ROLE OF EARLY ACOUSTIC EXPERIENCE IN DEVELOPMENT OF THE RAT

PRIMARY AUDITORY CORTEX

by

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Abstract

Neocortical architecture is established by both intrinsic, genetic factors and experience-dependent factors. Postnatal sensory experience plays a significant role in the maturation and refinement of cortical sensory fields, such as the primary auditory cortex (A1). In this thesis, I investigated the effects of manipulating postnatal acoustic experience on the functional and morphological properties of neurons in the thalamocortical auditory pathway of adult rats. In Experiment 1, I used two converging electrophysiological techniques to determine the effects of patterned acoustic deprivation (through exposure to continuous, moderate-level white noise; cWN) on the functional properties of neurons in the central auditory system. In Experiment 2, I used Golgi-Cox staining to visualize morphological correlates of experience-dependent changes in neuron functioning.

Long- and short-term plasticity mediate synaptic strengthening in sensory cortices in response to postnatal sensory experience. I assessed levels of long-term plasticity (using long-term potentiation; LTP) and short-term plasticity (using paired-pulse facilitation/depression; PPF/PPD) in vivo (under deep urethane anesthesia) in the A1 of normally reared rats and rats reared in the absence of patterned acoustic input through cWN exposure. Rats reared under cWN showed significantly greater LTP of field postsynaptic potentials (fPSPs) for thalamocortical, but not intracortical synapses in A1 compared to age-matched controls, indicative of immature, more plastic synaptic connectivity. Both groups showed similar, moderate levels of PPD (across interstimulus intervals ranging from 25 to 1000 ms) prior to LTP induction. Across groups, PPD was significantly enhanced after LTP induction, indicative of a presynaptic component of thalamocortical LTP in A1.
I also assessed the morphology of layer II/III pyramidal neurons in A1 using Golgi-Cox staining and two-dimensional neuron reconstruction. Morphological features, including dendritic length, arbor complexity, and spine density, did not differ significantly between rats reared under cWN and age-matched controls. Rats reared under cWN showed a significantly greater proportion of filopodia to mature spines on apical dendrites compared to age-matched controls.

Together, these data indicate that patterned acoustic experience results in a reduction of plasticity in A1, indicative of more mature, hard-wired synaptic connectivity. Furthermore, LTP in A1 in vivo is mediated in part by presynaptic mechanisms, such as increases in transmitter release probability at thalamocortical synapses.
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<tr>
<td>A1</td>
<td>Primary auditory cortex</td>
</tr>
<tr>
<td>AMPA(R)</td>
<td>α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (receptor)</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AP</td>
<td>Anteroposterior</td>
</tr>
<tr>
<td>Ca$^{2+}$</td>
<td>Calcium ions</td>
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<tr>
<td>cWN</td>
<td>Continuous white noise</td>
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<tr>
<td>dB</td>
<td>Decibel</td>
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<tr>
<td>EPSP</td>
<td>Excitatory postsynaptic potential</td>
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<td>fPSP</td>
<td>Field postsynaptic potential</td>
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<td>HFS</td>
<td>High frequency stimulation</td>
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<td>i.p.</td>
<td>Intraperitoneal</td>
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<tr>
<td>ISI</td>
<td>Interstimulus interval</td>
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<td>L</td>
<td>Lateral</td>
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<td>LTD</td>
<td>Long-term depression</td>
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<td>LTP</td>
<td>Long-term potentiation</td>
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<td>MD</td>
<td>Monocular deprivation</td>
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<td>Mg$^{2+}$</td>
<td>Magnesium ions</td>
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<tr>
<td>MGN</td>
<td>Medial geniculate nucleus</td>
</tr>
<tr>
<td>NMDA(R)</td>
<td>N-methyl-D-aspartate (receptor)</td>
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<td>P</td>
<td>Postnatal day</td>
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<td>PPD</td>
<td>Paired-pulse depression</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>PPF</td>
<td>Paired-pulse facilitation</td>
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<td>PPR</td>
<td>Paired-pulse ratio</td>
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<tr>
<td>s.c.</td>
<td>Subcutaneous</td>
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<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
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<td>SD</td>
<td>Standard deviation</td>
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<td>SPL</td>
<td>Sound pressure level</td>
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<td>STP</td>
<td>Short-term plasticity</td>
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<td>TBS</td>
<td>Theta-burst stimulation</td>
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<td>V</td>
<td>Ventral</td>
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<td>V1</td>
<td>Primary visual cortex</td>
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Chapter 1

Introduction

1.1 Neocortical Plasticity

The functional and morphological characteristics of the central nervous system result from a combination of genetic and environmental, experience-related factors. Although basic neocortical architecture is established by genetics and other biological factors prior to birth, the central nervous system is highly plastic, and its hardwiring is ultimately driven to a significant extent by natural sensory experience throughout postnatal development.

Neural plasticity is the ability of neurons and their functional connections to undergo changes in their anatomical, chemical, and physiological properties (Kolb & Whishaw, 1998). Such changes occur at all levels in the central nervous system, from molecular and synaptic modifications that underlie memory consolidation, to large-scale neocortical reorganization that underlies neocortical mapping in response to sensory experience.

Under normal environmental conditions, plasticity levels decrease throughout the brain over the course of development (Burke & Barnes, 2006). This developmental decrease in plasticity levels results in part from postnatal sensory experience, with external sensory stimuli promoting the maturation of neocortical areas responsible for processing sight, touch, and sound, through directing the refinement and consolidation of neural circuitry. For instance, postnatal visual stimulation alters the ocular dominance and orientation tuning of visual cortex neurons (Blakemore & Cooper, 1970; Hubel & Wiesel, 1970), and postnatal tactile stimulation alters the
responses of barrel cortex neurons to whisker stimulation (Glazewski & Fox, 1996; Simons & Land, 1987).

Neocortical development and plasticity levels can be modified through numerous experimental manipulations. Deprivation of sensory inputs during early postnatal development markedly disrupts the structural and functional maturation of neurons, and can result in global reorganization of the associated brain regions (Greenough, Black, & Wallace, 1987). For instance, the absence of visual, somatosensory, and auditory inputs during early postnatal development significantly delays the maturation of the respective neocortical fields and leaves these regions in a juvenile, highly plastic state. Deprivation of visual inputs through dark rearing or eye-lid suturing interrupts the morphological and functional development of the visual cortex (Cynader & Mitchell, 1980; Hubel & Wiesel, 1970). Similarly, deprivation of somatosensory inputs through vibrissal follicle destruction interrupts the morphological and functional development of the barrel cortex (Van der Loos & Woolsey, 1973). In both cases, sensory deprivation impedes normal neocortical hardwiring, resulting in maintenance of heightened plasticity levels in the cortical region responsible for processing the sensory stimuli. In some cases, chronic sensory deprivation, such as that caused by congenital blindness or deafness, can result in the adoption of the deprived cortex by other sensory modalities through a phenomenon known as cross-modal plasticity (Finney, Fine, & Dobkins, 2001; Sadato et al., 1996).

Interestingly, numerous studies have shown that deprivation of patterned sensory inputs, such as that caused by rearing in restricted sensory environments, is sufficient to interrupt the normal maturation of sensory cortices and leave these regions in highly plastic states (Chang &
Merzenich, 2003; Wilson & Riesen, 1966). For example, Wilson & Riesen (1966) deprived monkeys of patterned visual stimuli from birth, using plastic ocular contacts that allowed for only diffuse light stimulation. Both 20- and 60-day-old visually deprived monkeys exhibited learned and untrained visual behaviours similar to those seen in newborn monkeys. Upon exposure to patterned visual stimulation, the learned and untrained visual behaviours progressively developed in a manner consistent with that of normal postnatal development. These findings highlight the importance of patterned sensory experience in driving the postnatal maturation of sensory systems. Across sensory systems, the extent to which typical development can resume upon exposure to patterned sensory inputs depends on the onset, duration, and termination of the sensory restriction (Cynader, Berman, & Hein, 1976; Wiesel & Hubel, 1965).

1.2 Critical Periods in Development

Most experience-dependent neocortical changes occur during specific temporal periods when the functional and structural properties of neurons are particularly susceptible to modification, known as critical or sensitive periods (Hensch, 2004). During such periods, neural circuitry is refined and sensory systems mature. Although the onset and duration of critical periods vary depending on the organism and the neural system, plasticity levels tend to be highest throughout the brain during early postnatal life and progressively decrease over development as neural circuits are consolidated.

One of the most extensively studied critical periods is that for the refinement and stabilization of ocular dominance columns in the primary visual cortex (V1). During this critical period, the ocular dominance organization of V1 is highly susceptible to the effects of monocular
deprivation (MD), with dramatic column reorganization resulting from brief periods of MD (Hubel & Wiesel, 1970). After the closure of this critical period, the same MD has a much less pronounced effect on V1 organization. Across species, the critical period for ocular dominance column maturation and stabilization corresponds to the period during which the eye is first used and the thalamic projections to V1 are refined (Gordon & Stryker, 1996; Hubel & Wiesel, 1970; Issa, Trachtenberg, Chapman, Zahs, & Stryker, 1999).

Although plasticity levels progressively decline with developmental stabilization of neuronal circuitry, the adult brain maintains a considerable degree of plasticity. It is now widely accepted that several aspects of neocortical organization and function can be modified by experience in adulthood, after critical period closure. For instance, the adult brain can undergo dramatic reorganization following manipulations of peripheral sensory input. Merzenich and colleagues (Merzenich et al., 1983; Merzenich et al., 1984) assessed the spatial representation of sensory inputs in primary sensory areas of the adult monkey neocortex before and after surgical digit amputation or deafferentation. The authors showed that the neocortical representation of the intact digits significantly increased and expanded to regions previously responsive to inputs from the amputated or deafferentated digit. Similarly, Kaas et al. (1990) showed that neocortical neurons in the adult monkey brain that normally have receptive fields corresponding to a lesioned region of the retina become responsive to inputs from the intact retinal regions surrounding the lesion. Furthermore, these receptive field changes corresponded to significant reorganization of the spatial representation of the retina in the primary and secondary visual cortices. Such findings suggest that neocortical representations of the sensory environment are
particularly sensitive to sensory inputs during early postnatal development, but plasticity continues to be expressed in the central nervous system after critical periods of brain maturation are completed. Importantly, the specific factors that regulate the onset and duration of critical periods for sensory system maturation are not yet fully understood.

1.3 Plasticity in the Primary Auditory Cortex

The functional and morphological characteristics of the auditory cortex are strongly influenced by acoustic experience during early postnatal development, and thus the auditory cortex is a primary region to study neural plasticity. The mammalian auditory cortex is located in the superior temporal lobe and is interconnected with brainstem and thalamic auditory centers, including the inferior colliculus and the medial geniculate nucleus (MGN) of the thalamus, as well as to other parts of the cerebral cortex. The auditory cortex is functionally divided into primary, secondary, and tertiary regions, which form concentrically with the primary cortex in the center (Palomero-Gallagher & Zilles, 2004).

The primary auditory cortex (A1) exhibits tonotopic organization, meaning that neighboring neurons are sensitive to neighboring frequencies, reflecting the organization of the cochlea (Lauter, Herscovitch, Formby, & Raichle, 1985). One of the most extensively studied critical periods in the auditory system is that for the refinement and stabilization of A1 tonotopy. In rats, this period begins following the onset of low-threshold hearing at postnatal day (P) 9-10, peaks between P11 and P13, and is completed by P50 (Chang & Merzenich, 2003; Zhang, Bao, & Merzenich, 2001). During this period, two main changes occur: the total cortical region responsive to tonal stimuli contracts, and the juvenile overrepresentation of high frequencies is
converted to a mature, more balanced representation of high and low frequencies (Zhang et al., 2001. It is worth noting that the precision of A1 tonotopic organization has recently come into question, with more recent studies demonstrating less sharply-tuned receptive fields for A1 neurons, or heterogeneous local populations within A1 (see Rothschild, Nelken, & Mizrahi, 2010 for details). These apparent discrepancies are likely due to differences in the techniques used to assess tonotopic organization, with earlier studies analyzing tone-evoked spiking patterns and later studies analyzing tone-evoked (subthreshold) synaptic responses of A1 neurons.

Plasticity in A1 is largely restricted to early development and is markedly diminished upon critical period closure. For instance, repeated exposure to a single frequency tone during the critical period for tonotopic organization results in a long-lasting, competitive overrepresentation of that frequency in A1 in rats, while the same monotone exposure has no such effect after this period (de Villers-Sidani, Chang, Bao, & Merzenich, 2007; Zhang et al., 2001). Zhang et al. (2001) demonstrated that exposure to a single frequency during early postnatal A1 development (P9-28) resulted in a profound, long-lasting increase in the A1 region responsive to that frequency, but this effect was not seen with the same monotone exposure occurring after this developmental period. Similarly, de Villers-Sidani et al. (2007) showed that monotone exposure between P11 and P13 resulted in a long-lasting expansion of the A1 representation of that frequency, but this same exposure had no such effect after P13.

Although receptive field plasticity levels in A1 decrease over development under normal acoustic conditions, patterned acoustic deprivation during early postnatal life appears to suspend the developmental critical period, and allows this region to remain in an immature, highly plastic
state. Chang and Merzenich (2003) showed that rearing rats in continuous white noise (cWN), with no particular frequencies being more prominent than others, delays A1 tonotopic maturation and neuronal receptive field refinement. Once this patterned sensory restriction is removed and the brain receives patterned acoustic inputs, A1 rapidly matures. The results of this and similar studies (Hogsden & Dringenberg, 2009b; Speechley, Hogsden, & Dringenberg, 2007; Xu, Yu, Cai, Zhang, & Sun, 2010) support the idea that patterned acoustic experience is an important factor in regulating the duration of critical periods of A1 maturation in rats.

Interestingly, studies have demonstrated that the adult A1 tonotopy remains highly plastic even under normal rearing conditions. Adult A1 plasticity is influenced by numerous factors, such as the behavioural and physiological salience of auditory stimuli. Kilgard and Merzenich (1998) showed that the tonotopic organization of the A1 of adult rats can be altered through appetitive conditioning, by pairing tones of particular frequencies with electrical stimulation of the nucleus basalis. The nucleus basalis is a heterogeneous forebrain nucleus, composed of cholinergic, glutamatergic, and GABAergic projection neurons (Semba, 2000), that is activated in response to behaviourally salient stimuli through its connections with limbic and paralimbic structures (Levey, Hallanger, & Wainer, 1987). This pairing paradigm resulted in a dramatic reorganization of A1, with receptive fields associated with particular frequencies being increased or decreased depending on the specific pattern of tone-nucleus basalis stimulation pairings. These results were paralleled in the study conducted by Weinberger (2004), in which the tonotopic organization of the adult rat A1 was altered by making particular frequencies behaviourally salient through pairing bar-pressing in response to those frequencies with a water
reward. In each study, A1 organization shifted toward competitively over-representing the conditioned, salient frequencies, with the magnitude of the representational shift reflecting the level of stimulus significance. The results of these studies demonstrate that critical period-like levels of plasticity can be reinstated in the adult A1 when passive exposure to acoustic stimuli is no longer sufficient to drive neocortical changes.

In addition to A1 tonotopy and neuronal receptive field plasticity, other forms of plasticity in A1 are sensitive to manipulations of acoustic experience, including long- and short-term synaptic plasticity and structural plasticity.

1.4 Long- and Short-Term Synaptic Plasticity

1.4.1 Long-Term Potentiation

It is widely accepted that sensory experience alters the long and short-term dynamics of neocortical synapses throughout postnatal development. Donald Hebb proposed the first theoretical model for such experience-dependent changes, suggesting that the connections between neurons that are concurrently activated by sensory experience are selectively strengthened, and that this strengthening results in an enhancement of synaptic efficacy within active neural circuits (Hebb, 1949). In 1973, Bliss and Lomo provided empirical support for this theory by applying brief bursts of high frequency stimulation (HFS), to the perforant path of the hippocampus (Bliss & Lomo, 1973). The stimulation applied to these hippocampal afferents generated excitatory postsynaptic potentials (EPSPs) in the dentate gyrus granule cells, resulting
in a long-lasting enhancement of synaptic transmission within the circuit. This phenomenon is now known as long-term potentiation (LTP).

1.4.2 Mechanisms of Long-Term Potentiation

Most forms of cortical LTP are dependent on the N-methyl-D-aspartate (NMDA) glutamate type receptor (NMDAR; Lynch, 2004). Unlike most neurotransmitter receptors, the NMDAR requires more than the binding of its ligand for its activation. At rest, ion movement through the NMDAR is blocked by a Mg$^{2+}$ ion present in the pore. This Mg$^{2+}$ blockade is displaced by depolarization of the postsynaptic cell membrane. This depolarization results from activation of the non-NMDA type ionotropic glutamate receptor, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA, R), which results in an influx of Na$^{+}$. Based on the requirement for presynaptic glutamate release to be coupled with postsynaptic cell depolarization, NMDARs can be thought of as detectors of concurrent pre- and postsynaptic cell activity (Coan & Collingridge, 1985). NMDAR activation results in an influx of postsynaptic Ca$^{2+}$ (MacDermott, Mayer, Westbrook, Smith, & Barker, 1986), which activates Ca$^{2+}$-dependent enzymes that lower the postsynaptic cell’s threshold for future activations (Xia, Dudek, Miranti, & Greenberg, 1996). LTP maintenance is achieved by changes in gene transcription and protein synthesis that result in structural changes in synaptic connectivity (Lamprecht & LeDoux, 2004).

1.4.3 Effects of Sensory Experience on Long-Term Potentiation

Under normal environmental conditions, there is a developmental-decrease in the magnitude of LTP that can be reliably induced in sensory systems (Crair & Malenka, 1995;
Hogsden & Dringenberg, 2009a; Kirkwood, Lee, & Bear, 1995). The time frame for this decrease coincides with that of the decrease in the effects of sensory experience on synaptic connectivity and receptive field tuning (Fagiolini, Pizzorusso, Berardi, Domenici, & Maffei, 1994; Woolsey & Wann, 1976; Zhang et al., 2001). Thus, the magnitude of LTP that can be induced in a given neural circuit is a reliable indicator of synaptic maturity and plasticity levels.

LTP has been shown to be highly sensitive to manipulations of sensory experience. For example, acoustic deprivation through rearing under cWN results in an enhancement of LTP in the thalamocortical auditory cortex of adult rats in vivo (Speechley et al., 2007). These and similar findings (Feldman, Nicoll, & Malenka, 1999; Hogsden & Dringenberg, 2009b) are consistent with the hypothesis that LTP-like mechanisms mediate experience-dependent plasticity in sensory systems (Malenka & Bear, 2004).

1.4.4 Short-Term Plasticity

Unlike long-term synaptic strengthening or weakening that can last for hours to days, some synaptic changes occur at faster timescales, lasting milliseconds to minutes. Such changes are known as short-term plasticity (STP). One common measure of STP is paired-pulse stimulation. In paired-pulse stimulation, two successive stimulation pulses are applied presynaptically, and the two resultant field postsynaptic potentials (fPSP) are directly compared. The difference in magnitude between the first and second fPSPs is reported as a paired-pulse ratio (PPR) and serves as an index of change from the first to the second fPSP. In response to paired-pulse stimulation, synapses can show a transient increase (facilitation) or decrease (depression) in transmission efficacy known as paired-pulse facilitation (PPF) or paired-pulse
depression (PPD), respectively (Zucker, 1989). A PPR value greater than 1.0 is indicative of PPF, whereas a PPR value less than 1.0 is indicative of PPD.

The primary determinant of whether a synapse will express PPF or PPD is the probability of transmitter release from the presynaptic terminal. Mature synapses, characterized by strong neuronal coupling and high probability of release from presynaptic terminals, are more likely to exhibit depression of release with successive stimulation pulses. Immature synapses, characterized by weak neuronal coupling and low release probability, are more likely to exhibit facilitation of release with successive stimulation pulses (Atzori et al., 2001). Reflecting this, there is an inverse relationship between initial synaptic strength and PPRs, with PPR values decreasing with increasing initial EPSP amplitude (Jiang & Abrams, 1998). Therefore, paired-pulse responses serve as measures of synaptic maturity, which in turn serves as an indicator of synaptic strength and plasticity levels.

Short-term synaptic facilitation is generally considered a presynaptic phenomenon. The most widely accepted hypothesis for short-term facilitation is the residual Ca\(^{2+}\) hypothesis (Katz & Miledi, 1968). Transmitter release results from an influx of Ca\(^{2+}\) through voltage-gated Ca\(^{2+}\) channels that open when an action potential invades the presynaptic terminal. With successive action potentials, Ca\(^{2+}\) accumulates within the presynaptic terminal. This causes a greater amount of active Ca\(^{2+}\) to be available to trigger vesicle secretion and results in facilitation of responses to successive stimulations. Unlike PPF, PPD likely involves both pre- and post-synaptic mechanisms. The most widely accepted mechanism of PPD is depletion of the readily releasable pool of synaptic vesicles (Zucker & Regehr, 2002).
Because the PPR is a highly sensitive index of the synaptic strength (Thomson, 2000), it can be used as a measure of sensory experience-dependent synaptic maturation (or the lack thereof). For example, given that visual deprivation results in a disruption of visual cortex maturation (Cynader & Mitchell, 1980; Hubel & Wiesel, 1970), it would be expected that visual cortex synapses of dark-reared rats would fail to develop mature release probability and express higher levels of PPF compared to those of age-matched controls.

1.4.5 The Paired-Pulse Ratio and Mechanisms of Long-Term Potentiation

In addition to providing insights into short-term synaptic dynamics, the PPR can be used to determine the mechanisms underlying LTP. Despite the vast amount of LTP research conducted, there continues to be an active debate regarding the relative importance of pre- and postsynaptic contributions to LTP induction and maintenance (Malenka & Bear, 2004). Although most LTP research has focused on postsynaptic mechanisms, such as insertion and modification of membrane receptors (Malenka & Nicoll, 1999), it is now apparent that LTP also involves some presynaptic mechanisms, such as an increase in transmitter release probability (Dolphin, Errington, & Bliss, 1982; Skrede & Malthe-Sorensen, 1981).

Since responses to paired-pulse stimulation are strongly influenced by vesicle release probability, changes in the PPR following LTP induction can be used to indicate presynaptic mechanisms of LTP expression (Schulz, Cook, & Johnston, 1995). Changes in the PPR have been shown following LTP in various brain regions. For example, LTP induction was associated with a decrease in the PPR in visual cortex slices, with the magnitude of change being correlated with the amplitude of LTP induced (Pan, Yang, Han, & Xie, 2004).
1.5 Structural Plasticity

In his theoretical model of synaptic enhancement, Hebb asserted that a functional, activity-based change in synaptic connectivity is only one stage of the process of experience-dependent synaptic strengthening. He suggested that functional changes occur immediately in response to concurrent neuronal activation and carry on until structural changes take place, and it is these structural changes that allow for the synaptic enhancement to be maintained (Hebb, 1949). It has been clearly demonstrated that the external environment exerts significant effects on neuronal morphology throughout development (Markham & Greenough, 2004). Such effects include changes in dendritic length and arbor complexity (Bose et al., 2010; Volkmar & Greenough, 1972; 1973), as well as the amount, size, and shape of dendritic spines (Alvarez & Sabatini, 2007).

1.5.1 Neuronal Morphology

Dendritic arbor complexity is tightly linked to the number of synaptic connections that can be made by a neuron, and thus the amount of information processing and integration that can occur within it (Sidiropoulou, Pissadaki, & Poirazi, 2006). Reflecting the tight link between dendrite structure and neuron function, dendritic morphologies are highly distinctive for different neuronal subtypes, and highly consistent within individual subtypes. For instance, pyramidal neurons have a triangular cell body surrounded by a basal dendritic arbor and a single apical dendrite that projects toward the cortical surface and branches into a terminal tuft (Spruston, 2008). This characteristic morphology is maintained across distinct brain regions and cortical
layers, which allows for morphological analyses of these neurons under varying experimental conditions.

Under normal environmental conditions, the dendritic arbor of pyramidal neurons in sensory cortices is remarkably stable in the mature brain. For instance, layer V pyramidal neurons in the barrel cortex (Trachtenberg et al., 2002) and layer II/III pyramidal neurons in the visual cortex (Lee et al., 2006) exhibit no discernable addition or retraction of apical or basal dendrites over periods of weeks in the mature brain.

Despite widespread stability under normal environmental conditions, the dendritic arbor of cortical neurons is markedly affected by manipulations of sensory experience. Sensory deprivation results in a reduction of dendritic length and arbor complexity in the cortical region responsible for processing the sensory stimuli. For example, Bose et al. (2010) showed that acoustic deprivation resulted in reduced apical dendritic length in layer II/III A1 pyramidal neurons in rats. Similarly, Coleman and Riesen (1968) showed that dark rearing resulted in a reduced number and total length of dendrites of layer IV V1 stellate cells in cats. Conversely, sensory enrichment is associated with an increase in dendritic length and arbor complexity. For example, Volkmar and Greenough (1972; 1973) showed that rearing in an enriched environment resulted in greater higher-order dendritic branching in pyramidal neurons of the rat visual cortex compared to rearing in standard social or isolated environments.

1.5.2 Dendritic Spines

Most neurons have a high density of small, membranous dendritic protrusions called spines. Spines are the functional units of neuronal connectivity, and most excitatory synaptic
connections in the CNS occur on spines (Harris & Kater, 1994). Spines exhibit a high degree of structural diversity. For example, the volume of dendritic spines on apical dendrites of layer 5 pyramidal neurons in the neocortex of adult mice typically ranges from 0.015 to 0.77 µm³ (Knott, Holtmaat, Wilbrecht, Welker & Svoboda, 2006). During early postnatal development, there is a conversion from motile dendritic precursors, called filopodia, to stable dendritic spines with detectable postsynaptic densities (Schachtele, Losh, Dailey, & Green, 2011). There is considerable variation in the shape of mature dendritic spines. Thin spines have a small head and a narrow neck, mushroom spines have a larger head and a narrow neck, and stubby spines have no distinctive neck (Nimchinsky, Sabatini, & Svoboda, 2002).

Dendritic spines are highly dynamic and show clear evidence of structural plasticity in a number of brain areas. For instance, dendritic spines on neocortical pyramidal neurons appear, disappear, and change shape over a period of hours to days in vivo (Alvarez & Sebatini, 2007). Such structural dynamics are increased by manipulations of sensory inputs. For example, visual stimulation causes an increase in spine density, while visual deprivation causes a reduction in spine density on visual cortex pyramidal neurons (Parnavelas, Globus, & Kaups, 1973; Valverde, 1967). This pattern of results is also seen in the auditory cortex following acoustic enrichment and deprivation (Bose et al., 2010). Interestingly, the time frame for changes in dendritic spine density following sensory manipulations coincides with that of changes in neuronal receptive fields (Trachtenberg et al. 2002), highlighting the link between dendritic spine morphology and neuron function.
1.6 Golgi-Cox Staining

Many studies examining dendritic morphology have used the Golgi method, developed by Camillo Golgi in 1873 (Golgi, 1873). This method allows for visualization of neuronal architecture through staining entire neurons using metallic impregnation. One commonly used variation of the Golgi method that improved neuron staining is the Golgi-Cox technique (Cox, 1891). Golgi methods are useful for examining individual neurons in isolation because they randomly stain a small percentage of neurons in a given population. The factors responsible for determining the selectivity of neuron staining by Golgi methods remains unknown, but there is no evidence that neuron maturity or complexity systematically affect neuron staining.

1.7 The Present Thesis

The cellular mechanisms underlying experience-dependent plasticity in the neocortex include both functional and structural modifications. The focus of this thesis centers on characterizing the functional and structural effects of early acoustic experience on development of the adult central auditory system. The impact of patterned acoustic deprivation on plasticity in the thalamocortical auditory system of adult rats was investigated using convergent electrophysiological and histological techniques.

To the extent that early postnatal exposure to patterned sensory stimuli drives neocortical development and reduces plasticity levels, the absence of patterned acoustic stimuli should interrupt A1 development and result in maintenance of critical period-like levels of plasticity in this region. Each of the experiments in this thesis involves a unique method of examining the
effects of patterned acoustic deprivation achieved by rearing under cWN on plasticity in the adult thalamocortical auditory system.

In Experiment 1, the effects of rearing under cWN on long- and short-term plasticity in the thalamocortical auditory system were assessed using two electrophysiological measures: LTP induction and paired-pulse stimulation, respectively. Because patterned sensory deprivation during early postnatal development delays neocortical maturation (Chang & Merzenich, 2003; Wilson & Riesen, 1966), it would be expected that the A1 of adult rats reared under cWN will be significantly more immature and plastic than that of rats reared under normal acoustic conditions. Based on this, it was hypothesized that a significantly greater level of LTP will be induced in the thalamocortical auditory system of rats reared under cWN compared to age-matched controls. With respect to short-term plasticity, it was hypothesized that the thalamocortical synapses of rats reared under cWN will exhibit significantly greater levels of PPF, or lower levels of PPD, compared to age-matched controls, indicative of a weaker, more immature level of synaptic connectivity (Atzori et al., 2001). Further, it was hypothesized that LTP induction will result in a shift from PPF to PPD expression in rats reared under cWN and will significantly enhance the degree of PPD exhibited by age-matched controls.

In Experiment 2, the effect of cWN exposure on structural plasticity in the A1 of adult rats was examined using Golgi-Cox staining and morphological measures of neuronal complexity. In this experiment, dendritic length, arbor complexity, and spine density served as indices of maturity for layer II/III A1 pyramidal neurons. Because sensory deprivation appears to reduce dendritic length and arbor complexity (Bose et al., 2010; Coleman & Riesen, 1968), as
well as spine density (Parnavelas, Globus, & Kaups, 1973; Valverde, 1967), it was hypothesized that the dendritic length, arbor complexity, and spine density of layer II/III A1 pyramidal neurons will be significantly lower in rats reared under cWN compared to age-matched controls.
Chapter 2

Long- and Short-Term Plasticity

2.1 Experiment 1

Postnatal acoustic experience guides the functional maturation of synapses in the developing thalamocortical auditory system. Although deprivation of patterned acoustic input during early postnatal development markedly alters neuronal receptive field maturation and LTP levels in the adult thalamocortical auditory system, the effects of such deprivation on short-term synaptic dynamics in this system have not been previously explored.

The primary objective of this experiment was to investigate the effects of deprivation of patterned sound on long- and short-term plasticity in the adult thalamocortical auditory system. This was accomplished using two electrophysiological tests. First, the effects of rearing under cWN on theta burst stimulation (TBS)-induced LTP in the thalamocortical pathway in vivo were examined and compared to those found in previous studies that demonstrated an enhancement of LTP following rearing under cWN utilizing the same cWN and LTP-induction protocols (Hogsden & Dringenberg, 2009b; Speechley et al., 2007). Next, paired-pulse responses in the thalamocortical pathway of rats reared under cWN were examined and compared to those of age-matched controls to characterize the effects of patterned acoustic deprivation on short-term synaptic plasticity. Finally, the effects of LTP induction of paired-pulse responses were examined to determine the mechanisms underlying LTP in the thalamocortical auditory system.

It was hypothesized that a significantly greater level of LTP will be induced in the thalamocortical auditory system of rats reared under cWN compared to age-matched controls,
indicative of immature, more plastic synaptic connectivity. It was also hypothesized that the thalamocortical synapses of rats reared under cWN will exhibit significantly greater PPF (or less PPD) compared to age-matched controls, indicative of more immature, plastic synaptic connectivity. Finally, it was hypothesized that LTP induction will result in a significant shift from PPF to PPD, or an enhancement of PPD, in rats reared under cWN, and will significantly enhance PPD in age-matched controls, indicative of increased maturity and reduced plasticity of thalamocortical synapses.

2.2 Methods

2.2.1 Animals

Experiments were conducted on adult (200-400 g) male and female Long-Evans rats (Charles River Laboratories, QC, Canada). All rats were housed in a temperature (21 ± 2°C) and humidity (40-70%) controlled colony room, maintained on a reversed 12-hour light/dark cycle (lights off between 0700 and 1900 h) with food and water access ad libitum. All experiments were conducted between 0800 and 1800 h. All procedures were conducted in accordance with guidelines established by the Canadian Council on Animal Care, and were approved by the Queen’s University Animal Care Committee.

Untimed (approximately 19 days) pregnant female Long-Evans rats were housed individually either in the standard colony room or in sound-attenuated chambers, depending on the environmental condition to which they were randomly assigned. The sound-attenuated chambers were located in the standard colony room and maintained under the same conditions.
Pups were housed with their mother until weaning at P23, at which time they were separated by sex and divided into groups of three to five.

2.2.2 White Noise Apparatus

Two white noise chambers (114 x 61 x 66 cm, aluminum-lined plywood, fitted with a fan and time-controlled light) were located in the colony room. Each chamber contained two speakers (one 8 inch woofer and one 3.25 inch tweeter, with frequency ranges of 45 Hz to 5 kHz and 2 to 35 kHz, respectively; American Legacy Series 2 Speakers, Legacy Audio, NY, USA). Speakers were connected to a custom-made white noise generator (Department of Psychology, Queen’s University). Spectral analysis of the white noise generated showed that the signal covered a frequency range up to approximately 35 kHz, with power gradually declining in the range of 30 to 37.5 kHz (Speechley et al., 2007). Sound attenuation across the chamber wall was 27 dB for measurements made immediately outside a chamber containing a 80 dB sound pressure level (SPL) white noise signal.

The cWN exposure began at P5, approximately five days before the onset of low-threshold hearing in rats (Zhang et al., 2001). The sound volume was increased incrementally from 65 to 80 dB SPL over four days to limit stress experienced by the mother. The volume was subsequently maintained at this level until P50-60, when electrophysiological and histological procedures were conducted. This exposure paradigm has been shown to inhibit neuronal maturation and associated declines in synaptic plasticity levels (Hogsden & Dringenberg, 2009b; Speechley et al., 2007). White noise rearing of rats using similar parameters has been shown to
delay A1 tonotopic refinement (Chang & Merzenich, 2003) and alter neocortical LTP (Hogsden & Dringenberg, 2009b; Speechley et al., 2007).

2.2.3 Surgical Preparation

Rats were deeply anesthetized with urethane (Sigma-Aldrich, ON, Canada; 1.5 g/kg, given intraperitoneally (i.p.) as three 0.5 g/kg doses, once every 20 minutes and top-ups when necessary). In addition to maintaining highly stable, surgical-level anesthesia, urethane preserves aspects of spontaneous neocortical activity in intact preparations. With the dosing described above, urethane minimizes spontaneous high-frequency, low-amplitude activity in the electrocorticogram (Dringenberg, Hamze, Wilson, Speechley, & Kuo, 2007). The local anesthetic bupivocaine (Marcaine, 2 mg/kg; Hospira Healthcare Corporation, QC, Canada) was administered subcutaneously (s.c.) to the scalp 15 minutes prior to the start of the surgery. Throughout the experiment, body temperature was monitored and maintained at 36-37°C.

Rats were mounted in a stereotaxic apparatus (David Kopf Instruments, CA, USA). An incision was made to expose the skull and small burr holes were drilled over the MGN (from bregma, AP -5.5, L +4.0, and V -5.4 to -6.4) and the ipsilateral A1 (from bregma, AP -4.5, L +7.0, and V -3.2 to -5.4). Two additional holes were drilled over the frontal cortex to secure ground and reference connections.

2.2.4 Electrophysiology Procedure

A concentric, bipolar stimulating electrode (SNE-100, Rhodes Medical Instruments, David Kopf, CA, USA) was used to stimulate the MGN (0.2 ms pulse duration). The stimulating
electrode was connected to a stimulus isolator (ML 180 Stimulus Isolator, AD Instruments, ON, Canada) that provided a constant current output. A monopolar recording electrode (125 µm diameter, Teflon-insulated, stainless steel wire) was lowered into the middle cortical layers (IV-V) of A1. The final ventral depths of both electrodes were adjusted to yield maximal A1 fPSP amplitudes in response to single-pulse MGN stimulation. The recording electrode was connected to an amplifier (Model 1800, A-M Systems Inc., WA, USA; filter settings at 0.3 Hz to 1 kHz) and A/D converter (PowerLab/4s system, Scope software v. 4.0.2, AD Instruments, ON, Canada) that digitized (10 kHz) and stored the recorded signal for offline analyses.

Input-output curves were generated by stimulating the MGN at increasing intensities (0.1-1.0 mA in 0.1 mA increments). The MGN stimulation intensity yielding 50-60% of the maximal fPSP amplitude in A1 was used for the remainder of the experiment. One fPSP was recorded every 30 seconds, until 60 stable baseline fPSPs were obtained. Three episodes of theta-burst stimulation (TBS) were then applied to the MGN, each followed by 60 minutes of single-pulse MGN stimulation every 30 seconds. TBS was applied as four repeated trains of 10 bursts per train (bursts repeated at 5 Hz, each burst containing 5 pulses repeated at 100 Hz) repeated once every 10 seconds for a total of 40 bursts. Previous work has shown optimal LTP induction with this stimulation procedure (Dringenberg et al., 2007). A typical recording consisted of 60 baseline and 360 post-TBS fPSPs.

Paired-pulse stimulation was run prior to recording baseline fPSPs and again at the end of the experiment. Two single-pulse stimulations, separated by interstimulus intervals (ISI) of 25,
50, 75, 100, 125, 250, 500 and 1000 ms, were applied to the MGN. Ten episodes of paired-pulse stimulation were completed for each ISI, and episodes were separated by 5000 ms intervals.

2.2.5 Data Collection

All electrophysiological data were collected using Scope software (v. 3.6.5; AD Instruments, ON, Canada). For the long-term plasticity measure (LTP), amplitudes of the two negative-going fPSP peaks (occurring approximately 5-7 and 13-15 ms after the stimulation artifact) were computed offline by calculating the voltage difference between activity prior to stimulation and the peak. Peak amplitudes were averaged over 10-minute intervals (20 fPSPs in total) and normalized by dividing this value by the average baseline amplitude for each rat.

For the short-term plasticity measure (paired-pulse responses), a PPR was calculated for each animal at each ISI by dividing the peak amplitude (computed as described above) of the second waveform by that of the first. Only the first peak of each fPSP was used for PPR analysis.

Offline data collection and analysis was conducted by an experimenter who was unaware of the rats’ rearing conditions.

2.2.6 Histology

After completion of the experiment, rats were perfused through the heart with 0.9% saline (40 ml) followed by 10% formalin (40 ml). Brains were removed and stored in 10% formalin for a minimum of 24 hours before sectioning coronally into 40 µm slices using a cryostat. Slices were examined with a digital microscope to verify electrode placements, using a rat brain atlas for reference (Paxinos & Watson, 1998). The accuracy of placements was assessed.
by an experimenter who was unaware of the corresponding electrophysiological data or rearing conditions. Data from rats with inaccurate placements were omitted from the analyses.

2.2.7 Statistical Analysis

All analyses were performed using SPSS (version 21.0, SPSS Inc., IL, USA). A minimum criterion of \( p < .05 \) was used to determine statistical significance.

For analysis of LTP levels, individual fPSPs were analyzed with mixed-model ANOVAs and pairwise comparisons when appropriate. The magnitude of LTP induction served as an index of plasticity levels. For analyses of PPRs, the amplitude of the first negative peak of each fPSP was averaged and analyzed for each ISI before and after LTP-induction. The PPR was calculated by dividing the peak amplitude of the second fPSP by the amplitude of the first. Statistical comparisons of PPRs (both before and after LTP induction) for rats reared under cWN and age-matched controls were conducted using mixed-model ANOVAs. The PPR served as an index of synaptic maturity and plasticity levels. The effect of LTP induction on PPRs was analyzed using a mixed-model ANOVA and pairwise comparisons.

2.3 Results

2.3.1 Histological Analyses

Typical placements of a stimulation electrode in the MGN and a recording electrode in the A1 are shown in Figure 2.1.
Figure 2.1. Electrode placements. Typical placements of the recording electrode in A1 (A) and the stimulating electrode in the MGN (B) for both LTP induction and paired-pulse stimulation experiments. Atlas images adapted from Paxinos and Watson (1998); A1: Bregma -5.8 mm; MGN: Bregma -6.3 mm.
2.3.2 The Effect of cWN Exposure on Long-Term Potentiation

The effects of rearing under cWN on long-term plasticity in the thalamocortical auditory system were examined using LTP induction \textit{in vivo}. Figure 2.2 illustrates a typical fPSP recorded in A1 following MGN stimulation before (in blue) and after (in red) LTP induction. The waveform consists of two negative-going peaks occurring at approximately 7 and 15 ms after the MGN stimulation artifact. Previous work has shown that MGN stimulation elicits an initial current sink in deeper, thalamo-recipient cortical layers (with a maximal depth of approximately 700-800 µm), and a subsequent, more superficial current sink (with a maximal depth of approximately 500-600 µm; Hogsden & Dringenberg, 2009b; Kaur et al., 2005). The distribution and latencies of these sinks indicate that the first and second, negative-going peaks likely reflect initial excitation of direct (layer IV) thalamocortical synapses, and subsequent excitation of intracortical synapses (layer II/III), respectively. This has been confirmed by pharmacological characterization of the peaks, with selective inhibition of intracortical activity resulting in strong suppression of only the second peak (Hogsden, Rosen, & Dringenberg, 2011).
**Figure 2.2.** Typical A1 fPSP. Typical fPSPs recorded in the A1 of an adult rat reared under cWN following a single pulse stimulation of the MGN. The blue and red traces represent fPSPs before and after LTP induction, respectively.

The levels of TBS-induced LTP of thalamocortical (peak 1) and intracortical (peak 2) synapses in rats reared under cWN (n = 15 for peak 1 and 14 for peak 2) and age-matched controls (n = 16) were analyzed using two mixed-model ANOVAs. For each test, the factors entered were rearing condition (between subjects; two levels: cWN or control), sex (between subjects; two levels: male, n = 17 for peak 1 and 18 for peak 2, or female, n = 14 for peak 1 and 12 for peak 2), and time interval (within subjects; 21 levels: 10-minute recording epochs 1 through 21).

For the first fPSP peak, there was no significant main effect of sex and no significant interaction effects involving sex, ps > .05. There was a significant main effect of time, $F(3.43,$
92.55) = 30.25, \( p < .001 \), such that fPSP amplitude increased over time intervals (there was a significant linear increase, \( F(1, 27) = 62.57, \ p < .001 \)). There was a significant main effect of condition, such that rats that were reared under cWN showed significantly higher LTP following TBS of the MGN (with an average fPSP amplitude during the last 10 minutes of recording of 33% above baseline), compared to age-matched controls (with an average fPSP amplitude during the last 10 minutes of recording of 15% above baseline), \( F(1, 27) = 6.11, \ p = .020 \). There was a significant condition by time interaction, such that the difference in LTP levels between conditions increased over time intervals, \( F(3.43, 92.55) = 3.57, \ p = .013 \).

For the second fPSP peak, there was no significant main effect of sex and no significant interaction effects involving sex, \( ps > .05 \). There was a significant main effect of time, \( F(5.25, 136.55) = 12.29, \ p < .001 \), such that fPSP amplitude increased over time intervals (there was a significant linear increase, \( F(1, 26) = 45.50, \ p < .001 \)). There was no significant main effect of rearing condition on LTP levels and no significant condition by time interaction (effect of condition, \( F(1, 26) = .05, \ p > .05 \); condition by time interaction, \( F(5.25, 136.55) = 1.23, \ p > .05 \)), with average fPSP amplitudes during the last 10 minutes of recording of 24% and 20% above baseline for rats reared under cWN and age-matched controls respectively (see Figure 2.3).
Figure 2.3. The effect of cWN exposure on thalamocortical LTP levels. The effect of rearing under cWN on LTP levels of the 1st (A) and 2nd (B) fPSP peak of adult rats following TBS of the MGN (indicated by arrows). Rats that were reared under cWN ($n = 15$ for peak 1 and 14 for peak 2) had significantly greater levels of LTP of thalamocortical synapses (peak 1) compared to age-matched controls ($n = 16$). There was no significant effect of cWN exposure on LTP of intracortical synapses (peak 2). Asterisks represent statistical significance, $p < .05$. 
2.3.3 The Effect of cWN Exposure on Paired-Pulse Responses

Responses to paired-pulse stimulation (with ISIs ranging from 25 to 1000 ms) of the MGN were examined in rats reared under cWN ($n = 12$ for pre-LTP and $n = 11$ for post-LTP) and age-matched controls ($n = 14$ for pre-LTP and $n = 11$ for post-LTP). Figure 2.4 illustrates a typical recording of two successive fPSPs in response to paired-pulse stimulation of the MGN.

![Typical fPSPs elicited in A1 following paired-pulse stimulation.](image)

**Figure 2.4. Typical fPSPs elicited in A1 following paired-pulse stimulation.** Typical fPSPs recorded in the A1 of adult rats reared under cWN following paired-pulse stimulation of the MGN. The dashed lines mark the amplitude of the first peak of each fPSP, and the arrow marks the difference in peak amplitude between the two. The trace depicted was elicited with an ISI of 125 ms, and illustrates an example of PPD, with the peak amplitude of the second fPSP being smaller than that of the first.

The PPRs for rats reared under cWN and age-matched controls (obtained prior to recording baseline fPSPs and again at the end of the experiment) were analyzed using two
mixed-model ANOVAs. Rats were excluded from the post-LTP analysis if they did not show LTP induction. The factors entered in each analysis were condition (between subjects; two levels: cWN or control), sex (between subjects; two levels: male, $n = 15$ for pre-LTP and $n = 13$ for post-LTP, or female, $n = 11$ for pre-LTP and $n = 9$ for post-LTP), and stimulus interval (within subjects; 8 levels: 25, 50, 75, 100, 125, 250, 500, and 1000 ms). Prior to LTP induction, there was no significant main effect of sex and no significant interaction effects involving sex, $p > .05$. The effects of condition, stimulus interval, and the condition by stimulus interval interaction were non-significant (condition, $F(1, 22) = .20, p > .05$; stimulus interval, $F(2.86, 62.81) = .75, p > .05$; condition by stimulus interval interaction, $F(2.86, 62.81) = .29, p > .05$).

Similarly, following LTP induction, there was no significant main effect of sex and no significant interaction effects involving sex, $p > .05$. The effects of condition and the condition by stimulus interval interaction were non-significant (condition, $F(1, 18) = 1.08, p > .05$; condition by stimulus interval interaction, $F(2.08, 37.39) = .35, p > .05$). There was a significant main effect of stimulus interval, $F(2.08, 37.39) = 3.81, p = .030$, such that PPRs increased with increasing stimulus intervals (there was a significant linear increase, $F(1, 18) = 4.84, p = .041$).
Figure 2.5. The effect of cWN exposure on PPRs before and after LTP induction. The effect of rearing under cWN on PPRs before (A) and after (B) LTP induction of adult rats (P50-60) following paired-pulse stimulation of the MGN (ISIs ranging from 25 to 1000 ms). There was no significant effect of rearing condition on PPRs before or after LTP induction.
2.3.4 The Effect of LTP Induction on Paired-Pulse Responses

Because there was no significant effect of rearing condition on PPRs before or after LTP induction, the two groups were collapsed. The effect of LTP induction on PPRs for rats in the combined group (cWN and control rats; \( n = 22 \)) was analyzed using a mixed-model ANOVA and pairwise comparisons. The factors entered were time (within subjects; two levels: pre-LTP and post-LTP), sex (between subjects; two levels: male, \( n = 13 \), or female, \( n = 9 \)), and stimulus interval (within subjects; 8 levels: 25, 50, 75, 100, 125, 250, 500, and 1000 ms). There was no significant main effect of sex and no significant interaction effects involving sex, \( p > .05 \). There was a significant main effect of time, \( F(1.00, 20.00) = 18.42, p < .001 \), as well as a significant time by stimulus interval interaction, \( F(2.67, 53.34) = 4.15, p = .013 \). Pairwise comparisons revealed that there was a significant enhancement of PPD for the first six of eight ISIs following LTP induction (\( p < .05 \)). See figure 2.6. There was no significant main effect of stimulus interval, \( F(2.51, 50.11) = 1.32, p > .05 \)
Figure 2.6. The effect of LTP induction on PPRs. The effect of LTP induction in adult rats on responses to paired-pulse stimulation of the MGN (ISIs ranging from 25 to 1000 ms). Rats \( n = 22 \) showed a moderate level of PPD prior to LTP induction. LTP induction resulted in a significant enhancement of PPD. Asterisks represent statistical significance, \( p < .05 \).

2.4 Discussion

The present experiment examined the effects of patterned acoustic deprivation through rearing under cWN on functional properties of synapses in the thalamocortical auditory system of adult (P50-60) rats. The fPSPs recorded in A1 following MGN stimulation obtained in the current experiment were similar in shape and latency to those obtained in previous studies (Hogsden & Dringenberg 2009a, b; Hogsden et al., 2011). As noted in section 2.3.2, the first, negative-going peak of the fPSP (with latency to peak of approximately 7 ms) reflects activation of direct thalamocortical synapses located in cortical layer IV, whereas the second, negative-
going peak (with latency to peak of approximately 15 ms) reflects activation of intracortical synapses located in cortical layers II and III.

The results of this experiment show that rearing rats under continuous, moderate-level white noise markedly alters long-, but not short-term synaptic plasticity in the adult thalamocortical auditory system.

2.4.1 cWN Exposure and LTP

As hypothesized, rats that were reared under cWN exhibited significantly greater levels of LTP induction in the thalamocortical auditory system in vivo, compared to age-matched controls. This effect has also been shown in previous work investigating the effects of patterned acoustic deprivation on LTP in the adult thalamocortical auditory system (Hogsden & Dringenberg, 2009b; Speechley et al., 2007).

Previous studies have established LTP as a reliable indicator of synaptic maturation and plasticity. Under normal environmental conditions, LTP levels decrease over development. For example, Hogsden and Dringenberg (2009a) showed an age-related reduction in LTP in the thalamocortical auditory system, with a significantly greater magnitude of LTP being induced in rats aged P30-35 compared to rats aged P40-45 and P100-110. Similar age-related reductions in LTP have been shown in the visual cortex (Jang et al., 2009; Kato, Artola, & Singer, 1991) and somatosensory cortex (Crair & Malenka, 1995). Thus, the present finding that a greater level of LTP can be induced in rats reared under cWN compared to age-matched controls indicates that rearing under cWN disrupts normal synaptic maturation and results in immature, more plastic synaptic connectivity in the adult thalamocortical auditory system.
Interestingly, there was no significant effect of rearing under cWN on LTP at intracortical synapses. This finding differs from those of previous experiments using the same cWN exposure protocol, which demonstrated that rearing under cWN results in increases in LTP induction at both thalamocortical and intracortical synapses (Hogsden & Dringenberg, 2009b). The reason for this discrepancy is unknown. One possible explanation is general variability in LTP levels induced in vivo. Alternatively, this discrepancy may reflect a difference in the sensitivity of thalamocortical and intracortical synapses to acoustic experience, with intracortical synapses requiring less acoustic stimulation for their maturation and thus being less sensitive to the effects of rearing under cWN.

2.4.2 cWN Exposure and PPR

Contrary to the hypothesis presented above, rearing under cWN did not alter the PPRs of thalamocortical synapses in vivo. Rather, rats in both rearing conditions showed a similar, moderate level of PPD prior to and following LTP induction. Given that changes in PPRs are generally assumed to reflect alternations in presynaptic functioning (e.g., vesicle release probability or Ca\(^{2+}\) influx into the presynaptic terminal; Atzori et al., 2001; Katz & Miledi, 1968), the present results likely indicate that patterned acoustic input does not play a dominant role in the maturation of the presynaptic component of the thalamocortical pathway. This hypothesis is supported by previous work identifying postsynaptic modifications associated with synaptic maturation under normal sensory conditions (Quinlan, Philpot, Huganir, & Bear, 1999; Ramoa & Prusky, 1997).
2.4.3 Acoustic Experience and Postsynaptic Mechanisms of Synaptic Maturation

Sensory-dependent synaptic maturation is associated with modifications at the postsynaptic cell membrane. For example, under normal acoustic conditions, there is a change in the expression of particular subunits of NMDARs, post-synaptic receptors that are required for LTP induction. NMDARs are heteromeric ion channels assembled from NR1 and NR2 subunits. The NR2 subunit contains the glutamate binding site and is found as four different isoforms: NR2A-NR2D (Monyer et al., 1992; Monyer, Burnashev, Laurie, Sakmann, & Seeburg, 1994). Much experimental evidence has characterized a developmental increase in the ratio of NR2A to NR2B NMDAR subunits at excitatory synapses in the neocortex and thalamus (Sheng, Cummings, Roldan, Jan, & Jan, 1994; Stocca & Vicini, 1998), driven by postnatal sensory experience (Ramoa & Prusky, 1997; Quinlan, Olstein, & Bear, 1999; Quinlan et al., 1999). For example, under normal acoustic conditions, there is a developmental increase in relative NR2A subunit expression in A1 over postnatal development, while NR2B expression is initially high and remains relatively stable or progressively decreases (Bi et al., 2006; Hsieh, Chen, Leslie, & Metherate, 2002).

Interestingly, Sun, Tang, & Allman, (2011) showed that, in rats reared under cWN, NR2B subunit expression remains elevated into adulthood compared to age-matched controls, whereas NR2A subunit expression does not differ between groups. Thus, following rearing under cWN, the adult NR2A:NR2B ratio more closely resembles that observed at immature synapses than mature synapses. Given that immature synapses exhibit higher plasticity levels, the maintenance of a more immature-like NMDAR subunit ratio may underlie the preservation of
LTP levels seen following rearing under cWN. Consistent with this hypothesis, NR2B subunit antagonism reverses the enhancement of LTP at both thalamocortical and intracortical synapses following rearing under cWN (Hogsden & Dringenberg, 2009b).

Based on these results, the increased LTP induction following rearing under cWN observed in this experiment was likely caused, at least in part, by a disruption of the developmental change in NMDAR subunit expression by patterned acoustic deprivation.

2.4.4 PPR and Mechanisms Underlying LTP

Extensive research has been conducted to determine the precise mechanisms underlying LTP. Although postsynaptic changes following LTP induction have been extensively examined (Malenka & Bear, 2004), presynaptic changes have also been shown following LTP induction. For example, Schulz et al. (1995) showed that LTP induction in hippocampal slices was associated with changes in the PPR. In their study, PPF decreased following LTP induction in slices that initially had high levels of PPF (and low release probability), whereas PPF increased following LTP induction in slices that had initially low levels of PPF (and high release probability).

In this experiment, LTP induction resulted in a significant enhancement of PPD expressed by thalamocortical synapses in A1. This effect reflects the relationship between synaptic maturity and paired-pulse responses, such that greater synaptic maturity is associated with greater depression of responses to trains of stimulation (Atzori et al., 2001). This change in PPRs following LTP induction suggests that LTP in A1 in vivo is mediated, in part, by
presynaptic mechanisms, such as increases in transmitter release probability, as well as postsynaptic mechanisms at thalamocortical synapses.

2.4.5 Future Directions

Previous work has demonstrated that neocortical development and synaptic maturation resume upon exposure to normal acoustic stimulation following rearing under cWN (Chang & Merzenich, 2003; Hogsden & Dringenberg, 2009b). Based on this, an interesting extension to the present study would be to examine methods of accelerating normal synaptic maturation in the thalamocortical auditory system upon exposure to patterned acoustic inputs following rearing under cWN. In this investigation, exposing rats to an enriched acoustic environment, such as that used by Bose et al. (2010), could be used to accelerate recovery of synaptic maturation following rearing under cWN. Alternatively, auditory discrimination learning tasks could be used to accelerate synaptic maturation. Enhancing the behavioural salience of auditory stimuli has been shown to drive neuronal receptive field changes in A1 more effectively than passive exposure to auditory stimuli (Kilgard & Merzenich, 1998). Thus, it is possible that auditory training could be used to accelerate synaptic maturation following rearing under cWN. This could be accomplished though the use of operant conditioning involving different frequency tones or sweeps as stimuli, followed by electrophysiological measures of synaptic maturity and plasticity.

Another interesting extension of the current study would be to investigate the effects of individual components of patterned sound on synaptic maturation and plasticity levels in the thalamocortical auditory system. Natural acoustic stimuli are composed of frequency and amplitude patterns, as well as complex temporal structures and behavioural salience. Despite
this, previous research has not explored the individual contributions of these components to synaptic maturation in the thalamocortical auditory system. Future experiments could attempt to separate these components to determine which, if any, are sufficient to drive synaptic maturation in isolation. Given the tonotopic organization of A1 (Lauter et al., 1985; Zhang et al., 2001), frequency patterns are likely the most instructive in guiding synaptic maturation in the thalamocortical auditory system. This hypothesis could be tested by rearing rats under continuous, moderate-level white noise with controlled, intermittent exposure to a set of pure tones within a predetermined frequency band. Alternatively, rats could be reared under cWN that randomly covers a range of amplitudes to determine whether amplitude patterns alone are effective in driving synaptic maturation. In adulthood, electrophysiological measures including LTP induction and paired-pulse stimulation could be used to assess synaptic maturity and plasticity levels following the particular acoustic stimulation.

Finally, future studies could characterize the differential mechanisms that underlie synaptic maturation in the thalamocortical auditory system due to natural acoustic experience and LTP. In this experiment, the change in paired-pulse responses following LTP induction indicated that thalamocortical LTP is mediated, in part, by presynaptic mechanisms, as well as postsynaptic mechanisms (Schulz et al., 1995). Conversely, the lack of change in paired-pulse responses following rearing under cWN indicated that, in contrast to postsynaptic plasticity mechanisms, the maturation of presynaptic components of the thalamocortical auditory system is less dependent on acoustic experience. It would be interesting to characterize the postsynaptic changes that occur during natural acoustic experience and determine how these changes are
disrupted by patterned acoustic deprivation. This would further elucidate the mechanisms by which patterned sensory deprivation disrupts synaptic maturation and extends developmental critical periods in sensory systems.

2.4.6 Conclusion

The results of the present experiment demonstrate that patterned acoustic deprivation through rearing under cWN disrupts synaptic maturation and leads to an enhancement of long-term synaptic plasticity in the thalamocortical auditory system of adult rats. The results of the present experiment also demonstrate that natural acoustic stimulation and LTP drive synaptic maturation through somewhat different mechanisms. In the absence of natural acoustic stimulation, postsynaptic neurons remain in a more immature, plastic state than under natural acoustic conditions. LTP induction acts, at least in part, through modifications of the presynaptic neuron, which do not appear to rely as strongly on acoustic experience as postsynaptic modifications, to undergo normal maturation. Future work is needed to further characterize these mechanisms of synaptic maturity.
Chapter 3

Structural Plasticity

3.1 Experiment 2

Given the well-defined relationship between neuron structure and function, it is expected that a functional change in long-term synaptic properties in the thalamocortical auditory system following patterned acoustic deprivation would be associated with structural changes. For example, LTP is associated with morphological changes at the level of dendritic spines, including increases in spine density and modifications of spine shape (Yuste & Bonhoeffer, 2001). In Experiment 1, I observed that cWN rearing was associated with greater levels of LTP induction in the thalamocortical auditory system of adult rats, and I hypothesized that this was due to a disruption of postsynaptic mechanisms underlying synaptic maturation. Such disruptions may be reflected in changes in dendrite and dendritic spine morphology.

The primary objective of this experiment was to characterize the effects of patterned acoustic deprivation through rearing under cWN on neuron morphology in the adult rat A1. This was accomplished through Golgi-Cox staining and two-dimensional reconstruction of layer II/III A1 pyramidal neurons. First, the effects of rearing under cWN on dendritic length and arbor complexity were examined by comparing the length and branch structure of basal and apical dendrites of rats reared under cWN and age matched controls. Next, the effect of rearing under cWN on dendritic spine density was measured by comparing average spine densities for basal and apical dendrites between rats reared under cWN and age-matched controls.
It was hypothesized that layer II/III A1 pyramidal neurons of rats reared under cWN will exhibit significantly lower dendritic length and arbor complexity, as well as spine density compared to age-matched controls. Specifically, it was hypothesized that this effect would only be seen at apical dendrites, as basal dendrites have been shown to be unaffected by acoustic deprivation (Bose et al., 2010).

3.2 Methods

See section 2.2 for details. Rearing was carried out as outlined in Chapter 2; however, histological rather than electrophysiological analyses were conducted on P50-60.

3.2.1 Golgi-Cox Staining

On P50-60, rats were deeply anesthetized with isoflurane and rapidly decapitated. Brains were removed and processed using the FD Rapid Golgistain™ kit (FD NeuroTechnologies Inc., MD, USA). Brains were immersed in FD Rapid Golgistain™ impregnation solution (5-7 ml per cubic centimeter of tissue) for 14 days. This time period was determined in pilot experiments by processing test sections at regular intervals between 10 and 14 days. The brains were stored at room temperature in the dark and the solution was refreshed at least once 24 hours following immersion. The brains were then immersed in solution C (5-7 ml per cubic centimeter of tissue) and stored at 4°C in the dark for five days. The solution was refreshed at least once 24 hours following immersion. After processing, brains were rapidly frozen in isopentane on dry ice and stored at -80°C until sectioned. Brains were sectioned coronally into 100 µm slices using a cryostat. Sections were mounted on glass slides coated with 1% gelatin in dH2O using solution C.
and allowed to dry for at least seven days. Sections were then rinsed in dH$_2$O, stained, dehydrated in graded ethanol, and cleared in Xylene. Slides were coverslipped and stored at room temperature in the dark until analyzed.

### 3.2.2 Morphological Analysis

Light microscopy was conducted using a Nikon Eclipse 80i light microscope (Nikon, Tochigi, Japan) equipped with a Microfire digital camera (Optronics, OK, USA). Neuron imaging and reconstruction was performed using Neurolucida and morphological analyses were performed using Neuroexplorer (MBF Bioscience, VT, USA).

Layer II/III A1 pyramidal neurons were identified by the presence a characteristic triangular soma, a basal dendritic arbor, and a single apical dendrite projecting toward the cortical surface and branching into a terminal tuft. Three criteria were used for neuron selection: (1) dark and consistent staining across the entire dendritic field, (2) relative isolation from neighboring stained neurons, and (3) non-truncated basal and apical dendrites. Three to seven layer II/III A1 pyramidal neurons were traced per rat at 20x objective magnification to create a two-dimensional reconstruction of each neuron.

Sholl analysis (Sholl, 1953) was performed by applying concentric rings (separated by 20 µm intervals, centered at the soma) over each neuron reconstruction. The following variables were measured separately for basal and apical dendrites: the number of dendritic intersections per sholl ring, the total dendritic length between each ring, the number of primary-order dendrites, the total and mean dendritic length, the number of nodes (bifurcation points), and the
number of ends (terminal branches). Values for each measure were averaged for each animal and then for each condition.

Spine density was quantified by tracing the dendritic segment within the most distal 25 µm squared area possible and counting the number of dendritic protrusions from the segment (completed using 100x objective magnification). All protrusions from the selected segment were counted. Spine density was averaged for three segments per neuron, then for each animal, and then for each condition. Separate measures of spine density (expressed as the number of spines per µm of dendrite) were calculated and averaged for basal and apical dendrites.

All data collection and analysis was conducted by an experimenter who was unaware of the rats’ rearing conditions.

3.2.3 Statistical Analysis

All analyses were performed using SPSS (version 21.0, SPSS Inc., IL, USA). A minimum criterion of \( p < .05 \) was used to determine statistical significance. Measures of dendritic length and arbor complexity were analyzed with mixed-model ANOVAs and independent-samples t-tests. Spine density was analyzed using independent-samples t-tests.

3.3 Results

3.3.1 Neuron Morphology

An example of a typical staining of the adult rat neocortex (including A1) obtained with the Golgi-Cox procedure is shown in Figure 3.1
Figure 3.1. Adult rat neocortex stained using the Golgi-Cox method. A typical image of the stained neocortex (including A1) of adult rats. The tissue was prepared using the Golgi-Cox specifications described in section 3.2.2 and imaged at 4x objective magnification using light microscopy and Neurolucida (MBF Bioscience, VT, USA).

The effects of rearing under cWN on morphological characteristics of layer II/III pyramidal cells in the adult A1 were examined using sholl and branch structure analysis, as well as spine density analysis. Figure 3.2 shows a two-dimensional reconstruction of a typical layer II/III A1 pyramidal neuron with a sholl ring (20 μm) overlay. The reconstruction consists of a flattened trace of the entire visible neuron and circular markers indicating the location of bifurcation nodes.
Figure 3.2. Typical layer II/III A1 pyramidal neuron reconstruction with sholl rings. Two-dimensional reconstruction of a typical layer II/III A1 pyramidal neuron with sholl rings placed at 20 µm intervals centered at the soma. Circular markers represent bifurcation nodes.

Basal and apical dendritic length and arbor complexity were compared between rats reared under cWN ($n = 7$) and age-matched controls ($n = 10$). A total of 92 neurons (cWN: $n = 38$, control: $n = 54$) was analyzed. The total length and number of intersections at each sholl ring for basal and apical dendrites were compared between conditions using four mixed-model ANOVAs. The independent variable in each analysis was condition (between subjects; two levels, cWN or control) and the dependent variables were basal length at each sholl ring (within subjects; 26 levels), basal intersections at each sholl ring (within subjects, 25 levels), apical length at each sholl ring (within subjects, 30 levels), and apical intersections at each sholl ring
(within subjects, 29 levels). No significant effect of condition was found for any of the dependent variables, $ps > .05$ (see Table 1 for $F$ and $p$ values).

Branch structure was analyzed using an independent-samples t-test for each variable. The independent variable in each analysis was condition and the dependent variables were: number of primary-order basal dendrites, number of basal nodes, number of basal ends, total basal length, mean basal length, number of apical nodes, number of apical ends, and total apical length. There was no significant effect of condition on any of the dependent variables, $ps > .05$ (see Table 2 for means and standard deviations).

### Table 1. $F$ and $p$ values for ANOVA analyzing effect of rearing condition on sholl data

<table>
<thead>
<tr>
<th></th>
<th>$F(1, 15)$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apical Intersections</td>
<td>1.50</td>
<td>.239</td>
</tr>
<tr>
<td>Apical Length</td>
<td>1.58</td>
<td>.229</td>
</tr>
<tr>
<td>Basal Intersections</td>
<td>1.12</td>
<td>.742</td>
</tr>
<tr>
<td>Basal Length</td>
<td>0.22</td>
<td>.647</td>
</tr>
</tbody>
</table>

### Table 2. Mean (standard deviation) dendritic length and arbor complexity

<table>
<thead>
<tr>
<th></th>
<th>Mean (Standard Deviation)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
</tr>
<tr>
<td></td>
<td>White Noise</td>
</tr>
<tr>
<td>Primary Dendrites</td>
<td>5.28 (.55)</td>
</tr>
<tr>
<td>Nodes</td>
<td>10.86 (1.78)</td>
</tr>
<tr>
<td>Ends</td>
<td>16.17 (2.14)</td>
</tr>
<tr>
<td>Length ($\mu m$)</td>
<td>1017.49 (213.11)</td>
</tr>
<tr>
<td>Mean Length ($\mu m$)</td>
<td>201.67 (31.73)</td>
</tr>
</tbody>
</table>

### 3.3.2 Spine Density

A typical second-order basal dendritic segment with spines is shown in Figure 3.3.
Figure 3.3. Typical basal dendritic segment of a layer II/III A1 pyramidal neuron. A typical second-order basal dendritic branch imaged at 100x objective magnification using light microscopy and Neurolucida (MBF Bioscience, VT, USA), with a 25 x 25 μm grid overlay.

Average dendritic protrusion densities for rats reared under cWN (n = 7) and age-matched controls (n = 10) were compared using two independent-samples t-tests. For comparison of total dendritic protrusion density, a total of 69 neurons (cWN: n = 31, control: n = 38) was analyzed. There was no significant difference in average dendritic protrusion density between the groups for either basal or apical dendrites, ps > .05 (see Table 3 for means and standard deviations, as well as t and p values).

The mean spine density values for apical and basal dendrites of control rats found in this experiment fall within the range previously reported for sensory cortex neurons in rodents (Clemo & Meredith, 2012; Lee, Chen, Chuang, & Wang, 2009).
Spine density was then reanalyzed for a portion of the rats (cWN: \( n = 4 \), control: \( n = 6 \)), by separately marking filopodia and spines and computing average density values using only the spine counts. A total of 38 neurons (cWN: \( n = 16 \), control: \( n = 22 \)) was analyzed. There was no significant difference in average spine density between the groups, \( ps > .05 \). There was a significant difference in the proportion of filopodia on apical dendrites, such that rats that were reared under cWN showed significantly higher proportions of filopodia on apical dendrites compared to age-matched controls, \( t(8) = 2.92, p = .019 \). There was no significant difference in the proportion of filopodia on basal dendrites, \( p > .05 \) (see Table 3 for means and standard deviations, as well as \( t \) and \( p \) values).

### Table 3. Mean (standard deviation), \( t \), and \( p \) values for dendritic spine density

<table>
<thead>
<tr>
<th></th>
<th>Basal</th>
<th></th>
<th></th>
<th>Apical</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White Noise</td>
<td>Control</td>
<td>( t )</td>
<td>( p )</td>
<td>White Noise</td>
<td>Control</td>
</tr>
<tr>
<td>Total Protrusions per ( \mu m )</td>
<td>.89 (.07)</td>
<td>.86 (.09)</td>
<td>.87 .398</td>
<td></td>
<td>.82 (.10)</td>
<td>.86 (.11)</td>
</tr>
<tr>
<td>Spines per ( \mu m )</td>
<td>1.31 (.12)</td>
<td>1.32 (.18)</td>
<td>.16 .877</td>
<td></td>
<td>2.13 (1.40)</td>
<td>1.31 (.19)</td>
</tr>
<tr>
<td>Percentage of Filopodia</td>
<td>.13 (.09)</td>
<td>.04 (.04)</td>
<td>2.04 .076</td>
<td></td>
<td>.13 (.06)</td>
<td>.04 (.04)</td>
</tr>
</tbody>
</table>

### 3.4 Discussion

The present experiment examined the effects of patterned acoustic deprivation through rearing under cWN on the morphological characteristics of layer II/III pyramidal neurons in the A1 of adult (P50-60) rats. The results of this experiment show that rearing under continuous, moderate-level white noise does not result in any significant differences in dendritic length, arbor
complexity, or spine density, but did result in a significant increase in the proportion of immature dendritic precursors, or filopodia, on apical dendrites, compared to normal acoustic experience.

### 3.4.1 cWN Exposure on Dendritic Morphology

Contrary to the hypothesis presented above, rearing under cWN did not alter dendritic length or arbor complexity of layer II/III A1 pyramidal neurons. This was likely due to the fact that the analyses in this study were restricted to measures of primary-order dendrites and overall dendritic length and arbor complexity, and included limited branch structure analyses. Supporting this, the structural changes to sensory cortex neurons caused by manipulations of sensory experience have been shown to be largely restricted to changes in the number or length of higher-order dendritic branches (Volkmar & Greenough, 1972; 1973). The number and length of primary-order dendrites are likely unaffected by manipulations of sensory experience because they are typically fully developed by the time such manipulations begin.

Contrary to the hypothesis presented above, rearing under cWN did not alter overall dendritic protrusion or mature spine density of apical or basal dendrites of layer II/III A1 pyramidal neurons. However, rearing under cWN did significantly increase the proportion of immature spine precursors, or filopodia, on apical dendrites. Under normal acoustic conditions, there is a developmental reduction in the ratio of filopodia to spines (Schachtele, et al., 2011) and an overall decrease in filopodium density (Grutzendler, Kasthuri, & Gan, 2002). Thus, the increased proportion of filopodia on apical dendrites following rearing under cWN likely indicates that cWN exposure resulted in a disruption of neuronal maturation, marked by an immature ratio of spine precursors to mature spines.
The fact that rearing under cWN did not significantly alter the proportion of filopodia on basal dendrites may reflect a lower degree of sensitivity of basal dendrites than apical dendrites to the effects of patterned acoustic deprivation. This is consistent with the finding of Bose et al. (2010) that acoustic deprivation through deafening significantly decreased apical dendritic length, while leaving basal dendritic length unchanged. Alternatively, the lack of significant effect of rearing under cWN on the proportion of filopodia on basal dendrites may have been due to the small sample size used in this analysis. This effect may become statistically significant with larger sample sizes.

3.4.2 Limitations

The major limitation of the present experiment was the basic branch order analysis conducted. Sensory manipulations have been shown to result in structural changes at the level of higher-order dendritic branches (Volkmar & Greenough, 1972; 1973), while leaving primary-order branches unchanged. It is possible that a more thorough branch structure analysis, including characterization of total and mean length and branching patterns for second-order dendrites, third-order dendrites and so on, could reveal a significant effect of patterned acoustic deprivation on dendritic length and arbor complexity.

Similarly, the present experiment was limited in its use of two-dimensional, rather than three-dimensional neuron reconstruction. In this experiment, serial reconstruction was not used and no correction was applied for converting the three-dimensional neuronal structure to a two-dimensional trace. Although the same technique has been used in previous examinations of the effects of sensory manipulations on neuronal morphology (Volkmar & Greenough, 1972; 1973)
and the relative difference between neurons has been shown to remain essentially constant through the conversion of the three-dimensional structure to a two-dimensional reconstruction (Coleman & Riesen, 1968), it is possible that rearing under cWN influenced dendritic length or arbor complexity in ways not observable using the current neuron reconstruction procedure. Future studies should use three-dimensional, serial reconstruction to obtain a more accurate visualization of the effects of rearing under cWN on dendritic length and arbor complexity in layer II/III A1 pyramidal neurons.

Another limitation of the present experiment was the restriction of analyses to layer II/III A1 pyramid neurons. These neurons comprise the postsynaptic component of the intracortical synapses, which correspond to the second peak of the fPSPs measured in Experiment 1 that appeared to be unaffected by rearing under cWN (see peak 2 LTP levels in section 2.3.2). Future work should examine the effects of rearing under cWN on the morphological characteristics of different A1 cell populations, such as layer IV stellate neurons. These neurons comprise the postsynaptic component of thalamocortical synapses, which correspond to the first peak of the fPSPs measured in Experiment 1 that was affected by rearing under cWN (see peak 1 LTP levels in section 2.3.2). Thus, it is likely that the morphological characteristics of layer IV stellate neurons were more strongly affected by patterned acoustic deprivation than those of layer II/III pyramidal neurons.

A final limitation, which applies to both experiments 1 and 2, was that they did not address the possibility of systematic litter effects. Differences in stress levels or maternal care between litters could have systematically affected a large proportion of the animals in each
rearing condition and strongly influenced group averages for both electrophysiological and morphological measures in the current experiments. Such litter effects could be reduced in future studies by increasing the number of litters included in each rearing condition to reduce the influence of individual litters on group averages. Unfortunately, this is often not feasible given practical constraints.

3.4.3 Future Directions

In the current experiment, we did not examine the effect of sex on neuronal morphology following rearing under cWN. Due to the limited sample sizes used in the morphological analyses, further division of the groups by sex would have resulted in insufficient power to identify significant sex differences. Although we did not observe any significant sex differences in synaptic maturation or long-term plasticity following rearing under cWN in Experiment 1, sex differences in morphological characteristics of A1 pyramidal neurons may exist during postnatal development. Supporting this, Mufioz-Cueto, Garcia-Segura and Ruiz-Marcos (1990) showed sex differences in spine density at apical dendrites of layer V pyramidal neurons in V1, such that females aged P10-20 had significantly greater spine densities than age-matched males. Spine density progressively decreased in females and increased in males from P20 to P60, resulting in similar spine densities at P60. Interestingly, ovariectomy of females prevented this developmental decrease. These authors later showed that the locus of the sex difference in spine density was age-dependent, with spine density differences shifting from initially more proximal to progressively more distal segments of the dendritic tree with increasing age (Mufioz-Cueto, Garcia-Segura & Ruiz-Marcos, 1991). These findings suggest that there are sex differences in
pyramidal cell maturation in the neocortex during early postnatal development. Future analyses should be conducted to determine if similar sex differences are present in the developing A1, and whether sex influences the effects of patterned acoustic deprivation on dendritic morphology and dendritic spine density in A1 pyramidal neurons.

Based on the lack of significant effect of rearing under cWN on dendritic spine density, an interesting extension of the current experiment would be to analyze the effects of rearing under cWN on dendritic spine size or shape. Changes in dendritic spine size and shape have been demonstrated in vivo and in vitro (Alvarez & Sabatini, 2007), and such changes likely occur more readily than changes in overall spine density. As a result, the size and shape of spines may be more strongly affected by sensory experience than the overall amount of spines. Patterned acoustic deprivation may drive synaptic maturation through modifications of the size and shape of existing spines, such as enlargement of spine heads or shortening of spine necks, or through a change in the expression of certain types of spines, rather than through an alteration of the overall amount of spines. Future studies should investigate this possibility to further characterize the mechanisms by which patterned sensory deprivation disrupts synaptic maturation and extends developmental critical periods in sensory systems.

3.4.4 Conclusion

The results of the present experiment demonstrate that patterned acoustic deprivation does not alter dendritic length, arbor complexity, or spine density of layer II/III pyramidal neurons in the adult A1. However, patterned acoustic deprivation results in an increase in the proportion of filopodia to mature spines on apical dendrites, indicative of a lower level of
neuronal maturity. One should be cautious in interpreting these finding, however, as several methodological limitations may have influenced these results. Future work should be conducted to determine the accuracy and generalizability of the present findings.
Chapter 4

Summary

In summary, the experiments described in the present thesis investigated the effects of patterned acoustic deprivation during the critical period of A1 development on the functional and structural characteristics of neurons in the adult rat thalamocortical auditory system. The results of these experiments demonstrate that patterned acoustic deprivation through rearing under continuous, moderate-level white noise disrupts synaptic maturation and prolongs periods of heightened plasticity in the rat thalamocortical auditory system. This finding highlights the importance of patterned sensory experience in postnatal sensory system development.

The results of this study also demonstrate that natural acoustic stimulation and LTP drive synaptic maturation in the thalamocortical auditory system through somewhat different mechanisms. Specifically, natural acoustic experience appears to cause synaptic maturation through modifications of the postsynaptic neuron, whereas LTP appears to do so through modifications of the presynaptic neuron.

Finally, the results of this study demonstrate that patterned acoustic deprivation results in reduced maturation of layer II/III A1 pyramidal neurons at the level of dendritic spines.

Future work is required to better understand the mechanisms by which patterned sensory experience instructs synaptic maturation, and how patterned sensory deprivation disrupts this maturation and extends developmental critical periods in sensory systems.
References


heterogeneity, and roles in vigilance. *Behavioural Brain Research, 115*(2), 117-141.


Valverde, F. (1971). Rate and extent of recovery from dark rearing in the visual cortex of the mouse. *Brain Research, 33*(8), 1-11.


in kittens. *Journal of Neurophysiology, 28*(6), 1060-1072.


