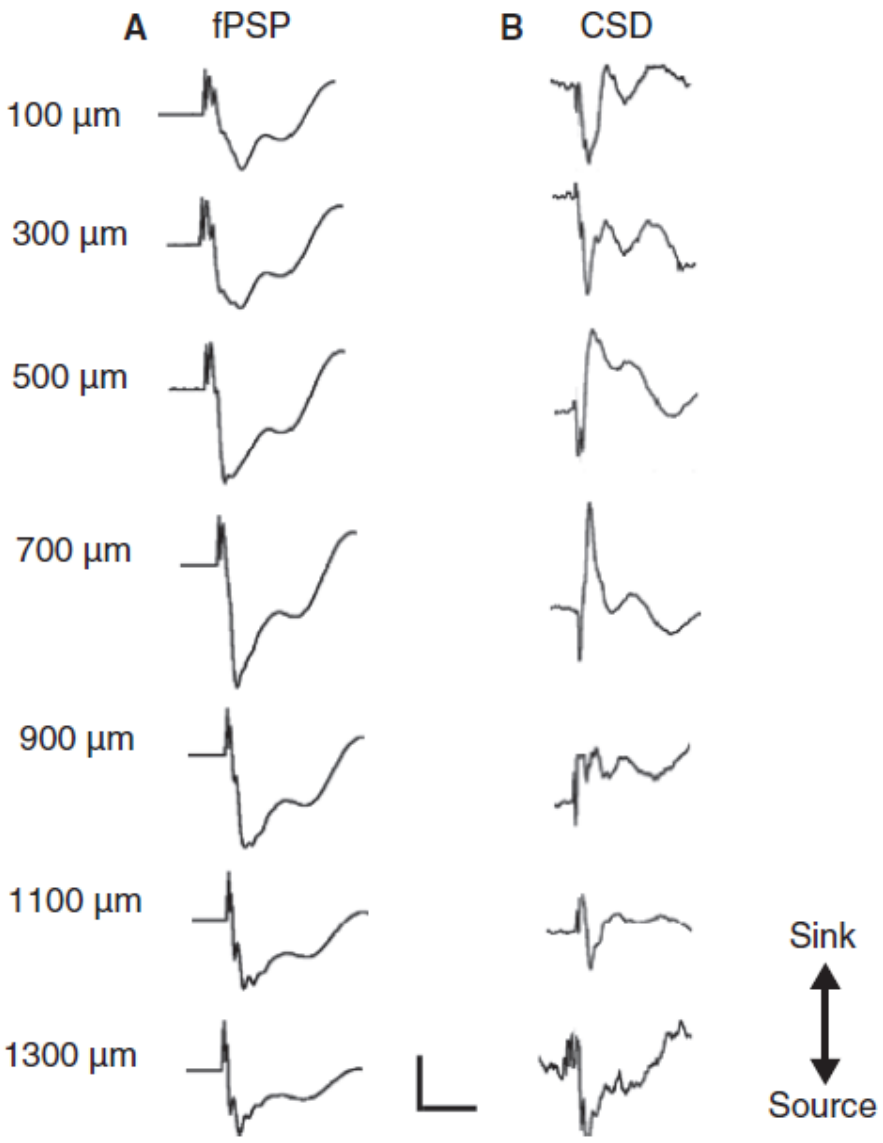

Figure 3.1 Field postsynaptic potentials (fPSPs) recorded in the primary auditory cortex (A1) in response to single pulse stimulation of the medial geniculate nucleus. (A) fPSPs recorded at different depths of A1 (depths, in micrometers, are indicated to the left of the fPSPs; recordings were made every 100 µm; recordings every 200 µm are shown). Scale bars are 1 mV and 10 ms. (B) Corresponding current source density (CSD) profiles derived from fPSP recordings in A1. Current sinks and sources are indicated by upwards-going and downwards-going deflections, respectively. Note the large-amplitude current sinks at depths of 500 and 700 µm.

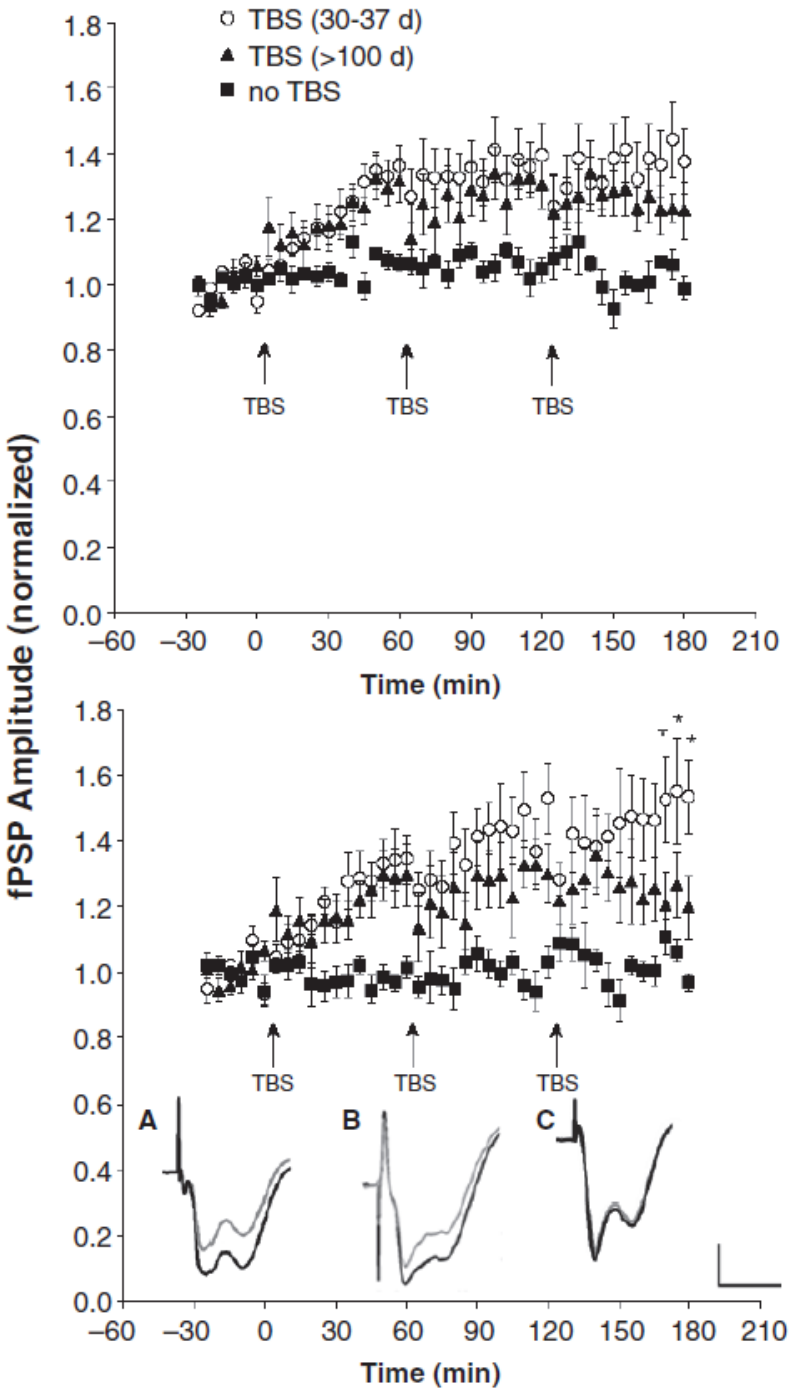

Figure 3.2 The effect of repeated theta-burst stimulation (TBS) (at arrows) of the medial geniculate nucleus on the amplitude of the first peak (top) and second peak (bottom) of field postsynaptic potentials (fPSPs) in the primary auditory cortex. Juvenile (30–37 days old, *n* =

6) and adult (> 100 days old, n = 8) animals showed long-term potentiation of both fPSP peaks, with significantly greater potentiation of the second peak in juveniles (age × time interaction for second peak, $F_{41,492}$ = 1.5, P = 0.03; interaction and main effect of age not significant for first peak, P > 0.3). A control group of adults not subjected to TBS (n = 4) did not show significant changes in fPSP amplitude over time, and exhibited significantly lower amplitude than the two TBS groups (group × time interaction: first peak, $F_{82,615}$ = 1.9, P < 0.001; second peak, $F_{82,615}$ = 2.2, P < 0.001; *significant simple effects, P < 0.05, between juveniles and adults given TBS). Inserts depict typical fPSPs (averaged over 20 individual fPSPs) recorded during baseline (pre-TBS; grey) and the final hour of the experiment (black; A, TBS, 30–37 days; B, TBS, > 100 days; C, no TBS). Scale bars are 0.2 mV and 20 ms.

Comparisons of amplitude changes of the first and second peaks showed that TBS resulted in similar levels of synaptic potentiation, with maximal LTP reaching 132 and 131% of baseline, respectively (Fig. 3.2). Furthermore, it appeared that a single episode of TBS resulted in maximal, or near maximal, levels of LTP for both peaks of the fPSP (Fig. 3.2). Interestingly, juvenile rats (30-37 days, n = 6, aCSF applied) showed greater LTP in response to thalamic TBS than adult animals, with maximal levels of LTP for the first and second peaks reaching 141 and 154%, respectively (Fig. 3.2). Consequently, levels of LTP appear to provide a sensitive index of the maturational state of A1 under the present experimental conditions.

Some pharmacological characteristics of LTP were studied in additional groups of adult animals that received local, cortical drug application at the A1 recording site by means of reverse microdialysis. These experiments showed that application of the NMDA receptor antagonist APV (10 mM, n = 6) completely blocked LTP induction for both the first and second peaks of the fPSP, which reached a maximum of only 100 and 102% of baseline, respectively (Fig. 3.3). In contrast, local application of the NR2B subunit antagonist Ro 25-6981 (10 mM, n = 8) did not significantly suppress LTP following TBS, with maximal potentiation of the two peaks reaching 129-135% in the presence of either drug concentration (Fig. 3.3). Application of the different antagonists in A1 did not produce major changes in baseline (i.e. non-potentiated) fPSPs. Average amplitudes of the first and second fPSP components were 112 and 82% of aCSF levels during APV application, and 114 and 99% of aCSF levels in the presence of Ro 25-6981.

3.4.3 Effect of white noise rearing on LTP: pharmacological characterization

Rats reared in continuous white noise from PD 5 to PD 50-60 (n = 7, aCSF applied) showed significantly greater levels of LTP for both fPSP peaks following TBS of the MGN relative to age-matched controls reared in unaltered sound environments (n = 8, aCSF applied; Fig. 3.4). White noise-reared animals showed maximal potentiation of 146 and 176% for the first

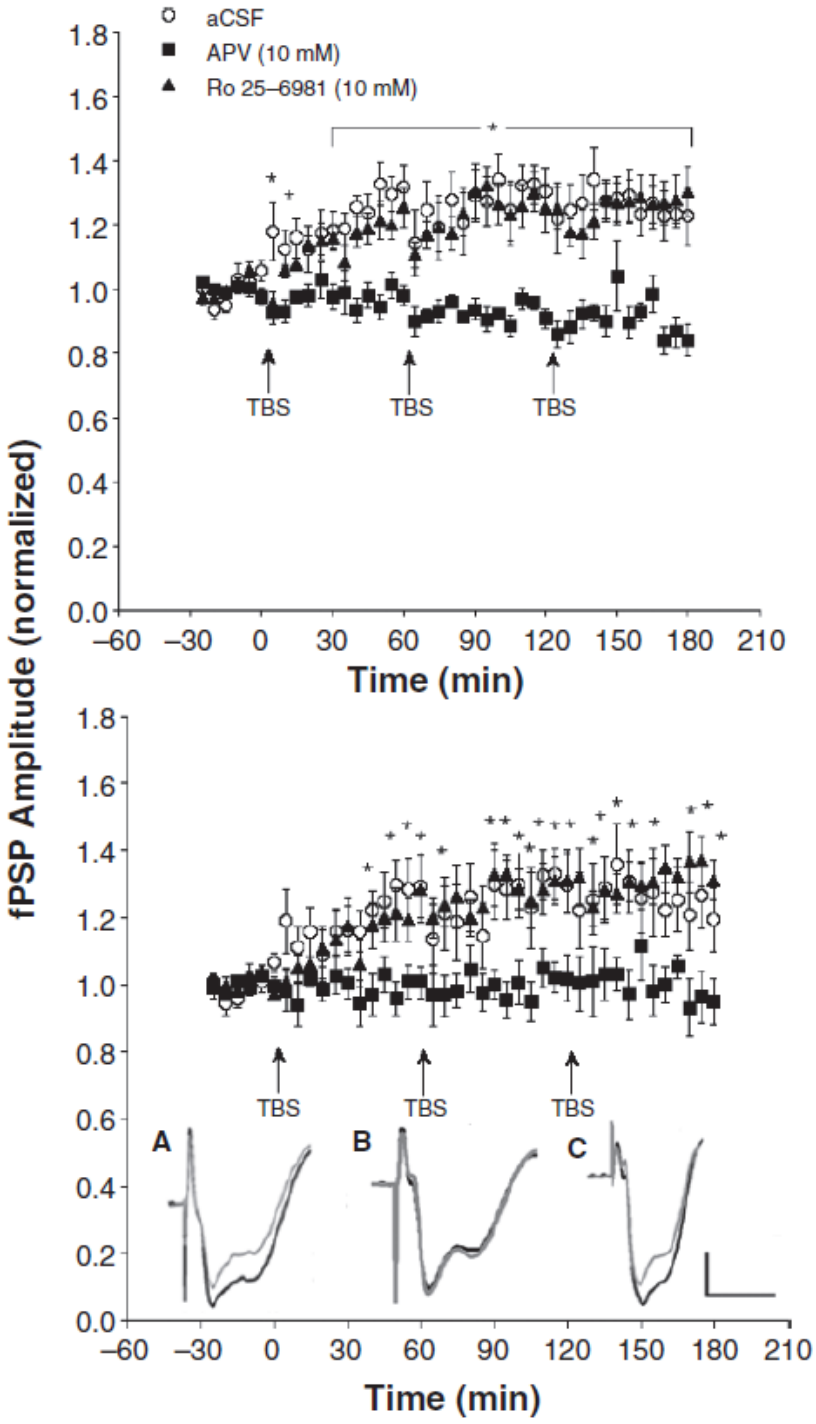

Figure 3.3 The effect of repeated theta-burst stimulation (TBS) (at arrows) of the medial geniculate nucleus on the amplitude of the first peak (top) and second peak (bottom) of field postsynaptic potentials (fPSPs) recorded in the primary auditory cortex during local,

cortical application of artificial cerebrospinal fluid (aCSF) (*n* = 8), APV (10 mM, *n* = 6), or Ro 25-6981 (10 mM, *n* = 8). TBS elicited long-term potentiation (LTP) of both peaks during aCSF application, an effect that was not reduced by local Ro 25-6981 application (ANOVA comparing aCSF and Ro 25-6981: first peak – effect of drug, $F_{1,14}$ = 0.3, *P* = 0.614; interaction, $F_{41,574}$ = 0.9, *P* = 0.624; second peak – effect of drug, $F_{1,14}$ = 0.001, *P* = 0.974; interaction, $F_{41,574}$ = 0.9, *P* = 0.679). In contrast, APV application completely blocked LTP induction (ANOVA comparing aCSF and APV: first peak – effect of drug, $F_{1,12}$ = 18.3, *P* = 0.001; drug × time interaction, $F_{41,492}$ = 4.0, *P* < 0.001; second peak – effect of drug, $F_{1,12}$ = 6.1, *P* = 0.029; drug × time interaction, $F_{41,492}$ = 1.7, *P* = 0.006; *significant simple effects, *P* < 0.05, for the comparison between aCSF and APV). Inserts depict typical fPSPs (averaged over 20 individual fPSPs) recorded during baseline (pre-TBS; grey) and the final hour of the experiment (black; A, aCSF; B, APV; C, Ro 25-6981). Scale bars are 0.2 mV and 20 ms.

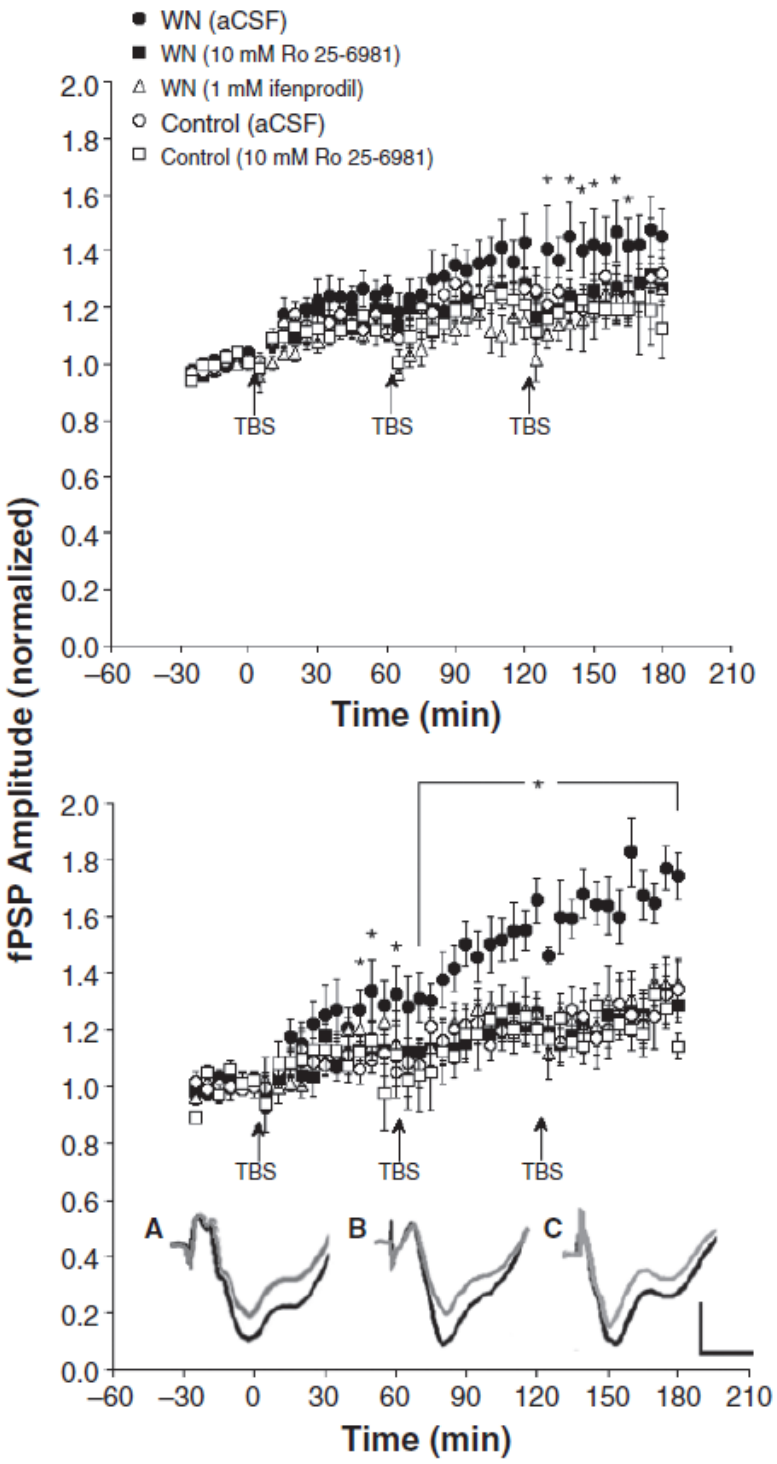

Figure 3.4 The effect of white noise (WN) rearing on long-term potentiation (LTP) elicited by repeated theta-burst stimulation (TBS) (at arrows) of the medial geniculate nucleus on

the amplitude of the first peak (top) and second peak (bottom) of field postsynaptic potentials (fPSPs) recorded in the primary auditory cortex. Rats reared in continuous white noise [n = 7, artificial cerebrospinal fluid (aCSF) applied] showed greater LTP than age-matched controls reared in unaltered acoustic environments (n = 8, aCSF applied: first peak – group × time interaction, $F_{41,533} = 1.6$, P = 0.011; second peak – interaction, $F_{41,533} = 4.5$, P < 0.001). The white noise-induced LTP enhancement was prevented by cortical application of Ro 25-6981 (10 mM, n = 10) or ifenprodil (1 mM, n = 6) [(ANOVA comparing white noise aCSF and white noise Ro 25-6981: first peak – effect of group by time interaction, $F_{41,615} = 1.5$, P = 0.019; second peak – interaction, $F_{41,615} = 6.1$, P < 0.001) (ANOVA comparing white noise aCSF and white noise ifenprodil: first peak – interaction, $F_{41,451} = 3.0$, P < 0.001; second peak – interaction, $F_{41,451} = 3.6$, P < 0.001)]. In rats reared in unaltered acoustic environments, application of Ro 25-6981 (10 mM, n = 3) did not suppress LTP relative to age-matched controls during aCSF application (first peak – drug × time interaction, $F_{41,369} = 0.6$, P = 0.984; second peak – interaction, $F_{41,369} = 0.5$, P = 0.991; *significant simple effects, P < 0.05, for the comparison between white noise (aCSF) and control (aCSF). Inserts depict typical fPSPs (averaged over 20 individual fPSPs) recorded during baseline (pre-TBS; grey) and the final hour of the experiment (black; A, white noise, aCSF; B, control, aCSF; C, white noise, Ro 25-6981). Scale bars are 0.25 mV and 10 ms.

and second fPSP peaks, respectively, and controls exhibited maximal LTP of 131 and 133% (Fig. 3.4). The fact that levels of LTP in white noise-reared animals were more similar to those in juveniles (not exposed to white noise; see above) than normally reared adults suggests that the absence of patterned sound results in developmental arrest, as indicated by the maintenance of more juvenile-like plasticity in A1.

The average amplitudes of baseline (pre-TBS) fPSPs of white noise-reared and age-matched control rats were also compared, with white noise-reared rats showing slightly larger amplitudes than controls for the first peak (0.58 and 0.50 mV, respectively) and slightly smaller amplitudes for the second peak (0.27 and 0.31 mV, respectively).

Local cortical application of Ro 25-6981 (10 mM, n = 10) resulted in complete reversal of the white noise-induced LTP enhancement for both fPSP peaks (Fig. 3.4). During Ro 25-6981 application, maximal levels of potentiation for the first and second fPSP peaks were 133 and 131%, respectively, values that were not significantly different from those obtained in age-matched control animals receiving local aCSF application (131 and 133%, see above). This pattern of results was replicated with local application of a second NR2B subunit antagonist, ifenprodil (1 mM, n = 6), which also blocked the white noise-induced LTP facilitation (Fig. 3.4; maximal potentiation of 128 and 136% for first and second peaks, respectively). In line with the results described above (see Fig. 3.3), application of Ro 25-6981 (10 mM, n = 3) in age-matched controls reared in unaltered sound environments did not suppress LTP of either peak of the fPSP following TBS (Fig. 3.4). Thus, blockade of the NR2B subunit of the NMDA receptor results in selective prevention of LTP enhancement following white noise exposure during early postnatal life.

3.4.4 Effect of white noise rearing on LTP: subsequent exposure to unaltered acoustic environments

We also examined whether white noise-induced LTP enhancement would still be apparent after removal of white noise-reared animals from the sound-attenuated chamber at PD 60 and placement of them into an unaltered acoustic environment for 40-50 days. When tested between PD 100 and PD 110, white noise-reared rats (n = 6) did not show enhanced LTP of either fPSP peak (127 and 130% for the first and second peaks, respectively) as compared with adult animals (> 100 days, n = 8, same group as above) reared exclusively in the unaltered auditory environment (Fig. 3.5; 132 and 131% for the first and second peaks, respectively). However, rats kept under continuous white noise until PD 100-110 (n = 7) and tested at that age did show LTP enhancement (158 and 148% for the first and second peaks, respectively) as compared with both age-matched controls and white noise-reared rats subsequently placed into unaltered acoustic environments for 40-50 days (Fig. 3.5).

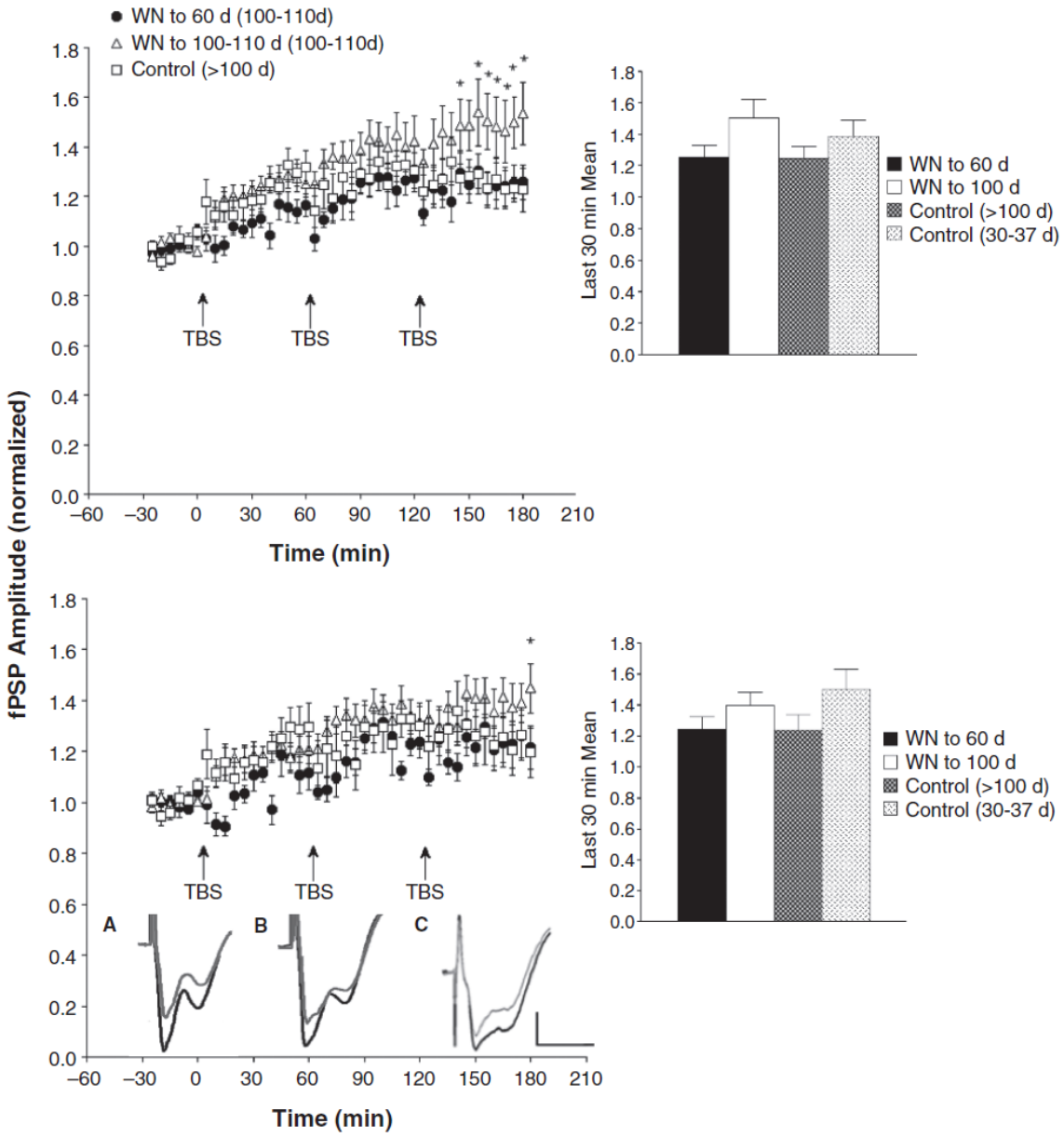

Figure 3.5 The effect of continuous white-noise (WN) rearing (to 100–110 days, *n* = 7) or initial white noise rearing (to 60 days) followed by subsequent exposure to an unaltered acoustic environment (*n* = 6) on long-term potentiation (LTP) of the amplitudes of the first peak (top) and second peak (bottom) of field postsynaptic potentials (fPSPs), assessed between 100 and 110 days. Theta-burst stimulation (TBS) (at arrows) of the medial geniculate nucleus elicited greater LTP in rats continuously maintained in white noise than in adult controls (> 100 days) never exposed to white noise (*n* = 8, same group as in Fig. 3.2)

or in rats placed in unaltered acoustic environments subsequent to white noise exposure; the latter two groups showed similar levels of LTP, indicative of a reversal of the initial effect of white noise exposure [(ANOVA comparing white noise 100–110 days with control, group × time interaction: first peak – $F_{41,574} = 2.3$, $P < 0.001$; second peak – $F_{41,574} = 1.5$, $P = 0.023$) (ANOVA comparing white noise 60 days and control, group × time interaction: first peak – $F_{41,492} = 0.98$, $P = 0.506$; second peak – $F_{41,492} = 1.0$, $P = 0.394$) (main effects of group also non-significant, $P > 0.05$; *significant simple effects, $P < 0.05$, for comparison of white noise 100–110 days with control)]. Inserts depict typical fPSPs (averaged over 20 individual fPSPs) recorded during baseline (pre-TBS; grey) and the final hour of the experiment (black; A, white noise 100–110 days; B, white noise 60 days; C, control). Scale bars are 0.2 mV and 20 ms. Bar graphs show fPSP amplitude means over the last 30 min of the experiment; young (30–37 days) control animals are the same as in Fig. 3.2. Note the similar levels of LTP between young rats and rats maintained under continuous white noise for 100 days.

3.5 Discussion

Both age and sensory experience can profoundly modulate synaptic plasticity. NMDA receptor activation is an important induction mechanism for many types of LTP, and it is thought that changes in NMDA receptor subunit composition influence the level of plasticity maintained throughout the course of development and into adulthood (Kudoh & Shibuki, 1994; Quinlan et al., 1999; Philpot et al., 2001; Bear, 2003; Speechley et al., 2007). The results of the present study suggest a critical role of the NR2B subunit of the NMDA receptor in the LTP enhancement seen following the masking of patterned sound input by means of continuous white noise exposure during early postnatal development.

Application of TBS to the MGN was effective in eliciting potentiation of fPSPs in A1 of adult animals reared in unaltered acoustic environments. This form of *in vivo* auditory cortex LTP was dependent on cortical NMDA receptor activation, as LTP was abolished by local application of APV to A1, consistent with previous work *in vivo* and *in vitro* (Kudoh & Shibuki, 1994; Speechley et al., 2007). In contrast, blockade of the NR2B subunit of the NMDA receptor with local application of the NR2B subunit antagonist Ro 25-6981 did not suppress LTP in normally reared animals. Thus, activation of this subunit is not necessary for LTP in A1 of adult rats, and local application of this drug to A1 does not appear to dampen general, cortical plasticity mechanisms. Our findings are in excellent agreement with prior *in vitro* studies showing that, in slices obtained from adult rats, NR2B subunit blockade did not suppress LTP in the hippocampus (Liu et al., 2004a), perirhinal cortex (Massey et al., 2004), or visual cortex (Huemmeke et al., 2004).

In line with previous work (Speechley et al., 2007), rats reared in continuous white noise from PD 5 to PD 50-60 showed greater LTP relative to age-matched controls reared in unaltered acoustic environments. Prior research has shown that degrading acoustic inputs during early

postnatal development by masking patterned sound with continuous white noise or unmodulated tone exposure prevents the emergence of an adult-like tonotopic organization and the appearance of frequency-selective neurons in A1 in rats (Chang & Merzenich, 2003; Chang et al., 2005; Zhou et al., 2008). Under these conditions, A1 retains a juvenile-like organization into adulthood, highlighting the importance of patterned acoustic information during critical periods of early development in the maturation of A1.

The greater levels of LTP noted here and in previous work (Speechley et al., 2007) are consistent with the notion that synapses in A1 of white noise-reared animals remain in a more immature, plastic state. Here, we observed significantly greater levels of LTP in juvenile rats than in adults, levels that were quite similar to those seen in older, white noise-reared animals. These data suggest that masking of patterned sound during early life slows the development of A1, resulting in the maintenance of more juvenile-like plasticity properties. Furthermore, these findings indicate that levels of LTP provide a sensitive index of age-dependent and sensory-dependent maturation of A1 in rats, in agreement with observations in other sensory systems [e.g. visual and somatosensory (Kato et al. (1991), Feldman et al. (1999), and Jiang et al. (2007)].

A number of important, neurochemical changes during the postnatal maturation of the neocortex, including A1, might underlie the decline in LTP seen with increasing age and appropriate sensory stimulation. Substantial changes in the relative expression of different NMDA receptor subunits occur in both A1 and the primary visual cortex during the first weeks of postnatal life. The level of NR2B subunit expression in A1 of rats is highest shortly after birth and then declines into adulthood, whereas NR2A subunits follow an opposite trend, with steadily increasing expression during postnatal life (Hsieh et al., 2002; Bi et al., 2006). Consequently, there is a major shift in the NR2A/NR2B subunit ratio in the rodent A1, with increases in the ratio being evident during postnatal development. A similar, developmental profile is seen in the

rodent visual cortex, where NR2B subunits dominate during early life, and this is followed by a substantial, visual experience-dependent increase in the NR2A/NR2B subunit ratio (Quinlan et al., 1999; Philpot et al., 2001; Chen & Bear, 2007). Relatively higher levels of NR2B subunit expression are thought to facilitate plasticity and LTP induction, owing to NMDAR currents of larger amplitude and longer duration, thus allowing greater total influx of calcium ions into the postsynaptic cell (Carmignoto & Vicini, 1992; Monyer et al., 1994; Flint et al., 1997; Kopp et al., 2007). Additionally, NR2B subunits have higher affinity than NR2A subunits for calcium/calmodulin-dependent protein kinase II, a signaling molecule that is important for synaptic plasticity (Barria & Malinow, 2005).

Given the fact that LTP in A1 depends on NMDA receptor activation (present results) (Kudoh & Shibuki, 1994; Speechley et al., 2007), it is possible that changes in the subunit composition of NMDA receptors mediate changes in plasticity during postnatal life. Alternatively, the maturation of inhibitory circuits also acts as an important regulator of critical periods and plasticity induction at cortical synapses (Hensch, 2004; Chang et al., 2005; Jiang et al., 2005). Hearing loss or exposure to white noise during early postnatal life disrupts the normal, developmental maturation of GABAergic release and postsynaptic receptor mechanisms in A1 (Chang et al., 2005; Kotak et al., 2005, 2008), suggesting that increases in the excitability of A1 neurons could also account for the enhanced LTP following white noise exposure.

Although it is likely that a combination of mechanisms are involved in co-regulation of cortical development and plasticity, we focused on the role of NMDA subunits, given that these have been shown to mediate sensory deprivation-induced enhancements of cortical plasticity in the rodent visual system (Quinlan et al., 1999; Philpot et al., 2001; Chen & Bear, 2007). We found that application of NR2B subunit antagonists (Ro 25-6981 or ifenprodil) blocked the LTP enhancement observed in white noise-reared animals, suggestive of an arrest of the normal,

developmental increase in the NR2A/NR2B subunit ratio in the absence of patterned sound stimulation. Whether this effect is due to the maintenance of higher NR2B subunit levels, attenuation of the increase in NR2A subunit levels, or a combination of these two, remains to be determined. It is noteworthy that other auditory deprivation techniques (ear plugging) do not appear to alter NR2B subunit expression at the age when we carried out the electrophysiological assessments of cortical plasticity in white noise-reared animals (Bi et al., 2006). At present, it is unclear whether this apparent discrepancy is due to the different deprivation techniques used, or whether suppression of NR2A subunits is responsible for lowering the NR2A/NR2B subunit ratio following sensory deprivation. Future experiments using subunit quantification techniques will provide a better understanding of the mechanisms mediating changes in NMDAR subunit expression following various forms of auditory deprivation.

The results of our CSD analyses are in excellent agreement with previous work using this approach *in vitro* and *in vivo* (Metherate & Cruikshank, 1999; Kaur et al., 2005). Kaur et al. (2005), using CSD analyses in urethane-anesthetized rats, showed an initial current sink at about 800 μm depth, followed by a delayed, more superficial sink between 400 and 600 μm, observations that are consistent with those made in our experiments. Importantly, this CSD profile relates to non-specific acoustic stimuli outside the preferred frequency range of a given, cortical recording site (Kaur et al., 2005). Arguably, electrical stimulation of the MGN, as was applied in the present study, provides a non-selective, broad excitation of large numbers of thalamocortical projections not related to the selective, characteristic frequency tuning of cortical cells near the recording electrode. Consequently, thalamocortical and intracortical mechanisms engaged by electrical MGN stimulation are probably similar to those mediating excitation of A1 neurons by acoustic signals outside their preferred frequency range (Kaur et al., 2005).

It is noteworthy that the laminar organization of thalamocortical projections to A1 is much more diffuse than that found in other sensory cortices (e.g. visual), with thalamic terminals being present in layers I, III, IV, V, and VI (e.g. Malmierca & Merchán, 2004; Wallace & Palmer, 2008). Thus, the depths of the two current sinks noted here and previously (Kaur et al., 2005) both correspond to terminal zones of thalamocortical axons in A1 (Malmierca & Merchán, 2004). However, the clear difference in latencies of the two sinks and the two corresponding fPSP peaks suggests that thalamic stimulation resulted in the initial activation of direct, thalamocortical synapses, followed by a more delayed excitation of intracortical synapses. Nevertheless, additional work using extracellular or intracellular recordings, or selective inhibition of intracortical synapses (Liu et al., 2007), is necessary to confirm the precise nature of synaptic activation that contributes to the various fPSP components observed under the present, experimental conditions.

We also investigated whether the white noise-induced LTP enhancement would still be evident following subsequent exposure to an unaltered acoustic environment. Animals reared in white noise (to PD 60) and then placed in regular environments until PD 100-110 showed levels of LTP comparable to those in adults that had never experienced white noise. Similarly, receptive field characteristics and tonotopic organization of A1 in white noise-reared rats can be normalized by subsequent exposure to regular housing conditions (Chang & Merzenich, 2003). In contrast, rats continuously reared in white noise to PD 100-110 still showed enhanced LTP as compared with both age-matched controls, and rats where white noise was followed by exposure to a regular environment. Consequently, the absence of patterned auditory input during early postnatal life appears to result in a developmental arrest, which can be overcome by appropriate sensory stimulation in later life. The fact that rats reared in white noise until 100-110 days still showed enhanced levels of LTP as compared with age-matched controls highlights the critical

role of sensory experience (in addition to age) in determining levels of cortical maturation and plasticity.

In summary, the findings of the present study highlight the importance of patterned sound exposure during early postnatal life, and implicate the relative upregulation of NR2B subunits in the maintenance of juvenile-like plasticity in white noise-reared rats. In addition to linking NMDA receptor characteristics to varying levels of plasticity during auditory cortex development, these findings may also suggest novel approaches for restoring heightened levels of plasticity in the mature cortex via the manipulation of NR2B subunits.

Chapter 4
Reinstatement of Juvenile-like Plasticity in the Auditory Cortex of Adult Rats

4.1 Abstract

Sensory cortices show a decline in synaptic plasticity (e.g., long-term potentiation, LTP) during postnatal maturation. We demonstrate a partial reversal of this decline in rat primary auditory cortex (A1) by pharmacological manipulations or modifications of the acoustic environment. In adult, anesthetized rats, field postsynaptic potentials (fPSPs) in A1 elicited by medial geniculate nucleus (MGN) stimulation consisted of two sequential peaks. The preferential inhibition of intracortical synapses with simultaneous application of a γ-Aminobutyric acid $(GABA)_A$ receptor agonist (muscimol) and $GABA_B$ receptor antagonist (SCH 50911) in A1 revealed that these two components largely reflect thalamocortical and intracortical synapses, respectively. Rats (postnatal day [PD]60-70) showed moderate LTP of fPSPs following theta-burst stimulation (TBS) of the MGN. Interestingly, repeated episodes (PD10-20 & 50-60) of patterned sound deprivation by continuous white noise exposure resulted in substantial LTP, an effect not seen with a single episode (PD10-20 or 50-60), or two episodes during adulthood (PD50-60 & 100-110). Thus, early sensory deprivation acts as a "prime", allowing subsequent deprivation to reinstate juvenile-like levels of LTP. Older (>PD200) rats that no longer exhibit LTP in A1 showed LTP of the first fPSP peak when TBS occurred during cortical zinc application. We conclude that juvenile-like plasticity in the mature A1 can be restored by pharmacological techniques or manipulations of the acoustic environment during specific periods of postnatal life.

4.2 Introduction

Sensory experience plays an important role in regulating levels of cortical plasticity throughout the lifespan of an animal. Developmentally regulated and sensory-dependent modifications of NMDA receptor (NMDAR) subunit composition can influence the ability of synapses to undergo plastic change (Bear 2003; Kopp et al. 2007). In primary visual (V1) and auditory cortices (A1), levels of NR2B subunits of the NMDAR are high at birth and remain high, whereas levels of NR2A subunits are initially low and increase with age and concomitant sensory experience (V1: Quinlan et al. 1999; Philpot et al. 2001; Chen and Bear 2007; A1: Hsieh et al. 2002; Bi et al. 2006). Given that excitatory postsynaptic currents of NR2B-containing NMDARs permit enhanced Ca^{2+} entry into postsynaptic cells, greater relative NR2B subunit levels are thought to facilitate plasticity (Monyer et al. 1994; Flint et al. 1997), which may act as one of several mechanisms underlying enhanced critical period plasticity (Bear 2003). Fittingly, induction thresholds for long-term potentiation (LTP) in various sensory cortices are significantly lower in young animals (Kato et al. 1991). For example, in A1, juvenile (30-35 day old) rats show the greatest levels of LTP, which declines into adulthood and can no longer be elicited in rats > 200 days of age (Hogsden and Dringenberg 2009a). Notably, the LTP enhancement in juvenile rats is greatly reduced with application of a NR2B antagonist (Ro 25-6981), highlighting the role of this subunit in the expression of greater levels of plasticity in young animals (Hogsden and Dringenberg 2009a).

Sensory deprivation during early postnatal life disrupts the typical, developmental alteration in NMDAR subunit composition. In rodents, visual deprivation by means of dark-rearing attenuates the developmental increase in NR2A subunits, resulting in the maintenance of a low NR2A:NR2B ratio, which is thought to extend the critical period for ocular dominance plasticity in V1 (Quinlan et al. 1999). Similarly, masking patterned sound during early postnatal

life via continuous white noise exposure arrests the development of highly organized, adult-like tonotopicity in the rodent A1 and leads to the persistence of heightened, juvenile-like LTP into adulthood (Chang and Merzenich 2003; Speechley et al. 2007; Hogsden and Dringenberg 2009b). Consequently, continuous white noise exposure can be considered a form of sensory deprivation, as it masks patterned acoustic inputs necessary for cortical development. Notably, white noise-induced LTP enhancements can be reversed with application of NR2B antagonists in A1 (Hogsden and Dringenberg 2009b), consistent with a role of sensory experience in regulating the levels of NMDAR subunits and plasticity in maturing sensory cortices. Together, these data are consistent with the notion that the ratio of NR2A:NR2B subunits is an important regulator of plasticity levels during postnatal life, with greater relative levels of NR2B promoting plasticity, while increasing levels of NR2A result in resistance to synaptic reorganization (Quinlan et al. 1999; Bear 2003).

Despite the fact that plasticity induction is hindered by the developmental processes described above, significant levels of plasticity can be expressed in fully matured sensory cortices (e.g., Weinberger 2004, 2007; Hofer et al. 2006a). Importantly, the susceptibility to synaptic modifications can be influenced by previous episodes of experience-dependent synaptic reorganization. Hofer et al. (2006b) demonstrated that a monocular deprivation regimen, found to be ineffective when applied only once to adult mice, resulted in an ocular dominance shift in the mature V1 when repeated episodes were administered over time. Remarkably, this effect was observed regardless of whether the first deprivation episode occurred during the critical period for ocular dominance plasticity or in adulthood (Hofer et al. 2006b). Similarly, realignment between spatial auditory and visual maps in the optic tectum of adult barn owls (induced by exposure to prism goggles that shifted the visual field) occurred more readily when owls had prior experience wearing the goggles as juveniles (Knudsen 1998). These findings are significant since they

suggest that plasticity events at an earlier time can prime the cortex for the expression of subsequent, synaptic rearrangements.

Given the findings reviewed above, we asked whether repeated episodes of patterned sound deprivation with white noise exposure can enhance and restore juvenile-like levels of LTP in A1. Our results show that a previous episode of white noise does, indeed, facilitate the effects of subsequent sensory deprivation to enhance LTP in adult rats, but only if the first episode occurs during the critical period for auditory development (de Villers-Sidani et al. 2007). In addition, we also examined the effect of intracortical zinc ($ZnCl_2$) application, known to act as an antagonist of NR2A subunits, on LTP in older (> 200 days old) rats. This range was chosen since rats at this age typically no longer show LTP in A1 and fall outside the temporal limits that appear to govern plasticity facilitation by prior auditory deprivation. Zinc application restored LTP in these rats, possibly by means of lowering the NR2A:NR2B ratio.

4.3 Materials and Methods

4.3.1 Animals

All subjects were obtained from Charles River (St. Constant, Quebec) and treated in accordance with the regulations on animal experimentation established by the Canadian Council on Animal Care. All experimental procedures were approved by the Queen's University Animal Care Committee.

Male Long-Evans rats were obtained (45-50 days old) and housed four per cage in a colony room under unaltered acoustic conditions until they reached the appropriate age for testing. The colony room was maintained on a 12-12 h reverse light cycle, lights on at 1900 h, with food and water available *ad libitum*.

For experimental groups that received white noise exposure during early postnatal life (from postnatal day [PD]10 to 20), untimed (~19 days) pregnant female Long-Evans rats were obtained. In a separate colony room, these pregnant rats were singly-housed in cages upon arrival (food and water available *ad libitum*). Cages were situated in a sound-attenuated chamber (114 x 61 x 66 cm, plywood lined with aluminum, fitted with a time controlled light, fan, and two equally spaced speaker boxes mounted on the ceiling of the box). Following birth, offspring were housed with their mothers until weaning (PD21), at which point the mothers and female offspring were removed from the cages, and males were separated into cages containing no more than four rats.

4.3.2 White Noise Exposure

Within the sound attenuated chamber, each of the two ceiling-mounted speaker boxes contained two speakers (one 8 inch woofer and one 3.25 inch tweeter, frequency ranges of 45 Hz-5 kHz and 2 kHz-35 kHz, respectively, American Legacy Series 2 Speakers, Legacy Audio, Brooklyn, NY). The speakers were connected to a custom-made white noise generator (Department of Psychology, Electronics Workshop, Queen's University). Previous spectral analyses of the sound signal recorded inside the chamber showed that white noise generated with this apparatus covers a frequency range up to ~35 kHz, with power gradually declining between 30 to 37.5 kHz (Speechley et al. 2007). Even though rats are sensitive to sounds at frequencies above 35 kHz, previous mapping studies have demonstrated that the large majority of neurons in A1 are optimally responsive to frequencies within the range generated by this apparatus (Kilgard and Merzenich 1999; Chang and Merzenich 2003). Sound attenuation across the chamber wall is ~27 dB sound pressure level (SPL) for a white noise signal of ~80 dB SPL generated inside the chamber (Hogsden and Dringenberg 2009b).

For rats reared on the premises, continuous white noise exposure began at PD5, which is approximately 5-6 days prior to the onset of low-threshold hearing in the rat (de Villers-Sidani et al. 2007). Over a five day period, white noise sound volume was increased incrementally from ~65 dB to ~80 dB SPL to minimize stress experienced by the mother. Subsequently, white noise was maintained at this level from PD10 to 20, at which point all rats were removed from the chamber and placed in a regular colony room under unaltered sound conditions. Some of these rats received a second white noise exposure from either PD50 to 60-70 or PD100 to 110-120. Electrophysiological procedures were then carried out in these groups between PD60-70 or PD110-120. In all cases where rats were exposed to white noise during adulthood (also see below), a three day acclimatization period was given (e.g., PD47 to 50) during which rats were housed in the sound attenuated chamber and white noise levels increased gradually from ~65 to ~80 dB SPL.

An additional group of rats was placed in the white noise chamber only during adulthood (PD50 to 60-70) and tested at PD60-70. Finally, one group of rats received repeated episodes of white noise exposure during adulthood (PD50 to 60 and PD100 to 110-120, tested at PD110-120). Between these two exposure periods, rats were returned to the unaltered sound environment.

4.3.3 Surgical Procedure

Rats were positioned in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA) under deep urethane anaesthesia (1.5 g/kg, i.p., administered as 3 × 0.5 g/kg doses every 15 min, with supplements provided as necessary). An incision was made to expose the skull and small holes were drilled over the medial geniculate nucleus (MGN; AP −5.5, L +4.0, V −5.4 to −6.4) and ipsilateral A1 (AP −4.5, L +8.0, V −3.2 to −5.4). Holes were also drilled above the contralateral frontal cortex and cerebellum for ground and reference electrode screws, respectively. All

stereotaxic measurements were based on the anatomical rat brain atlas of Paxinos and Watson (1998). Body temperature of the rats was maintained at 36-37°C throughout the experiment with the use of an electric heating pad and cotton wool blanket.

4.3.4 Electrophysiology

A recording electrode-microdialysis probe assembly was lowered into A1 to record field postsynaptic potentials (fPSPs) and permit local drug application using reverse microdialysis (see below). The electrode (125 μm diameter stainless steel wire insulated with Teflon) was positioned immediately adjacent to the dialysis probe (Mab. 2.14.4; 2 mm active polyether sulfone membrane, 35 kDA cut-off; S.P.E Limited, North York, Ontario, Canada), with the electrode tip placed in the center of the vertical extent of the active membrane. This assembly was lowered into A1 so that the recording electrode penetrated the middle cortical layers (layers IV-V, depth of about 400-600 μm). With this set-up, physiological recordings can be obtained directly at the site of maximal drug diffusion across the probe membrane into the surrounding neural tissue.

The dialysis probe was connected to a 2.5 mL Hamilton airtight microsyringe driven by a microdialysis pump (CMA/102, CMA Microdialysis, Solna, Sweden). The recording electrode was connected to an amplifier (Model 1800, A-M Systems Inc., Carlsborg, WA; half-amplitude filter settings at 0.3 Hz to 1 kHz) and A-D converter (PowerLab/4s system, running Scope software v. 4.0.2, AD Instruments, Toronto, ON, Canada), which digitized (at 10 kHz) and stored the recorded signal for offline analysis.

A concentric, bipolar stimulating electrode (SNE-100, Rhodes Medical Instruments, David Kopf, Tujunga, CA) was used to stimulate the MGN (0.2 ms pulse duration). The electrode was connected to a stimulus isolation unit (ML 180 Stimulus Isolator, AD Instruments) that provided a constant current output. Final ventral placements were adjusted for both the recording

and stimulating electrodes in order to achieve maximum fPSP amplitude in A1 in response to ipsilateral MGN stimulation.

4.3.5 Data Collection

Following final electrode placement, the brain was allowed to stabilize for 30 min before generating initial input-output curves by stimulating the MGN at successively increasing intensities (0.1 - 1.0 mA, in 0.1 mA increments). The stimulation intensity that produced approximately 50-60% of the maximal fPSP amplitude was chosen as the intensity used for the subsequent experiment.

For LTP experiments, baseline fPSPs were recorded every 30 s until 30 min (i.e., a total of 60 fPSPs) of stable recordings were attained. Theta burst stimulation (TBS) of the MGN was then applied as trains of 10 bursts (each burst consisting of 5 pulses at 100 Hz, bursts repeated at 5 Hz), with trains repeated every 10 s for a total of 4 trains. Following TBS, single pulse stimulation of the MGN continued every 30 sec for a 60 min recording period. The TBS protocol was then repeated twice more for a total of three induction episodes, each followed by 60 min of fPSP recordings. Multiple TBS episodes were used in order to characterize LTP saturation points. Previous experiments in our lab using this experimental preparation have shown that, in the absence of TBS delivery, fPSP amplitude remains stable throughout the course of the experiment (Speechley et al. 2007; Hogsden and Dringenberg 2009b).

4.3.6 Pharmacological Manipulations

Drugs were applied locally in A1 using reverse microdialysis, allowing drug molecules to cross the dialysis probe membrane to reach neural tissue by concentration driven diffusion. Using this technique, it is estimated that the drug concentration in tissue outside the probe membrane reaches ~10% of the concentration contained in the perfusion medium (e.g., Oldford and Castro-

Alamancos 2003). For all experiments, the probe was continuously perfused with artificial cerebral spinal fluid (aCSF; flow rate of 1.0 µl/min), consisting of (in mM): 124.0 NaCl, 4.4 KCl, 1.2 $MgSO_4$, 1.0 NaH_2PO_4, 2.5 $CaCl_2$, 26.0 $NaHCO_3$, and 10.0 glucose.

Previous work has shown that fPSPs in A1 elicited by stimulation of the MGN consist of two distinct, negative-going components with latencies to peak of ~7 and ~14 ms, thought to reflect deeper thalamo-recipient and more superficial intracortical synapses, respectively (Kaur et al. 2005; Hogsden and Dringenberg 2009b). In order to further characterize these fPSPs, we performed initial experiments employing a pharmacological method to isolate thalamocortical and intracortical synapses by application of a $GABA_A$ receptor agonist (muscimol) and $GABA_B$ receptor antagonist (SCH 50911). This cocktail results in strong inhibition of intracortical synapses (by muscimol) while concurrently protecting presynaptic, thalamic terminals in cortex due to the blockade of $GABA_B$ receptors located on these fibers (by SCH 50911; Liu et al. 2007). For these experiments, initial input-output curves were generated (0.1-1.0 mA in 0.1 mA increments, 10 fPSPs/intensity) in the presence of aCSF only. Subsequently, aCSF containing muscimol (2 mM) + SCH 50911 (3 mM; ratio of 1:1.5; see Liu et al. 2007) was applied for 30 min before another input-output curve was recorded in the presence of these drugs.

In order to assess the effects of intracortical zinc application on LTP, zinc chloride ($ZnCl_2$; 1 µM, 10 µM, or 1 mM) was dissolved in aCSF containing the heavy metal chelator tricine (10 mM) and applied locally to A1. Tricine is frequently used to control the concentration of free zinc ions (Zn^{2+}), as physiological solutions are known to contain contaminating concentrations of Zn^{2+} (Paoletti et al. 1997; Amar et al. 2001; Wilkins and Smart 2002). The amount of free Zn^{2+} in the brain using the above concentrations of zinc and buffered by 10 mM tricine is estimated to be in the range of 1 to 500 nM (see Chu et al. 2004). Drug application began immediately prior to the start of baseline recordings and continued for the entire duration

of the experiment. Control animals for this experiment received continuous aCSF + tricine (10 mM) application. All drugs were obtained from Sigma-Aldrich (Oakville, Ontario).

4.3.7 Histology

Following all experiments, rats were perfused through the heart with 0.9% saline, followed by 10% formalin. Brains were extracted and standard histological techniques used to examine the placements of stimulation electrodes and cortical electrode-dialysis probe assemblies. Data from experiments with inaccurate placements were discarded.

4.3.8 Data Analysis

The cortical fPSPs consisted of two negative-going peaks. Amplitudes of both peaks were computed offline, averaged over 10 min intervals, and normalized by dividing them by the average baseline fPSP amplitude recorded for each rat. All data are expressed as mean ± S.E.M. Statistical comparisons were made using analyses of variance (ANOVA) and, where statistically appropriate, simple effects tests using the CLR ANOVA software package (v.1.1, Clear Lake Research Inc., Houston, Texas).

4.4 Results

4.4.1 Histology

A typical placement of the recording electrode-probe assembly in A1 is shown in Fig. 4.1A. The placements of all stimulation electrodes in the MGN included in the experiments reported below are shown in Fig. 4.1B. The large majority of stimulation electrodes were located in the ventral division of the MGN, known to provide direct, monosynaptic innervation of the rat A1 (Malmierca and Merchán 2004).

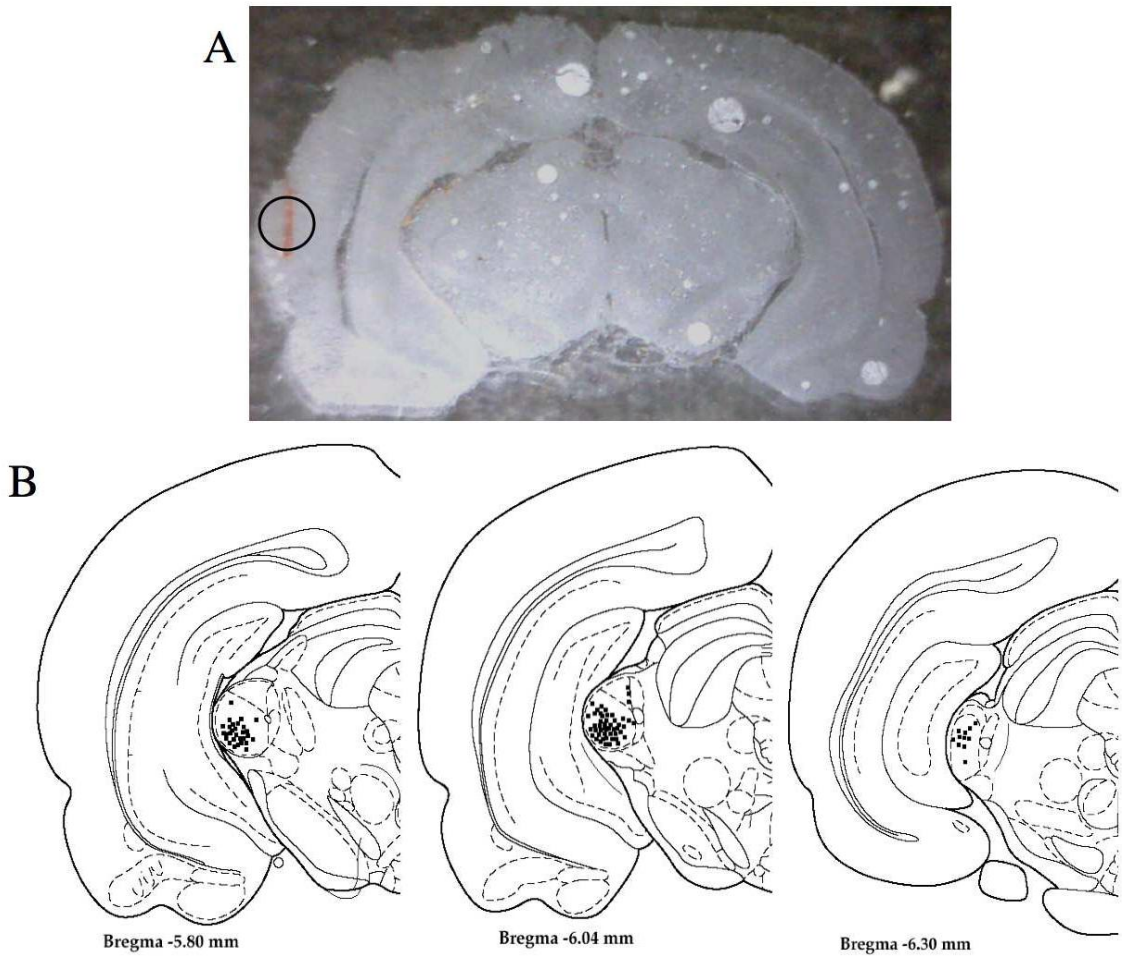

Figure 4.1 A typical placement of the recording electrode-dialysis probe assembly in the primary auditory cortex (A), and the location of all stimulation electrodes in the medial geniculate nucleus that were included in the Results (B). Note that the tip of the recording electrode is located about 1 mm above the tip of the dialysis probe. Numbers in B refer to the distance from bregma in mm. Drawings are adapted from Paxinos and Watson (1998).

4.4.2 Pharmacological Isolation of Thalamocortical Synapses

Stimulation of the MGN in adult rats (PD60-70, n = 6) elicited fPSPs recorded in the middle layers of A1 (depth of 400-600 μm) that consisted of two distinct peaks, with latencies to peak of about 7 and 14 ms (Fig. 4.2). Similar fPSP characteristics have been observed in previous work using the same experimental conditions (e.g., Hogsden and Dringenberg 2009a,b; also see Kaur et al. 2005 for a detailed analysis of fPSPs in A1). Input-output curves in the presence of aCSF application in A1 showed that the amplitude of both peaks increased with higher stimulation intensities (Fig. 4.2). For most rats, the stimulation intensity to elicit fPSPs with an amplitude 50-60% of maximum was in the range of 0.3-0.4 mA (Fig. 4.2).

Initial pharmacological experiments conducted to further characterize these two fPSP components revealed that simultaneous application of aCSF containing muscimol (2 mM) and SCH 50911 (3 mM) significantly reduced the amplitude of both peaks. However, this effect was much greater for the second peak, which was reduced to approximately 35%, while the amplitude of the first peak reached about 70% of aCSF levels (Fig 4.2). An ANOVA comparing fPSP amplitude in the presence of aCSF or aCSF with muscimol + SCH 50911 showed a significant effect of drug for both the first peak ($F_{(1,10)} = 11.03$, $p = 0.008$; effect of stimulation intensity, $F_{(9,90)} = 99.19$, $p < 0.0001$; interaction, $F_{(9,90)} = 4.19$ $p < 0.001$), and second peak ($F_{(1,10)} = 43.53$, $p = 0.0001$; effect of intensity, $F_{(9,90)} = 31.62$, $p < 0.0001$; interaction, $F_{(9,90)} = 6.43$, $p < 0.0001$). Further, post-hoc analyses confirmed a significantly greater suppression of the second compared to the first fPSP peak ($p < 0.05$, Newman-Keul's test). The presumptive preferential sensitivity of intracortical synapses to combined muscimol + SCH 50911 application (Liu et al. 2007) suggests that the second peak largely reflects intracortical synapses, with the first component resulting primarily from activation of direct, thalamocortical synapses from MGN to A1.

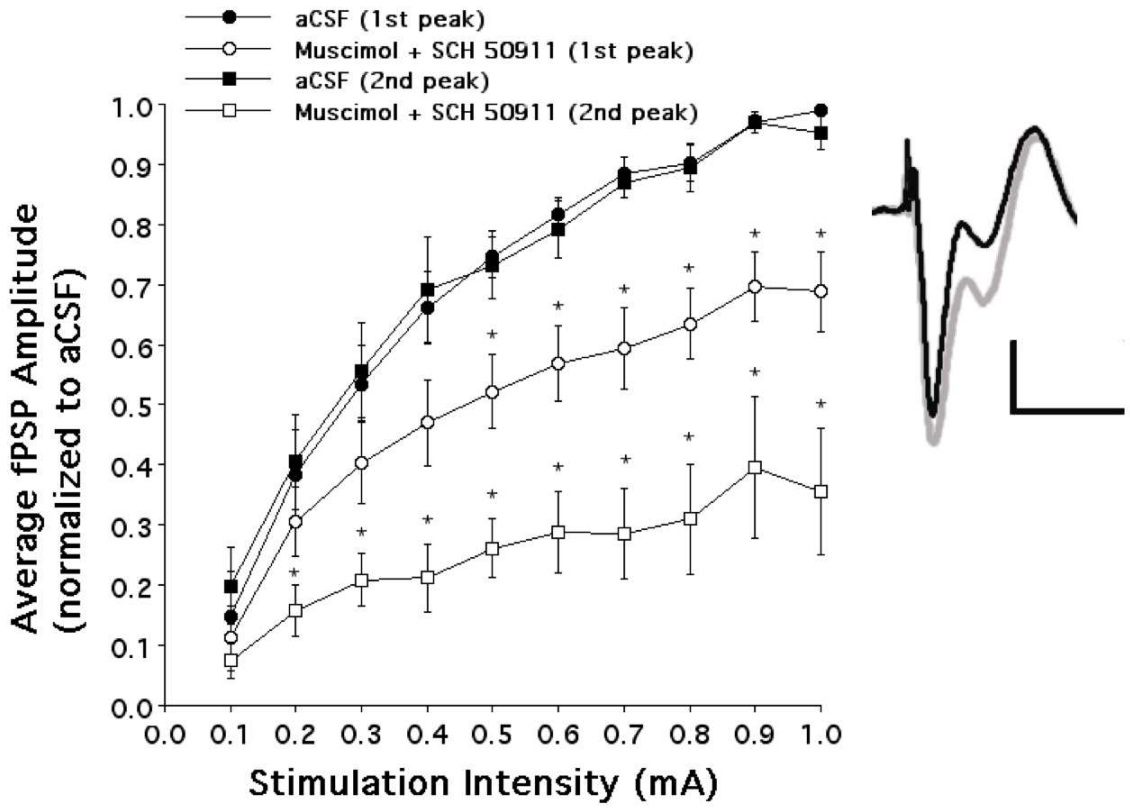

Figure 4.2 Amplitude of the first (circles) and second (squares) peak of field postsynaptic potentials (fPSPs) in A1 following single pulse stimulation of the medial geniculate nucleus (n = 6). For each animal, all amplitude values are normalized to the maximal fPSP amplitude of that animal during aCSF application. Simultaneous application of muscimol (2 mM) and SCH 50911 (3 mM) reduced the amplitude of both peaks, but this effect was much more pronounced for the second peak of the fPSP. The insert depicts typical fPSPs in the presence of aCSF (dark grey) or muscimol + SCH 50911 (black; scale bars are 0.25 mV and 20 ms). *significant simple effects tests, $p < 0.05$, for the comparison between muscimol + SCH 50911 vs. aCSF.

4.4.3 Effect of Repeated White Noise Exposure during Juvenile and Adult Life on LTP

Adult control rats (PD60-70, n = 8) never exposed to white noise showed moderate levels of synaptic potentiation when three episodes of TBS (spaced by 60 min intervals; stimulation intensity typically in the range of 0.3 to 0.4 mA) were delivered to these animals, with the first and second fPSP peaks reaching 123 and 118% of baseline, respectively (Fig. 4.3; values are averages over the final 10 min of the fPSP recordings). Rats tested at the same age that had experienced two episodes of continuous white noise exposure (from PD10 to 20 and PD50 to 60, n = 8) showed significantly greater LTP for both peaks as compared to control animals, with the first and second peak reaching 145% and 150% of baseline, respectively, during the final 10 min of recordings (Fig. 4.3; first peak: effect of group, $F_{(1,14)} = 5.83$, $p = 0.03$; group by time interaction, $F_{(20,280)} = 1.57$, $p = 0.058$; second peak: effect of group, $F_{(1,14)} = 10.79$, $p < 0.01$; group by time interaction, $F_{(20,280)} = 3.15$, $p < 0.001$).

Repeated exposure to white noise appeared to be critical for the observed LTP enhancement, as levels of LTP in rats that received white noise exposure only once, either between PD10 to 20 (n = 7) or PD50 to 60 (n = 7), failed to show this effect and did not differ from age-matched control rats (see above) never exposed to white noise (Fig. 4.3). Final levels of LTP for the first and second peak were 130% and 124%, respectively, for rats exposed from PD10 to 20, and 121% and 120% of baseline for rats exposed from PD50 to 60 (Fig. 4.3; comparison of controls vs. PD10 to 20, first peak: group by time interaction, $F_{(20,260)} = 0.86$, $p = 0.63$; second peak: interaction, $F_{(20,260)} = 1.22$, $p = 0.24$; comparison of controls vs. PD50 to 60, first peak: interaction, $F_{(20,260)} = 1.24$, $p = 0.22$; second peak: interaction, $F_{(20,260)} = 0.98$, $p = 0.48$).

Next, we asked whether the effect of repeated episodes of white noise to enhance LTP occurs with longer time intervals between the initial, juvenile exposure and a second exposure in

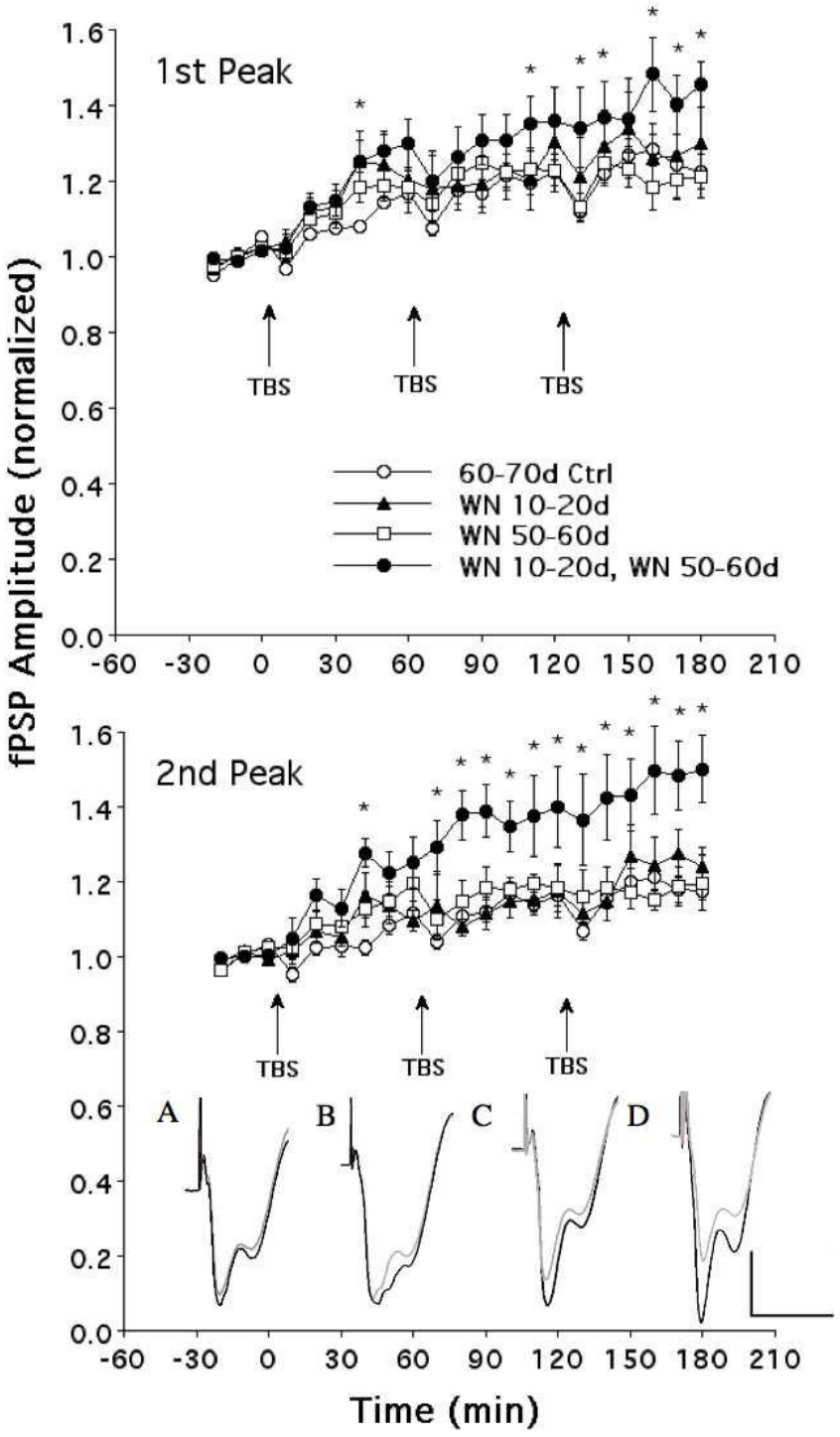

Figure 4.3 The effect of single and repeated episodes of white noise (WN) exposure on long-term potentiation (LTP) of field postsynaptic potentials (fPSPs) recorded in A1 induced by theta burst stimulation (TBS; at arrows) of the medial geniculate nucleus of adult rats 60-70

days (d) of age. Control rats (Ctrl; n = 8) never exposed to WN showed moderate levels of LTP of the first (top) and second (bottom) fPSP peaks. Rats that experienced two episodes of WN (from PD10 to 20 and 50 to 60; n = 8) showed significantly greater LTP for fPSP peaks. Multiple episodes of WN exposure appeared to be necessary for this LTP enhancement, as levels of potentiation in rats experiencing only one period of WN exposure during either early postnatal life (PD10 to 20; n = 7) or adulthood (PD50 to 60; n = 7) did not significantly differ from controls. Inserts depict typical fPSPs before (grey) and after (black) the final episode of TBS in the following conditions: A, Ctrl, 60-70d; B, WN 10-20d; C, WN 50-60d; D, WN 10-20d and 50-60d (fPSPs shown are averages of 20 individual fPSPs recorded during the last 10 min of baseline and of the experiment, respectively; scale bars are 0.25 mV and 20 ms). *significant simple effects tests, $p < 0.05$, for the comparison between WN 10-20d + 50-60d vs. Ctrl.

adulthood. Rats (n = 6) were exposed to white noise between PD10 to 20 and PD100 to 110 and compared to age-matched controls (n = 7) never exposed to white noise. Interestingly, levels of LTP in white noise-exposed rats were comparable to controls (Fig. 4.4; controls, first and second peak: 123 and 116%; white-noise, first and second peak: 124 and 119%), suggesting that the effects of an earlier exposure are lost with longer time intervals (80 days) to a second white noise episode (first peak: group by time interaction, $F_{(20,220)} = 0.60$, $p = 0.91$; second peak: interaction, $F_{(20,220)} = 0.39$, $p = 0.99$).

Finally, we addressed the question of whether the initial exposure to white noise has to occur during the critical period for postnatal A1 development, or whether two exposure periods during adulthood produce a similar effect to enhance LTP. Rats (n = 10) were exposed to white noise from PD60 to 70 and again from PD100 to 110. In these animals, levels of LTP did not significantly differ from age-matched controls never exposed to white noise (same group as above, n = 7), with white noise-exposed rats showing first and second peak amplitudes of 120% and 119% of baseline, respectively (Fig. 4.5; first peak: group by time interaction, $F_{(20,300)} = 0.26$, $p = 0.99$; second peak: interaction, $F_{(20,300)} = 0.53$, $p = 0.95$). Consequently, it appears that the effect of an initial period of patterned sound deprivation to "prime" the cortex for subsequent white noise exposure is restricted to an early period of postnatal A1 development.

4.4.4 Effect of Intracortical Zinc Application on LTP

In a further set of experiments, we examined whether intracortical application of zinc can modulate LTP at an age when rats no longer show significant levels of LTP in A1 (Hogsden and Dringenberg 2009a) and are well beyond the temporal limits that restrict LTP priming by previous white noise exposure (see above). Consistent with this previous work, > 200 day old rats (n = 6; aCSF + 10 mM tricine) failed to show LTP in response to three episodes of TBS (1 hour

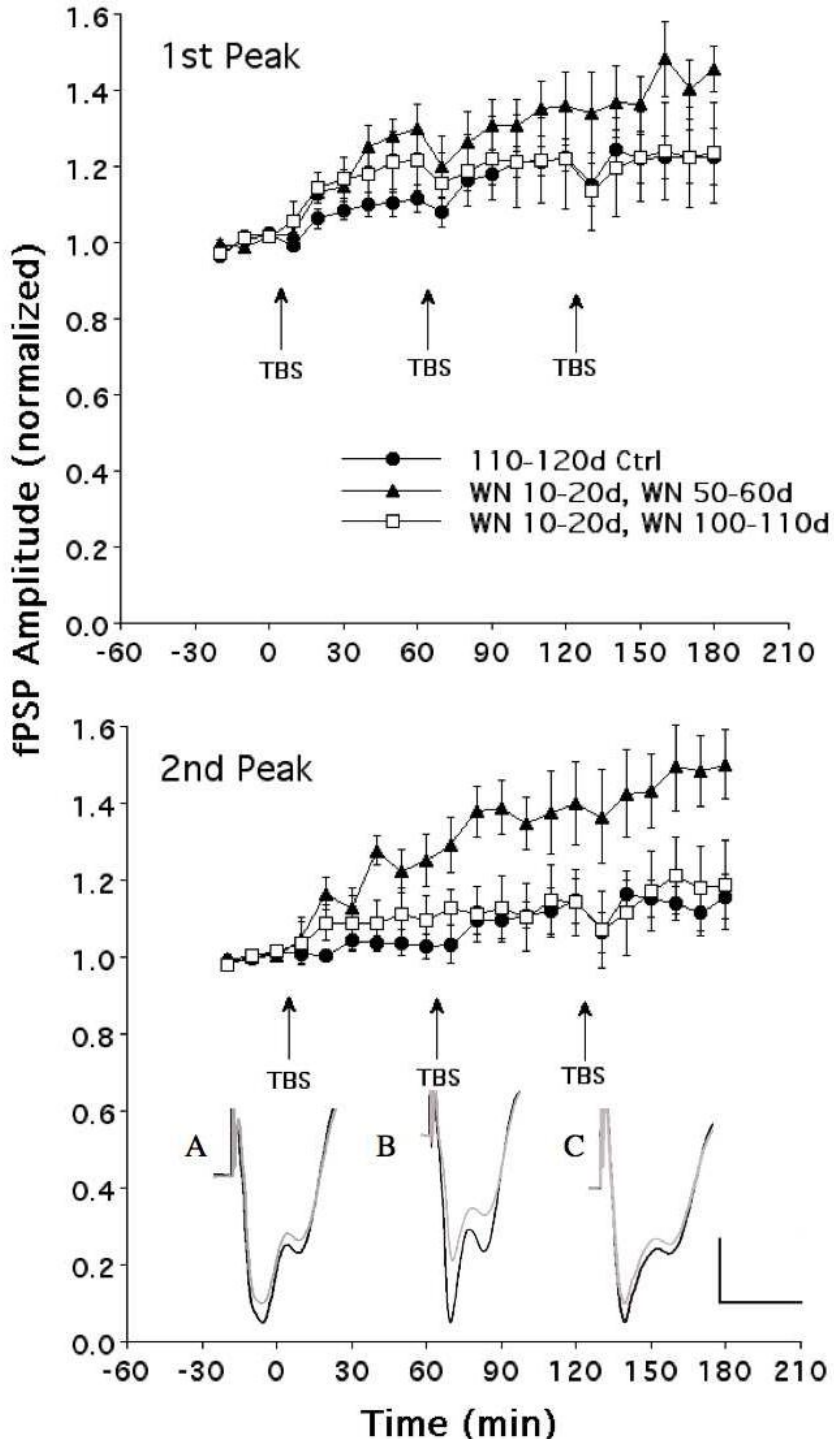

Figure 4.4 The effect of a longer interval between juvenile and adult white noise (WN) exposure on long-term potentiation (LTP) elicited by theta burst stimulation (TBS; at arrows) of the medial geniculate nucleus on amplitude of the first (top) and second (bottom)

peak of field postsynaptic potentials (fPSPs) recorded in A1 in rats aged 110-120 days (d). Rats that had an 80 day period between the two WN exposure episodes (WN from 10-20d and 100-110d; n = 6) did not differ in levels of LTP of either fPSP peak relative to age-matched controls (Ctrl; n = 7) reared exclusively in an unaltered acoustic environment. For comparison purpose, the WN 10-20d, WN 50-60d group (n = 8; same as Fig. 4.2) is shown to demonstrate the LTP enhancement seen with shorter time intervals between the two WN episodes. Inserts depict typical fPSPs before (grey) and after (black) the final episode of TBS in the following conditions: A, Ctrl, 110-120d; B, WN 10-20d, WN 50-60d; C, WN 10-20d, WN 100-110d (fPSPs shown are averages of 20 individual fPSPs recorded during the last 10 min of baseline and of the experiment, respectively; scale bars are 0.25 mV and 20 ms).

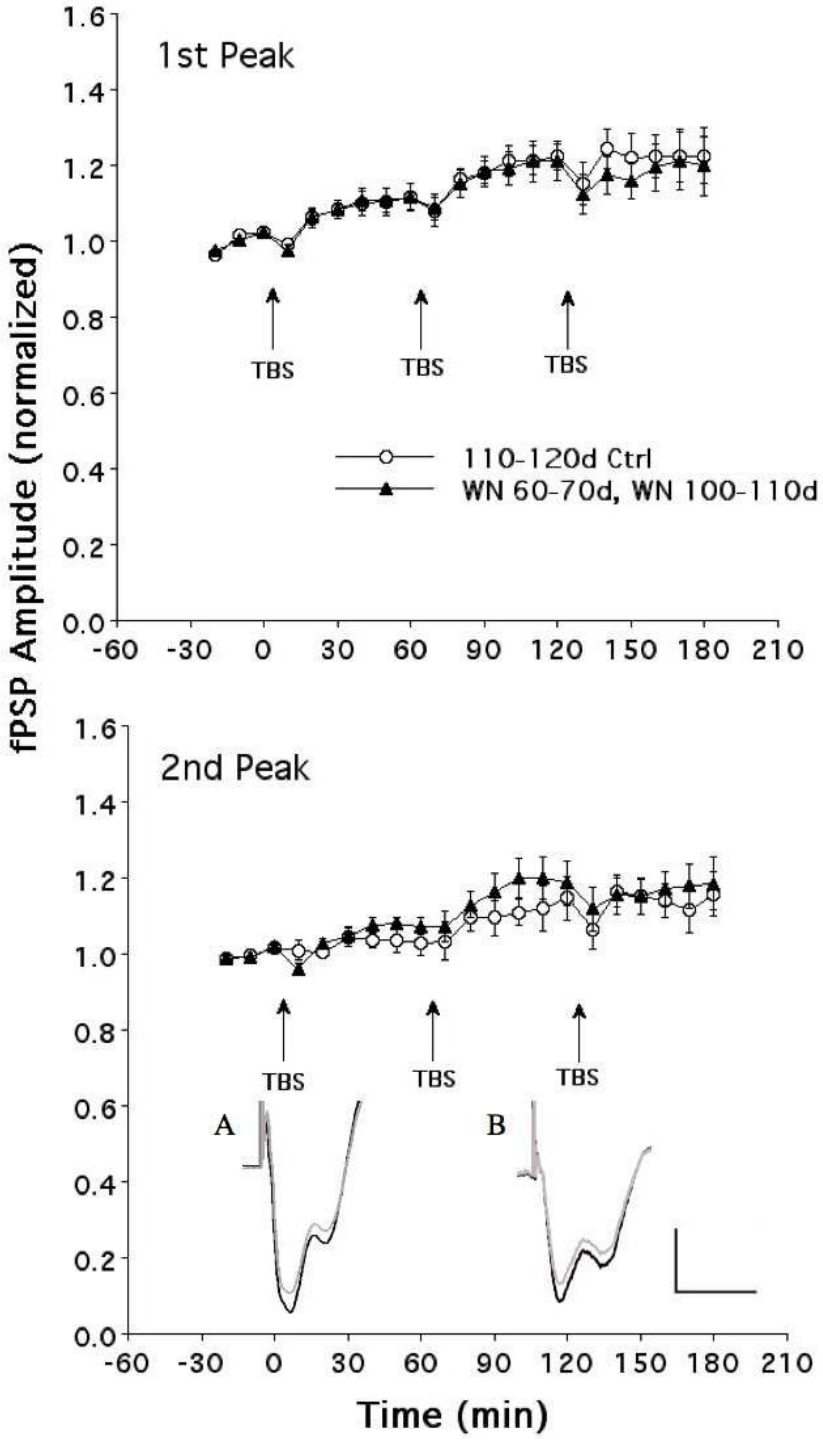

Figure 4.5 The effect of repeated episodes of white noise (WN) exposure during adulthood on long-term potentiation (LTP) elicited by theta burst stimulation (TBS; at arrows) of the medial geniculate nucleus on amplitude of the first (top) and second (bottom) peak of field

postsynaptic potentials (fPSPs) recorded in A1 in rats 110-120 days (d) of age. Rats that experienced two episodes of WN (from 60-70d and 100-110d; n = 10) did not differ in levels of LTP of either peak relative to age-matched controls (Ctrl; n = 7) that were reared exclusively in an unaltered acoustic environment. Inserts depict typical fPSPs before (grey) and after (black) the final episode of TBS in the following conditions: A, Ctrl, 110-120d; B, WN 60-70d, WN 100-110d (fPSPs shown are averages of 20 individual fPSPs recorded during the last 10 min of baseline and of the experiment, respectively; scale bars are 0.25 mV and 20 ms).

apart; Fig. 4.6). Interestingly, during local application of zinc, TBS resulted in a significant increase in fPSP amplitude of the first peak, which reached 123% of baseline for a concentration of 1 mM zinc (dissolved in aCSF + 10 mM tricine; n = 7). However, a facilitation of LTP by zinc was not apparent for the second fPSP peak (104%; Fig. 4.6; first peak: effect of drug, $F_{(1,11)}$ = 5.77, p = 0.035; drug by time interaction, $F_{(20,220)}$ = 1.36, p = 0.15; second peak: effect of drug, $F_{(1,11)}$ = 0.259, p = 0.62; interaction, $F_{(20,220)}$ = 0.52, p = 0.96). Amplitude of the first, but not the second peak, also appeared to be increased when lower concentrations of zinc (1 µM, n = 5; 10 µM, n = 6) were applied, but these groups were not significantly different from controls (Fig. 4.6 insets; first peak: 1 µM: effect of drug, $F_{(1,9)}$ = 1.24, p = 0.29; drug by time interaction, $F_{(20,180)}$ = 0.58, p = 0.92; 10 µM: effect of drug, $F_{(1,10)}$ = 3.2, p = 0.10; interaction, $F_{(20,200)}$ = 1.47, p = 0.09; second peak: 1 µM: effect of drug, $F_{(1,9)}$ = 0.002, p = 0.96; interaction, $F_{(20,180)}$ = 0.56, p = 0.94; 10 µM: effect of drug, $F_{(1,10)}$ = 0.47, p = 0.51; interaction, $F_{(20,200)}$ = 0.62, p = 0.89).

An additional group of animals was administered 1 mM zinc (n = 5; dissolved in aCSF + 10 mM tricine) in the absence of TBS to verify that the LTP enhancements noted above were not due to non-specific effects associated with zinc application. In these rats, fPSP amplitudes of the first and second peak did not differ from controls, reaching 97% and 93% of baseline, respectively (Fig. 4.6). Thus, it appears that drug application alone is not responsible for the LTP enhancements described above (first peak: effect of drug, $F_{(1,9)}$ = 0.52, p = 0.83; drug by time interaction, $F_{(20,180)}$ = 1.02, p = 0.44; second peak: effect of drug, $F_{(1,9)}$ = 0.67, p = 0.42; interaction, $F_{(20,180)}$ = 0.79, p = 0.73).

We also examined whether zinc application resulted in changes in baseline (i.e., non-potentiated) fPSP amplitude prior to TBS delivery. For animals receiving aCSF + 10 mM tricine, average amplitudes during the 30 min period prior to TBS delivery were 0.79 and 0.37 mV for the first and second fPSP component, respectively. Amplitude values during application of the

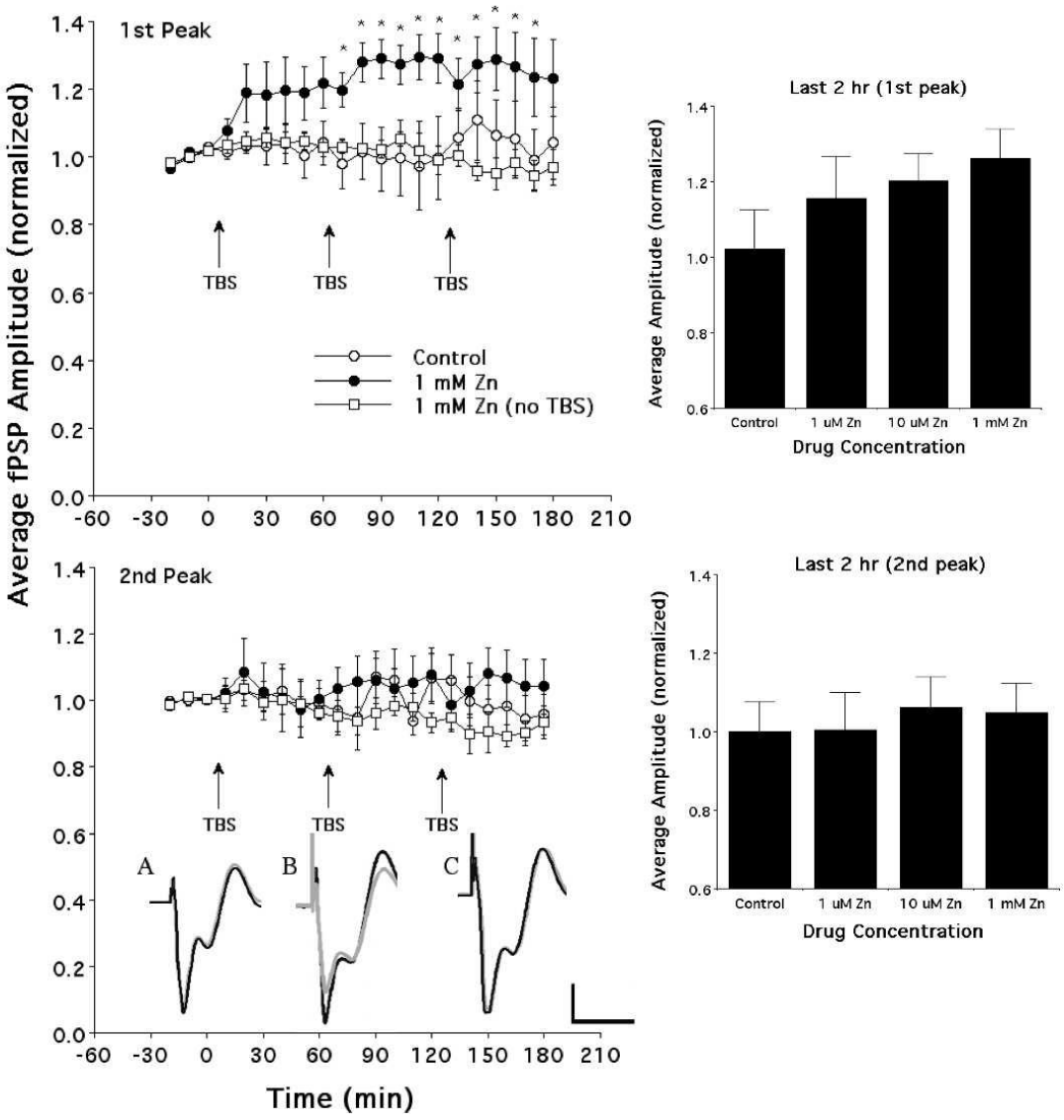

Figure 4.6 The effect of local, cortical zinc (Zn) application on long-term potentiation (LTP) elicited by theta burst stimulation (TBS; at arrows) of the medial geniculate nucleus on amplitude of the first (top) and second (bottom) peak of field postsynaptic potentials (fPSPs) recorded in A1 of rats > 200 days old. Control rats (n = 6, aCSF without Zn applied throughout the experiment) failed to show LTP of either fPSP peak. Application of aCSF containing Zn resulted in robust LTP of the first, but not the second peak, an effect that was

most pronounced for a Zn concentration of 1 mM (n = 7; 1 µM, n = 5; 10 µM, n = 6). Application of Zn (1 mM; n = 5) in the absence of TBS did not result in significant fPSP potentiation over the course of the experiment. Inserts depict typical fPSPs before (dark grey) and after (black) the final episode of TBS in the following conditions: A, Control; B, 1 mM Zn; C, 1 mM Zn without TBS (fPSPs shown are averages of 20 individual fPSPs recorded during the last 10 min of baseline and of the experiment, respectively; scale bars are 0.25 mV and 20 ms). Bar graphs show average fPSP amplitude of the first (top) and second (bottom) peaks during the final 2 hr of recording for all experimental groups.

highest zinc concentration (1 mM) were 0.67 and 0.48 mV for the two components, indicative of a minor, component-specific modulation of fPSPs during cortical zinc application.

4.5 Discussion

It is commonly held that the capacity of neural circuits to express plasticity declines with age. In the present study, we found that pharmacological (zinc application) or sensory deprivation techniques (white noise exposure) effectively enhance plasticity (LTP) in the A1 of adult rats. These findings highlight the potential of the mature cortex to express heightened levels of plasticity following pharmacological interventions or simple, non-invasive manipulations of the sensory environment experienced by the animals.

Application of the $GABA_A$ receptor agonist muscimol is widely used to inhibit neural activity, thereby silencing targeted regions of the nervous system (Martin and Ghez 1999; Majchrzak and Di Scala 2000). However, muscimol also activates presynaptic $GABA_B$ receptors, resulting in a reduction of presynaptic transmission (Yamauchi et al. 2000). Liu et al. (2007) devised a pharmacological technique of simultaneous application of muscimol and the $GABA_B$ receptor antagonist SCH 50911, which acts to silence intracortical transmission while largely preserving synaptic inputs from thalamic relay nuclei. Using this method to preferentially inhibit intracortical synapses, we found that the first peak of the fPSP recorded in A1 was moderately suppressed (by ~30%) after combined, intracortical muscimol + SCH 50911 application, suggesting that it largely reflects cortical excitation directly driven by thalamic terminals (see Liu et al. 2007). In contrast, the second peak was strongly reduced (by ~65%), indicating that this fPSP component is due primarily to activity at local, intracortical synapses. These findings confirm previous current source density experiments, reporting an initial current sink in deeper (~700-800 μm), thalamo-recipient layers, followed by a delayed, more superficial (~400-600 μm)

current sink, reflecting activation of intracortical synapses (Kaur et al. 2005; Hogsden and Dringenberg 2009b).

Previous work has shown that continuous white noise exposure during early postnatal life arrests the development of tonotopicity and results in the maintenance of heightened, juvenile-like levels of LTP in the rodent A1 (Chang and Merzenich 2003; Speechley et al. 2007; Hogsden and Dringenberg 2009b). Importantly, exposure to white noise commencing in adulthood does not result in LTP enhancements, indicating that appropriate timing of patterned sound deprivation during the critical period of postnatal development of A1 is required to produce this effect (Speechley et al. 2007). However, here, we show that white noise exposure during adulthood (from PD50 to 60) can result in the reinstatement of heightened levels of LTP in A1 when it is preceded by a period of white noise exposure in early life (PD10 to 20). This plasticity enhancement following repeated sensory deprivation is not due to a simple, additive effect of multiple exposure periods, since repeated episodes of white noise, both experienced during adulthood (from PD50 to 60 and PD100 to 110), did not result in a notable facilitation of LTP. It is important to note that rats reared under continuous white noise from PD5 to 100-110 show elevated levels of LTP in A1 (Hogsden and Dringenberg 2009b). This observation indicates that the failure of repeated white noise episodes in adulthood to facilitate LTP is unlikely to be due to an inability of A1 synapses at this age to express heightened levels of plasticity. Rather, it appears that for mechanisms mediating this "priming effect" to be engaged, the first white noise exposure must occur during the highly plastic, critical period of postnatal A1 development (de Villers-Sidani et al. 2007).

The data summarized above showing enhancements in plasticity with juvenile and adult patterned sound deprivation are consistent with prior work in the visual system, demonstrating ocular dominance plasticity in adult mice with repeated episodes of monocular deprivation, while

a single episode was ineffective (Hofer et al. 2006b). Remarkably, Hofer et al. (2006b) observed this effect regardless of whether the first deprivation episode occurred during early postnatal life or in adulthood. As mentioned, we did not observe plasticity enhancements with two episodes of white noise exposure when both episodes occurred in adulthood. Thus, it is possible that the mature A1 is more resistant to enhancements in plasticity induced by repeated sensory deprivation relative to V1, at least under the parameters used in the present study. However, it should be noted that monocular deprivation and the masking of patterned acoustic inputs by white noise exposure likely recruit different mechanisms, which may contribute to these differences. Future work could examine whether shorter periods between white noise episodes during adulthood are effective to engage the structural or biochemical mechanisms mediating these "priming effects" on cortical plasticity. Similarly, whether the same mechanisms mediating plasticity "priming" in V1 (e.g., persistent increases in cortical spine density following the first deprivation period; Hofer et al. 2009) are involved in the LTP enhancements in A1 seen here with juvenile + adult sensory deprivation remains to be determined.

Our experiments revealed that neither a single white noise exposure from PD10 to 20 or PD50 to 60, nor repeated exposure during adulthood (see above) is effective in enhancing plasticity of the adult, thalamocortical auditory system. The importance of timing of an initial experience with an altered sensory environment to enhance adult plasticity is in agreement with work in avian species. Previous work showed that the adjustment of auditory and visual maps in the optic tectum of adult owls induced by prism goggles occurs more readily with prior experience of goggles during juvenile life, an effect likely mediated by the reactivation of anatomical traces established during the initial exposure to the altered sensory environment (Knudsen 1998; Linkenhoker et al. 2005). However, our results suggest that the reactivation of such traces occurs only within specific temporal windows, since LTP enhancements were only

seen with a 30-day, but not an 80-day delay between juvenile and adult white noise exposure. Thus, at least for A1, there appear to be limits with regard to the duration of such "priming" effects, even when altered sensory environments are experienced during the critical period for sensory cortex development.

Given the apparent, temporal restrictions associated with repeated episodes of white noise exposure, pharmacological manipulations may serve as an alternative means to enhance plasticity in the mature auditory cortex. Consequently, we examined the influence of local, cortical zinc application on LTP in rats > 200 days of age, which normally do not show any significant levels of LTP in A1 following TBS of the MGN (Hogsden and Dringenberg 2009a). Remarkably, during zinc application, reliable LTP could be elicited in these rats, even though this effect was apparent only for the first peak of the fPSP. There findings are in agreement with previous work showing that low (micromolar) concentrations of zinc can facilitate NMDAR-dependent LTP in area CA1 of hippocampal slices (Takeda et al. 2009, 2010; but also see Takeda et al. 2008).

One of the well-characterized pharmacological properties of zinc is the high-affinity antagonism of the NR2A subunit of the NMDAR (Paoletti et al. 1997; Erreger and Traynelis 2008). Given the hypothesis that the NR2A:NR2B ratio acts as a potent regulator of cortical synaptic plasticity (Quinlan et al. 1999; Bear 2003), and the fact that the slower current kinetics of NR2B-containing NMDARs permit enhanced Ca^{2+} entry to facilitate plasticity (Monyer et al. 1994; Flint et al. 1997), it is possible that a ratio change by zinc (i.e., resulting in a greater contribution of NR2B subunits to overall current duration) mediates the effects on LTP seen here and elsewhere (Takeda et al. 2009, 2010). Recent data demonstrating that the cytoplasmic tails of NR2A and NR2B subunits carry inhibitory and excitatory signals for the induction of LTP (Foster et al. 2010) are consistent with this hypothesis. However, zinc can also influence signal transduction pathways important for synaptic plasticity through its action on several signaling

molecules, including protein kinase C (Baba et al. 1991), cyclic adenosine monophosphate (Klein et al. 2002), and calcium/calmodulin-dependent protein kinase II (Lengyel et al. 2000). Thus, further experimentation is required to determine the exact mechanisms underlying the zinc-induced LTP enhancements noted here. Notably, the current lack of highly selective NR2A antagonists (Neyton and Paoletti 2006; de Marchena et al. 2008) hinders corroboration of the role of NR2A subunit blockade in this effect using other NR2A antagonists.

We noted that the effect of zinc to enhance LTP was selective for the first of the two fPSP peaks, which appears to reflect synapses in deeper (thalamo-recipient) cortical layers (also see Kaur et al. 2005; Hogsden and Dringenberg 2009b). A possible explanation for this selectivity might be the well-characterized layer differences in NMDAR subunit distribution in A1. It appears that the ratio of NR2A:NR2B subunits favours NR2B in more superficial layers, while it is relatively equal in the deeper layers of A1 (Hsieh et al. 2002). It is tempting to speculate that these differences make deeper layers more sensitive to changes in the NR2A:NR2B ratio induced by NR2A antagonism. Alternatively, differences in the levels of endogenous zinc in different cortical layers of the visual and somatosensory cortices have also been reported, with a relative absence of zinc in layers IV and VI in adulthood (Dyck et al. 1993; Land and Akhtar 1999; Brown et al. 2003). Thus, the selective enhancement of the first peak observed here may reflect a greater sensitivity of deeper, thalamo-recipient layers to zinc application.

Together, the findings of the present study show that sensory manipulations or pharmacological tools provide effective means to promote plasticity in the fully matured A1 of adult rats. Repeated deprivation of patterned sound during early life and adulthood, or application of zinc, restores LTP to levels normally seen only in younger animals. Some of the findings reported here might guide the development of novel, therapeutic applications aimed at restoring plasticity in humans suffering from auditory deficits in adulthood or advanced age.

Chapter 5
General Discussion and Conclusions

5.1 A Sliding Modification Threshold for Plasticity

Several of the findings presented in this thesis can be understood within the framework of existing theories on the mechanisms and properties of synaptic plasticity. In what is now known as the Bienenstock, Cooper, and Munro (BCM) theory, Bienenstock et al. (1982) proposed that the history of neuronal activity modifies the threshold for synaptic plasticity (long-term potentiation (LTP) or long-term depression (LTD)). This theory built upon prior models of synaptic plasticity, which had suggested that active synapses are strengthened when postsynaptic activity is above, and depressed when it falls below the modification threshold (θ_m; Cooper et al., 1979). The BCM theory extended these ideas by proposing that the value of θ_m varies according to the prior synaptic activity, in that a period of decreased activity leads to a reduction in θ_m, whereas increased activity acts to increase θ_m (Bienenstock et al., 1982). This theory has important implications for experience-dependent plasticity. Conceptually, synapses would be more likely to favour the formation and strengthening of synaptic connectivity (LTP) during early postnatal life, when prior synaptic activity would be relatively minimal thus promoting a lower θ_m. A lower threshold would be advantageous during early life, permitting the organization of neuronal circuitry based on experience with the external environment. During the subsequent critical period, heightened synaptic activation with exposure to sensory stimuli would lead to a shift in θ_m, resulting in higher thresholds that would aid in the refinement and stabilization of newly formed connectivity. Thus, exposure to sensory experience likely plays an important role in the modulation of thresholds for synaptic plasticity during development.

5.2 Metaplasticity: The Plasticity of Synaptic Plasticity

Indeed, the bidirectional influence of sensory experience in determining the value of θ_m has been shown experimentally. Kirkwood et al. (1996) reported stable LTP in primary visual cortex (V1) slices of light-deprived rats using a low-frequency protocol, which was insufficient to induce LTP in controls. Additionally, LTD-inducing protocols were found to be less effective in light-deprived rats, an effect that could be reversed by two days of light exposure. These findings are indicative of a sliding-θ_m, which is lowered during periods of cortical inactivity (by visual deprivation) to favour LTP over LTD, but shifts with later increases in cortical activity due to sensory (visual) stimulation (Kirkwood et al., 1996). The modifiability of plasticity mechanisms based on prior sensory experience highlights the fact that synaptic plasticity itself is plastic. Abraham and Bear (1996) refer to this type of plasticity as "metaplasticity," wherein prior synaptic activity or inactivity results in a lasting change in the ability of synapses to later induce plasticity.

Notably, experience-dependent alterations to NMDAR subunit configuration have been proposed as a possible molecular mechanism underlying a shifting modification threshold for synaptic plasticity. In rat V1, the typical developmental increase in NR2A subunits is attenuated by dark-rearing, however levels increase rapidly with subsequent light exposure (Quinlan et al., 1999). Similarly, brief periods of binocular deprivation also results in a decreased NR2A:NR2B ratio and increases the duration of NMDAR-mediated synaptic currents. Notably, with both sensory deprivation regimes, the effects on NMDAR subunit composition and current duration are reversed within hours of subsequent light exposure (Philpot et al., 2001). Together, these findings strongly suggest that the ratio of NR2A:NR2B subunits reflect the recent activity of cortical synapses. It is thought that the increased current duration associated with greater relative levels of NR2B subunits results in enhanced postsynaptic calcium entry through NMDARs,

which acts to favour LTP during periods of inactivity resulting from sensory deprivation (Philpot et al., 2001; Bear, 2003; Yashiro & Philpot, 2008).

Several of the results described in the present thesis likely reflect metaplasticity in the adult rat A1. Enhancements in LTP were observed in rats reared in continuous white noise as compared to controls, suggesting that patterned sound deprivation results in the favouring of LTP in rat primary auditory cortex (A1). The fact that this white noise-induced LTP enhancement was reversed with application of NR2B antagonists is in line with prior hypotheses suggesting the modulation of plasticity thresholds by activity-dependent alterations to NMDAR subunit configuration (Quinlan et al., 1999; Philpot et al., 2001; Bear, 2003). Notably, LTP in these rats returned to control levels following subsequent exposure to an unaltered acoustic environment, which is suggestive of a sliding modification threshold (favouring LTP induction) with continuous white noise exposure.

White noise exposure is unique from other sensory deprivation paradigms in that sensory surfaces continue to receive a high level of acoustic stimulation, albeit containing very low levels of patterned information (Speechley et al., 2007). Thus, it appears that the pattern of sound, rather than overall sound intensity, is important in regulating plasticity thresholds in the rat A1 under the present experimental conditions, as thresholds appear to be lowered even with high levels of random, white noise stimulation. Importantly, previous work has shown that continuous white noise exposure of a comparable duration (40-50 days) beginning in adulthood does not result in enhanced LTP. Thus, it is unlikely that habituation to the high levels of stimulation is the mechanism by which activity levels are lowered to reduce thresholds and promote LTP, as adult rats exposed to continuous white noise do not appear to show a reduction in θ_m (Speechley et al., 2007). Rather, it may be the lack of meaningful, functional activation of cortical neurons during early postnatal life with white noise exposure that acts to maintain lower plasticity thresholds

(and promote plasticity) in these immature circuits. The fact that Speechley et al. (2007) did not observe LTP enhancements with continuous white noise exposure in adulthood suggests that the mature A1 is more resistant to activity-dependent changes to plasticity modification thresholds.

Interestingly, there are some discrepancies in the literature regarding the impact of sensory deprivation on synaptic plasticity. While Kirkwood et al. (1996) show LTP enhancements in V1 of dark-reared rats, Kotak et al. (2007) find that LTP induction is abolished in A1 of gerbils deafened by cochlear ablation. Inherent differences between the two deprivation protocols (e.g. removal of sensory experience vs. the surgical ablation of sensory organs) make direct comparisons of these results difficult. However, it is possible that pathological changes in the central auditory system associated with surgically induced hearing loss, such as neuronal atrophy, degeneration and altered synaptic transmission, may intrinsically limit the potential for LTP induction (Shepherd & Hardie, 2001; Russell & Moore, 2002). Nevertheless, future studies are required to pinpoint the factors contributing to the varied effects on plasticity evident with these different sensory deprivation techniques.

The results of the present thesis showing LTP enhancements following repeated episodes of white noise exposure (during juvenile life and early adulthood) also demonstrate metaplasticity in the adult rat A1. Here, the early experience with patterned sound deprivation resulted in relatively long-lasting changes in the properties of synaptic plasticity, thus favouring LTP following subsequent exposure to the same environment. However, it is unclear whether alterations to the NR2A:NR2B ratio contributed to lowering of LTP thresholds with this manipulation. Notably, a brief (3 day) period of dark exposure has been shown to result in the increased expression of NR2B subunits in V1 of juvenile rats (Chen & Bear, 2007). Thus, future studies could examine whether similar changes in subunit composition are apparent in A1 following brief episodes of white noise. The effect of NR2B antagonist application on LTP in rats

that experienced repeated episodes of white noise could also be tested to examine the role of NR2B subunit activation in the plasticity enhancements noted in these animals.

5.3 The Role of Inhibition

While much of this thesis has focused on the role of excitatory NMDARs in the expression of experience-dependent plasticity, it is possible that changes in inhibitory neurotransmission with maturation or following sensory deprivation also contribute to the presented findings. Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain, and exerts fast hyperpolarizations with the activation of GABA receptors (GABARs). Notably, the maturation of inhibitory synaptic connectivity occurs later than excitatory connectivity in the neocortex. This predominance of excitation during early postnatal life is thought to underlie the heightened levels of critical period plasticity, with the maturation of inhibition contributing to its cessation. Thus, this early imbalance of excitation and inhibition has been suggested to reflect the window during which experience-dependent plasticity can occur (Chang et al., 2005; Jiang et al., 2005).

The maturation of inhibitory circuitry is thought to contribute to the refinement of synaptic connectivity that typically occurs during development. The presence of sideband inhibitory receptive field (IRFs) is associated with narrower excitatory receptive field (ERFs) bandwidths in infant and adult rats, and application of $GABA_AR$ antagonists has been shown to broaden ERFs (Chang et al., 2005). Much like excitatory circuitry, sensory experience is also important for the maturation of inhibition. Rats reared in continuous white noise maintain more immature receptive field properties (broader ERF and IRF bandwidths), yet appear similar to controls upon re-exposure to an unaltered acoustic environment (Chang et al., 2005). Recently, continuous white noise rearing has been shown to alter levels of $GABA_ARs$ in rats, with a

decrease in the expression of α1 and β subunits, and increase in α3 subunits, which is characteristic of more juvenile-like $GABA_A$Rs (Xu et al., 2009b; Xu et al., 2010). Thus, it is possible that increases in excitability due to disrupted inhibitory maturation with sensory deprivation may have contributed to the LTP enhancements noted in our white noise-reared rats. Notably, white noise exposure beginning in adulthood does not affect $GABA_A$R expression, highlighting the importance of early sensory experience for the normal developmental increase in GABAergic inhibition in A1 (Xu et al., 2010). However, the effect of repeated episodes of white noise exposure (both in early life and in adulthood) on GABAR subunit expression has not been examined. Future studies could assess whether changes in the level of GABARs are evident following white noise exposure in adulthood when rats had prior experience with this form of sensory deprivation in early life.

Curiously, despite the vast amount of literature demonstrating changes in NMDAR subunit composition with sensory deprivation, Xu et al. (2009b) did not observe an effect of white noise rearing on NMDAR expression using quantitative immunoblot analyses. While white noise-reared rats appeared to have slightly higher levels of NR2B (~15-20% greater) and decreased NR2A:NR2B ratios (~15% lower), they were not found to be significantly different from controls. These findings appear to be in conflict with our demonstration of the role of NR2B subunits in the expression of enhanced LTP in A1 following continuous white noise rearing. However, differences between the deprivation protocols may have contributed to this discrepancy. The sound volume of white noise utilized by Xu et al. (2009b) was lower (65-70 dB sound pressure level; SPL) than what was used in the present thesis (80 dB SPL). Thus, the use of a higher sound volume may have resulted in significant changes in NMDAR subunit expression. Notably, all observations of enhanced LTP following white noise rearing in this thesis and elsewhere (Speechley et al., 2007) were made with the use of higher sound volumes (80-82 dB

SPL). Additionally, sample sizes used by Xu et al. (2009b) were relatively low (n = 4), thus it is possible that an effect may have emerged with the inclusion of additional animals.

5.4 Plasticity in Adulthood

Much of the work presented in this thesis demonstrates the potential for synaptic plasticity in the mature cortex. While plasticity in adulthood has been shown in the past with the use of behaviourally relevant stimuli or classical neuromodulators, the findings reported here suggest novel ways to promote plasticity in the mature A1. As previously noted, the use of white noise exposure to enhance plasticity in A1 may hold promise for therapeutic applications such as cochlear implants, perhaps by enhancing plasticity to promote the functional reorganization of connectivity following implantation. However, all experiments presented here were conducted with fully hearing rats, thus future studies could examine the effects of white noise exposure in animals with hearing deficits. The congenitally deaf white cat, for example, is an excellent model for prelingual deafness. The majority of these cats are fully deaf, yet some possess residual hearing, albeit with elevated thresholds for sound-evoked activity in brainstem and auditory nerve fibres (Heid et al., 1998; Ryugo et al., 1998). Thus, young white cats with some residual hearing could be exposed to white noise to determine whether such manipulations result in enhanced plasticity (LTP), or even improve the efficacy of later cochlear implantation. Given that fully deaf cats do not show sound evoked activity in auditory nerve fibres (Ryugo et al., 1998), the effect of delivering random, "white noise-like" electrical stimulation directly to the cochlea could be examined in these severely deaf animals. The use of these manipulations in animals with sound-induced hearing loss could also be considered.

In this thesis, the demonstration that zinc can enhance plasticity in older animals that typically no longer express LTP provides support for the notion that zinc can influence plasticity

and neuronal excitability. Zinc is an abundant essential element in the body and nervous system, and as such has many diverse functions including roles in the regulation of DNA synthesis, stabilization of enzymes and proteins, apoptosis, and brain development (Nakashima & Dyck, 2009). Much of research examining the influence of zinc on plasticity has focused on its action at NMDARs, given its high affinity to NR2A subunits and the known involvement of NMDARs in synaptic plasticity (Paoletti et al., 1997; Nakashima & Dyck, 2009). However, zinc can exert many different effects on postsynaptic neurons, including the inhibition of both NMDA and GABA receptors, or the potentiation of AMPARs. Additionally, upon entry into the postsynaptic cell through NMDA or calcium-permeable AMPA receptor channels, zinc can also modulate second messenger signaling molecules essential for neuroplasticity, such as protein kinase C and CaMKII (Baba et al., 1991; Lengyel et al., 2000). Consequently, given the diverse action that zinc can have in the nervous system, it is possible that the enhancements in plasticity observed in the present thesis are due to a complex combination of zinc-mediated effects on postsynaptic cells, and not exclusively due to its inhibition of NR2A subunits. Thus, further experimentation is required to determine the mechanisms by which zinc enhances plasticity in the mature A1. The current lack of highly selective NR2A antagonists (Neyton & Paoletti, 2006; de Marchena et al., 2008) makes verification of the role of NR2A blockade with the use of other NR2A antagonists difficult. Nevertheless, experiments could be performed to determine whether changes in NMDAR current duration are evident following zinc application in A1. Given that NR2A-containing receptors have faster current decay kinetics than NR2B-containing receptors (Monyer et al., 1994; Flint et al., 1997), prolonged decay kinetics would indicate that zinc selectively antagonized the faster NR2A-containing receptors at the doses tested (see de Marchena et al., 2008). Such findings would provide evidence for the involvement of NR2A subunit antagonism in the enhanced LTP noted here, given that the slower decay kinetics associated with a greater

relative contribution of NR2B-containing NMDARs to overall synaptic current are thought to lower induction thresholds for LTP (Philpot et al., 2001; Bear, 2003; Yashiro & Philpot, 2008).

Interestingly, developmental changes in levels of endogenous zinc have been reported in sensory cortex, possibly indicating a role for zinc in mediating changes in plasticity during maturation (Nakashima & Dyck, 2009). In visual and somatosensory cortices, vesicular levels of zinc are reported to increase in layers I, II/III, and V during early postnatal life, and remain high in adulthood. In contrast, zinc levels in layers IV and VI are moderate very early in development and quickly decline by PD 12-30, with zinc relatively absent in layer IV in adulthood (Dyck et al., 1993; Land & Akhtar, 1999; Brown et al., 2003). Notably, increases in levels of endogenous zinc have been observed in sensory cortex following sensory deprivation. In visual cortex, increases in vesicular zinc were evident in layer IV following short periods (24 hours) of monocular deprivation in vervet monkeys, which were localized to regions believed to be innervated by the deprived eye. However, levels of zinc appeared to decrease with longer 3-month deprivation periods (Dyck et al., 2003). In somatosensory cortex, increases in zinc have also been observed in layer IV of deprived barrels following 4-6 weeks of whisker trimming in rodents, but only when trimming began at birth (Land & Akhtar, 1999; Quaye et al., 1999). Together, these findings may suggest the involvement of zinc in plastic changes that occur following sensory deprivation. Future studies could characterize developmental changes in levels of vesicular zinc in cortical layers of A1, and following continuous white noise exposure, to examine whether zinc also contributes to the experience-dependent modulation of plasticity in auditory cortex. Given the known developmental changes in NMDAR subunit composition and levels of endogenous zinc, it would be interesting to test the effect of local zinc application in A1 of rats at different ages. Since relative levels of NR2A in A1 are lower in younger animals (Hsieh et al., 2002; Bi et al., 2006), it is possible that zinc application may not be as effective in enhancing LTP in juveniles as

was seen in the older animals used in the present thesis, assuming that this zinc-induced enhancement was mediated by the antagonism of NR2A subunits.

5.5 Functional Significance of Auditory Plasticity

While this thesis clearly shows the enhancement of plasticity in A1 of mature rats following white noise exposure or pharmacological manipulations, it remains unclear how such enhancements impact auditory processing. Future studies could address these questions by examining the performance of these rats in various auditory discrimination tasks. Considering the broadly-tuned organization of A1 in white noise-reared rats (Chang & Merzenich, 2003), it seems likely that these rats would perform more poorly on sound discrimination tasks than control rats that have had experience with more complex sound stimuli. However, it would be interesting to test the performance of rats that received repeated episodes of white noise (in early life and in adulthood), as they also show elevations in plasticity, yet have experience with unaltered acoustic environments between episodes and likely possess more refined tonotopic maps.

Interestingly, recent work has examined the influence of auditory enrichment on auditory discrimination learning in rats. Xu et al. (2009a) demonstrated that rats exposed to music from PD14 to 56 showed enhanced performance in signal-detection and sound discrimination tasks, as well as increased levels of NR2B subunits in A1. The authors speculated that these changes in NMDAR subunit composition may be the result of increased dendritic branching or synaptogenesis with auditory enrichment. Notably, elevations in NR2B levels were not observed when music exposure began outside of the critical period (PD28 and 56; Xu et al., 2009a). It would be interesting to examine levels of LTP in these rats exposed to auditory enrichment, and how they compare to our white noise-reared rats. Given the elevations in NR2B subunits reported by Xu et al. (2009a), it is possible that auditory-enriched animals would also show enhancements

in LTP. Taken together, it is clear that the developing auditory system is highly sensitive to sensory input, be it of low or high informational value. Paradoxically, it appears that both auditory deprivation and enrichment can result in enhanced plasticity when experienced during early postnatal life, although it is likely that the cellular mechanisms underlying these effects on plasticity differ, with an expanded dynamic range for synaptic modification probable with environmental enrichment (see Artola et al., 2006), rather than a lowering of modification thresholds within a set dynamic range as would be expected with sensory deprivation (Bienenstock et al., 1982).

5.6 Summary and Conclusions

Together, the results presented in this thesis provide a detailed characterization of changes in cortical plasticity in the rat A1 during development and following sensory deprivation. Clear, age-dependent declines in LTP were observed in A1 with maturation, which appeared to be partially mediated by changes in the relative levels of NR2B subunits of the NMDAR. The importance of sensory experience in modulating plasticity during development was evident with the maintenance of juvenile-like levels of LTP in A1 following sensory deprivation by white noise rearing. In the absence of appropriate sensory experience during early life, the cortex appears to remain in an immature, plastic state, yet can be later normalized with subsequent exposure to an unaltered acoustic environment. Like juvenile rats, the enhanced plasticity observed in white noise-reared rats was also sensitive to NR2B subunit blockade, further highlighting the involvement of NMDAR subunits in experience-dependent plasticity. Subsequent experiments examined the modifiability of cortical plasticity in the mature auditory cortex, which is thought to be more resistant to plastic modification. Interestingly, exposure to white noise in early life appeared to “prime” the cortex for later exposure to white noise in

adulthood, resulting in the facilitation of LTP in A1. The facilitation of LTP was also achieved with local, cortical zinc application in older animals that typically no longer elicit LTP, which may also be mediated by changes in the NR2A:NR2B subunit ratio. Nevertheless, further experimentation is required to fully understand the mechanisms underlying white noise- and zinc-facilitated LTP in the adult auditory cortex. Considered together, it is apparent that the expression of plasticity in A1 is highly regulated by sensory experience, yet can be modulated with sensory or pharmacological manipulations that (presumably) alter plasticity modification thresholds. This thesis highlights the importance of early sensory experience in cortical development, and the great potential for plasticity in the mature cortex that can be expressed with strategies that modulate plasticity machinery.

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