Experience-dependent plasticity in the adult rat auditory cortex induced by passive exposure to white noise

Maryse Elisabeth Thomas

Integrated Program in Neuroscience

McGill University, Montreal, Canada

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Abstract

The ability of the brain to change in response to its external environment is known as experience-dependent plasticity. Robust experience-dependent plasticity is typically restricted to early stages of life, when developing neural circuits are readily shaped by passive sensory experience. In the auditory system, for example, exposing juvenile but not adult rats to pure tones produces a functional over-representation of the tone frequency in the cortical tonotopic map. Recent studies have revealed the continued potential for passive experience to induce robust plasticity in the adult brain, however. In particular, chronic exposures to uninformative or disruptive sounds, such as white noise, have been shown to alter experience-dependent plasticity in the adult auditory cortex, returning the brain to a more plastic and juvenile state. This phenomenon provides an opportunity to study unprecedented cortical plasticity late in life, yet also reveals the brain's vulnerability to abnormal sensory environments. Tackling both issues, the present thesis uses white noise as a tool to probe experience-dependent plasticity in the adult rat auditory cortex in three studies. In the first study, passive exposures to non-traumatic white noise of varying amplitude modulation depths are used to show the importance of salient temporal inputs for mature auditory function. Exposure to unmodulated but not modulated noise induces juvenile-like plasticity and frequency over-representation in response to a second exposure to pure tones, demonstrating that white noise triggers plasticity by masking temporal inputs from the environment. Since greater functional representation is generally thought to improve perceptual discrimination, the hypothesis that noise-induced plasticity could be used to improve adult perceptual learning is tested in the second study. Contrary to our expectations, sound-exposed animals were worse at discriminating the over-represented frequency,

demonstrating that increased functional representation is not sufficient to improve discrimination. Finally, the third study investigates the possibility that changes in neural activity induced by noise exposure could be indicative of maladaptive plasticity leading to aberrant or unwanted perceptual consequences. Common neural and behavioral correlates of the auditory disorders tinnitus and hyperacusis were assessed in noise-exposed animals. Evidence of hyperacusis in exposed rats suggests that noise exposure opens windows of plasticity that may be understood as windows of vulnerability to maladaptive plastic changes. The results presented in this thesis help to elucidate the mechanisms and perceptual consequences of noise-induced plasticity in the adult rat auditory cortex. They describe the profound impact of noise on brain structure and function, advance our present understanding of experience-dependent plasticity in sensory circuits, and demonstrate how sensory environments may powerfully influence the brain throughout life.

Résumé

Plasticité cérébrale du cortex auditif chez le rat adulte induite par l'exposition passive au bruit blanc

La plasticité liée à l'expérience est un terme généralement utilisé pour désigner la capacité innée du cerveau à se modifier en réponse à son environnement externe. Ce type de plasticité est normalement limité aux premiers stades de la vie, lorsque les circuits neuronaux en développement sont plus facilement façonnés par l'expérience sensorielle passive. Des avancées scientifiques récentes ont toutefois révélé qu'il est également possible d'induire des changements plastiques dans le cerveau adulte à la suite d'une stimulation sensorielle passive. Plus spécifiquement, il a été démontré qu'une exposition chronique à des sons continus non informatifs ou perturbateurs, tels que le bruit blanc, induit un état plastique au sein du cortex auditif adulte, produisant ainsi des propriétés neuronales similaires à celles observées au sein du cerveau juvénile. Ce phénomène offre l'occasion d'étudier un nouveau type de plasticité corticale adulte, mais révèle toutefois la vulnérabilité du cerveau aux environnements sensoriels anormaux. Pour mieux comprendre ce phénomène, la présente thèse utilise la présentation chronique de bruit blanc comme outil pour sonder la plasticité liée à l'expérience dans le cortex auditif du rat adulte par le biais de trois études complémentaires. Dans la première étude, nous avons exposé les rats à du bruit blanc ayant différents niveaux de modulation d'amplitude pour montrer l'importance des signaux temporels saillants pour maintenir une fonction auditive adulte. Lorsque le bruit blanc n'est pas modulé temporellement, il induit une plasticité corticale semblable à celle observées chez les juvéniles, ce qui suggère que le bruit blanc déclenche la plasticité en masquant les signaux temporels de l'environnement. Une caractéristique

déterminante de la plasticité de type juvénile est la possibilité de produire une surreprésentation corticale fréquentielle suite à une exposition passive à un ton pur répété. Puisqu'il est généralement convenu qu'une plus grande représentation corticale est associée aux meilleures habiletés de discrimination perceptuelle, la seconde étude avait pour objectif de tester l'hypothèse selon laquelle la plasticité induite par la présentation chronique de bruit blanc pourrait être utilisée pour améliorer l'apprentissage perceptuel chez l'adulte. Contrairement à nos attentes, bien que les animaux aient développé une surreprésentation corticale spécifique à la fréquence du ton pur à laquelle ils ont été exposée, ils ont toute fois démontré des déficits de discrimination fréquentielle lorsque comparer à des pairs non-exposés. Enfin, la troisième étude avait pour but d'examiner la possibilité que les changements dans l'activité neuronale induits par une exposition chronique au bruit blanc puissent indiquer une plasticité inadaptée menant à des conséquences perceptuelles aberrantes ou indésirables. Des corrélats neuronaux et comportementaux standards associés aux troubles auditifs tels que l'acouphène et l'hyperacousie ont été évalués chez des animaux exposés chroniquement au bruit blanc. Les similitudes observées entre la plasticité induite par le bruit et l'hyperacousie suggèrent que l'exposition au bruit ouvre des fenêtres de plasticité qui sont potentiellement des fenêtres de vulnérabilité aux modifications plastiques inadaptées. Les résultats présentés dans cette thèse permettent de mieux comprendre les mécanismes et les conséquences perceptuelles de la plasticité induite par le bruit dans le cortex auditif du rat adulte. Ils décrivent l'impact profond du bruit sur la structure et la fonction du cortex auditif, font progresser notre compréhension actuelle de la plasticité liée à l'expérience au sein des circuits sensoriels et démontrent l'influence importante que peut exercer notre environnement sensoriel sur le cerveau tout au long de la vie.

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Contribution to Original Knowledge

The present thesis used passive exposure to moderate-intensity white noise as a tool to probe experience-dependent plasticity in the adult rat auditory cortex. This approach extends only a handful of previous studies that established the plasticity-inducing effects of noise, thereby greatly contributing to the literature on this subject. In three studies, the conditions of noise exposure that lead to juvenile-like plasticity, and the potential positive and negative aspects of this plasticity were explored. The first study published in *Cerebral Cortex* provided evidence that noise exposure triggers juvenile-like plasticity by masking salient temporal inputs from the acoustic environment. This finding underlines the important role of high-fidelity sensory experience in the maintenance of adult cortical circuits. The second study accepted for publication by *Journal of Neuroscience* assessed the perceptual consequences of juvenile-like plasticity induced by noise. Since the ability to alter cortical sensory maps in adulthood could have applications for learning and memory, this was an important first step in understanding the neurotherapeutic potential of noise exposure. Sound-exposed rats were observed to have impaired perceptual discrimination; however, this deficit was recovered with training, providing support for the use of cognitive training-based strategies to recover frequency-specific perceptual deficits. The final study published in *Frontiers in Systems Neuroscience* investigated the similarities between noise-induced plasticity and the central auditory conditions tinnitus and hyperacusis. Symptoms of hyperacusis in noise-exposed animals reveal the brain's increased vulnerability during periods of enhanced plasticity. These results demonstrate the profound impact of noise exposure on cortical plasticity, behavior, and perception and should encourage the continued investigation of the phenomenon of noise-induced plasticity.

Contributions of Authors

Chapter 2: Thomas M, Friedman N, Cisneros-Franco JM, Ouellet L, de Villers-Sidani E.

MT, JMCF, and EVS designed the study. MT performed the experiments and acquired the electrophysiological data. LO performed the histological staining and microscopy. NF contributed original scripts to analyze the data. MT analyzed the data. MT wrote the first draft of the manuscript. All authors reviewed the manuscript. Additional support came from Christina Chou, who assisted in some of the experiments and Patrice Voss, who edited the manuscript.

Chapter 3: Thomas M, Lane C, Chaudron Y, Cisneros-Franco JM, de Villers-Sidani E.

MT and EVS designed the study. MT performed the experiments. MT and CL acquired the electrophysiological data. MT, YC, and JMCF trained the animals. YC contributed original scripts to analyze the data. MT analyzed the data. MT wrote the first draft of the manuscript. All authors reviewed the manuscript. Additional assistance came from Patrice Voss, who edited the manuscript.

Chapter 4: Thomas M, Guercio G, Drudik K, de Villers-Sidani E.

GG, and EVS designed the study. MT, GG, and KD performed the experiments. GG and KD obtained the behavioral data. MT obtained the electrophysiological data. MT analyzed the data. MT wrote the first draft of the manuscript. All authors reviewed the manuscript.

All co-authors have approved the inclusion of each manuscript in the present thesis.

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List of Key Abbreviations

A1	Primary auditory cortex
AAF	Anterior auditory field
AM	Amplitude-modulated
ATD	Adaptive tone discrimination
BF	Best frequency
BW	Bandwidth
BW20	Bandwidth 20 decibels above threshold
CF	Characteristic frequency
CP	Critical period
CV	Coefficient of variation
EI	Excitatory/inhibitory
FP	False positive
GABA	Gamma-aminobutyric acid
GLMM	Generalized linear mixed model
GPIAS	Gap-prepulse inhibition of the acoustic startle
HPF	High-power field
IC	Inferior colliculus
ISI	Inter-spike interval
MGB	Medial geniculate body
PAF	Posterior auditory field
PNN	Peri-neuronal net
PPI	Prepulse inhibition
PSTH	Peri-stimulus time histogram
PV	Parvalbumin
SRAF	Supra-rhinal auditory field
SST	Somatostatin
V1	Primary visual cortex
VAF	Ventral auditory field
VIP	Vasoactive intestinal polypeptide

List of Publications

1. Voss P, Thomas M, Chou Y, Cisneros-Franco JM, Ouellet L, de Villers-Sidani E. (2016) Pairing cholinergic enhancement with perceptual training promotes recovery of age-related changes in rat primary auditory cortex. *Neural Plasticity*. 2016:1-18.

2. ^Voss P, ^Thomas M, Cisneros-Franco JM, de Villers-Sidani E. (2017) Dynamic brains and the changing rules of neuroplasticity: Implications for learning and recovery. *Frontiers in Psychology*. 8:1657. ^*These authors contributed equally to this work*.

3. Thomas M, Friedman N, Cisneros-Franco JM, de Villers-Sidani E. (2019) The prolonged masking of temporal acoustic inputs with white noise drives plasticity in the adult rat auditory cortex. *Cerebral Cortex*. 29(3):1032-1046.

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5. Guercio G, Thomas M, Cisneros-Franco JM, Voss P, Panizzutti R, de Villers-Sidani E. (2019) Improving cognitive training for schizophrenia using neuroplasticity enhancers: Lessons from decades of basic and clinical research. *Schizophrenia Research*. 207:80-92.

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7. Cisneros-Franco JM, Voss P, Kang MS, Thomas M, Côté J, Ross K, Gaudreau P, Rudko DA, Rosa-Neto P, de Villers-Sidani E. (2020) PET Imaging of Perceptual Learning-Induced Changes in the Aged Rodent Cholinergic System. *Frontiers in Neuroscience*. 13:1438.

8. Cisneros-Franco JM, Thomas M, Regragui I, Lane C, Ouellet L, de Villers-Sidani E. (2020) Chronic downregulation of parvalbumin-positive cell activity accelerates early perceptual learning but impairs end performance. *bioRxiv*.

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Chapter 1: General Introduction

Sections 1.1 and 1.2.1 are reproduced from Voss, Thomas, Cisneros-Franco, de Villers-Sidani. (2017) Frontiers in Psychology. 8:1657(2,4).

The ability of the brain to change in response to its external environment is known as experience-dependent plasticity. Early in life, sensory experiences via touch, taste, sight, smell, and hearing shape neural circuits through plastic mechanisms, ensuring the proper development of basic sensory areas of the brain as well as determining individual variability at a young age. As a result, abnormal or absent sensory experiences during this period can profoundly impact neural development. The continued and important influence of sensory experience on mature brain function has been somewhat overlooked, however, due to decades of scientific thinking that the adult brain was fixed once developed. In reality, a growing number of studies have shown that introducing statistical variations, such as noise, into the sensory environment can have unprecedented effects on adult sensory function. The present thesis will describe a series of studies in which acoustic noise is used to probe adult auditory cortical plasticity. Their results describe the profound impact of noisy inputs on brain structure and function and advance our present understanding of how sensory environments influence the brain throughout life.

1.1 Experience-dependent plasticity in the developing and mature brain

1.1.1 Critical periods for experience-dependent plasticity

Age is a key determinant of experience-dependent cortical plasticity. Important structural and functional changes tend to predominantly occur early in life during time-limited epochs of stimulus-driven plasticity known as critical periods (Knudsen 2004). A well-known example of

this limited time-window was provided by the classic monocular visual deprivation studies of Wiesel and Hubel (1963). Critical periods (CPs) have since been described in all major sensory systems and in a variety of animal species and their identification has been instrumental in the discovery of the cortical machinery involved in their regulation (see Hensch 2005 for a review). Many studies of CP plasticity have focused on the rat primary auditory cortex (A1) model, which displays a succession of partially overlapping CPs for various stimulus parameters during development (de Villers-Sidani and Merzenich 2011). For example, frequency tuning has the earliest and shortest CP in the auditory system (around days 11–14 of life), whereas CPs for more complex sound representations, such as frequency modulation tuning, tend to occur slightly later during early infancy (around days 25–33) (Insanally et al. 2009). Several sensitive periods have also been identified in humans, particularly as they relate to hearing restoration following prelingual deafness and language acquisition. Current evidence suggests that the optimal time for cochlear implantation is before 4 years of life and that implantations performed after 7 years are unlikely to produce satisfactory results (see Kral and Sharma 2012). Although typically associated with early developmental stages, there is a growing body of evidence demonstrating that CPs can be reopened later in life due to a variety of factors that are still being uncovered. These include damage to peripheral sensory organs (Chino et al. 1992; Diamond et al. 1993; Van Brussel et al. 2011) and changes in the sensory environment (He et al. 2006; Zhou et al. 2011). Recent work has shown that plastic changes in auditory cortex that normally occur within early CPs can even be observed in aging humans and rodents (de Villers-Sidani et al. 2010; Mishra et al. 2014). This suggests that the elements that regulate plasticity change throughout the lifespan and do not only operate around developmental CPs.

1.1.2 Plasticity inhibitors and cellular brakes

With CP closure, sensory representations are stabilized (Rice and Van der Loos 1977; Fagiolini et al. 1994; Zhang et al. 2002; de Villers-Sidani et al. 2007). This process requires the maturation of inhibitory (GABAergic) cellular networks and the maintenance of sufficient GABAergic tone in the cortex (Hensch 2005; Fritschy and Panzanelli 2014). Any further modification of these networks and associated cortical plasticity is regulated by a series of plasticity inhibitors and molecular brakes, so-called because of their role in limiting plasticity in the mature brain (see Hensch 2005; Bavelier et al. 2010 for reviews). Functional and structural elements that promote and constrain plasticity include the inhibitory activity of GABAergic interneurons such as parvalbumin positive (PV+) cells (Kuhlman et al. 2013), extracellular matrix components including perineuronal nets (PNNs) (Wang and Fawcett 2012), and myelin associated proteins (McGee et al. 2005). For a summary of these elements, see **Figure 1.1C**.

Throughout life, the proportion of GABAergic interneurons in the cortex remains relatively stable. However, the number of PV+ and somatostatin positive (SST+) interneurons decreases with age, indicating that different interneuron subtypes are differentially affected by aging (Stanley et al. 2012; Ouellet and de Villers-Sidani 2014). Furthermore, PV staining intensity has been shown to be positively correlated with the degree of experience-dependent plasticity (de Villers-Sidani et al. 2008; Zhou et al. 2011). Adult brain CP-like plastic remodeling can be induced by down-regulating cortical inhibition (Fagiolini and Hensch 2000) or disrupting PNNs (Pizzorusso et al. 2002; McRae et al. 2007; Wang and Fawcett 2012) or myelin (Kartje et al. 1999; McGee et al. 2005), which form structural barriers to limit plasticity and stabilize cortical representations. Loss of inhibition during aging could lead to a state of cortical instability where sensory representations are easily distorted by non-specific passive experiences as is the case during CPs (Zhou et al. 2011)

(**Fig. 1.1A-B**). Indeed, [our group] recently observed that experience-dependent plasticity is not only paradoxically enhanced, it is also unstable (i.e., producing plastic changes that decayed rapidly in time) in old rats compared to young controls, and was paralleled by a reduction in PV+ cell density, GABA concentration, and PNNs (Cisneros-Franco et al. 2018). We also found that passive distortions of the auditory map decayed rapidly, indicating an ongoing instability of A1 tuning in the aging cortex. These observations led us to propose that the inhibitory regulation of plasticity, rather than plasticity per se, is reduced in the aged brain. This finding has important repercussions for the development of rehabilitation strategies targeted toward aging and opposes the traditional view that aging is a period of limited plasticity.

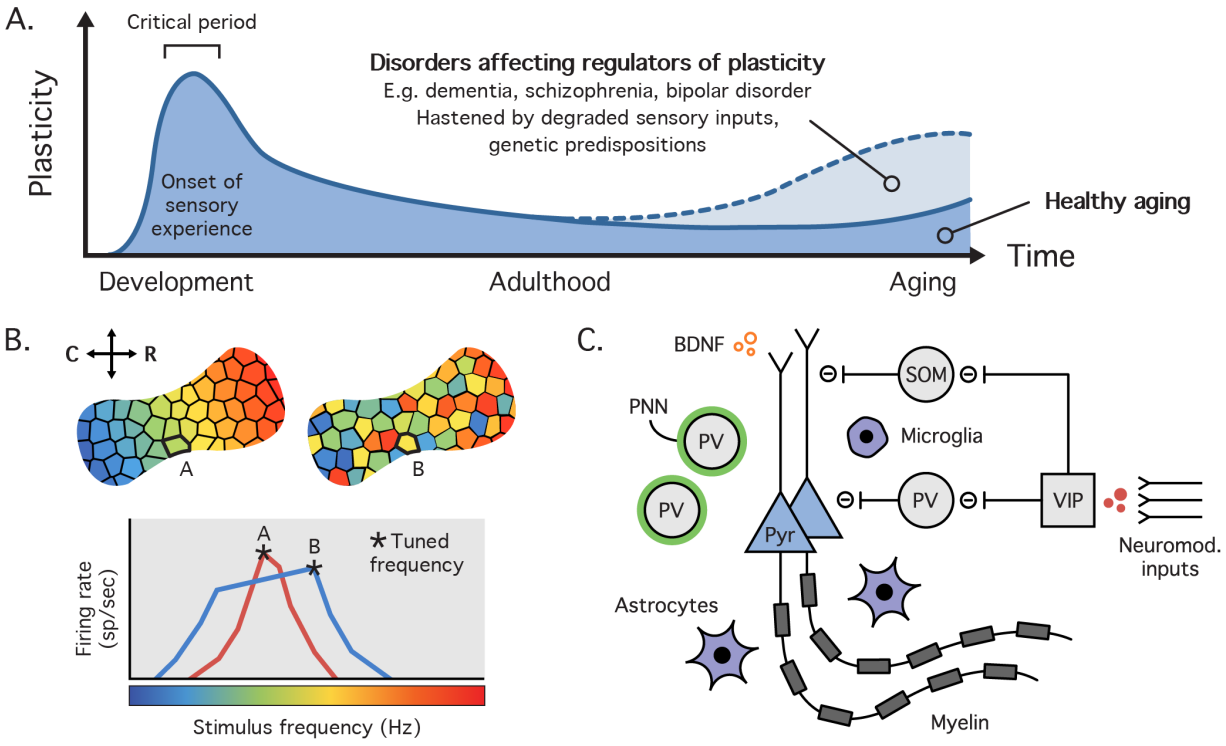

Figure 1.1: *Regulation of experience-dependent plasticity*. **A.** Trajectory of experience-dependent plasticity during the lifetime. The onset of sensory experience triggers the opening of critical period windows during which the sensory cortex is rapidly organized in response to passive stimulation from the external environment. With maturation, the critical period closes and sensory representations are stabilized. Plasticity continues to take place during adulthood but is tightly regulated by a variety of cellular and molecular processes. These mechanisms tend to decrease with age allowing for non-specific passive experience to elicit plasticity during aging. Disorders that affect regulators of

plasticity increase the likelihood for maladaptive plastic changes to take place in the brain. **B.** Auditory tonotopic map plasticity. Example of a mature tonotopic map from the rat primary auditory cortex (top left) and that of a rat demonstrating irregular plasticity (top right). The tonotopic map typically exhibits a smooth gradient with neurons in the most caudal (C) part of the cortex firing preferentially (or tuned to) low frequencies and neurons in the most rostral (R) part tuned to high frequencies. In unusual plastic states, such as aging and after long-term exposure to white noise, this functional gradient becomes disrupted as tuning of individual neurons becomes less selective (bottom). For example, a neuron's tuning may shift from being narrow and selective (site A – red line) to broad and flat peaked (site B – blue line), sometimes altering its tuning frequency (A,B based on Cisneros-Franco et al. 2018; Thomas et al. 2018). **C.** Some of the major regulators of plasticity in the auditory cortex. Plasticity regulators limit plasticity in the mature brain by controlling the activity of excitatory cells, primarily pyramidal (Pyr) neurons. They include cells such as inhibitory interneurons and glia, structural molecules like peri-neuronal nets (PNNs) and myelin associated proteins, neuromodulatory control from other brain regions, and neurotrophic factors. *Figure reproduced from Voss et al. 2017.*

1.2 Sensory inputs reaching the brain influence the rules of plasticity

1.2.1 The quality and quantity of sensory inputs affect the timing of CP windows

Studies of CPs have demonstrated the importance of sensory experience for normal neurodevelopment and sensory map acquisition. The quality and quantity of sensory experience, however, can have diverse effects on CP duration and outcome. [Both enriched sensory environments and deprived or unstructured noisy environments can prolong the CP into adulthood (Greifzu et al. 2014; Cynader and Mitchell 1980; Mower 1991). However, enriched environments stimulate dendritic growth (Leggio et al. 2005; Bose et al. 2010) and improve neuronal response properties (Engineer et al. 2004; Feldman 2005), whereas deprived environments prevent the development of mature response properties (Fagiolini et al. 1994)]. In general, the excess presence of a specific stimulus during the CP appears to result in its exaggerated incorporation into the sensory map. For instance, altering the visual environment of the kitten through striped surroundings (Sengpiel et al. 1999) or goggles (Tanaka et al. 2009) shifts the orientation selectivity of visual cortical neurons to prefer the dominant orientation of their environment. In auditory cortex, pure tone pips of a chosen frequency played continuously result in the overrepresentation

of that frequency within the tonotopic map (Zhang et al. 2001; de Villers-Sidani et al. 2007). However, there is evidence for hardwired preferences for ethologically relevant stimuli such as tone pips played at a temporal modulation rate similar to that of communication (Kim and Bao 2009) and vocalizations from members of the same species (Soha and Marler 2001). The quantity of salient stimuli present during development can also affect the timing of CP closure. Exposure to temporally modulated white noise produces a shorter than usual CP for spectral tuning in auditory cortex, whereas the masking of normal auditory inputs with continuous white noise keeps it open indefinitely (Zhang et al. 2002; Chang and Merzenich 2003, **Fig. 1.2**). [Similarly, pups raised in the presence of a continuous tone but not pulsed tones also stay in CP longer, irrespective of the tone's frequency (Zhou et al. 2008, **Fig. 1.2**). The contrasting effects between exposure to modulated (Zhang et al. 2002; Zhou and Merzenich 2012) and unmodulated noise (Chang and Merzenich 2003) listed above provide evidence that the temporal structure of noise has strong influence on auditory cortical processing. Finally, these effects can be restricted to functional regions of the cortex as exposure to bandlimited noise results only in the selective functional and inhibitory maturation of sectors of the tonotopic map outside the noise band] (de Villers-Sidani et al. 2008, **Fig. 1.2**).

1.2.2 The absence of sensory experience promotes plasticity in mature cortices

Just as normal sensory experience is important for neurodevelopment, the continued presence of sensory inputs is necessary for adult brain function. The most striking and intuitive examples of this are instances in which sensory experience is interrupted by injury to the sensory organs or peripheral denervation, leading to massive cortical change due to disconnect between the periphery and cortex. For instance, somatosensory cortical maps are reshaped following digit

amputation in adult owl monkeys (Merzenich et al. 1984), retinotopic cortical maps of adult cats shift in response to monocular retinal lesions (Chino et al. 1992), and functional reorganization of the barrel cortex has been observed in adult rodents after whisker trimming (Diamond et al. 1993; Maier et al. 2003). Common to these studies is the finding that neural activity in the 'disconnected' regions of cortical maps becomes driven by the spared sensory structures. This plasticity is likely facilitated in the short-term by disinhibition (Kelly et al. 1999) and in the long-term by structural changes in synapses that strengthen or generate new horizontal connections (Fox 2002). Both cortical sprouting and synaptogenesis have been observed following either peripheral or central deafferentation (Florence et al. 1998; Yamamoto et al. 2000; Chen et al. 2002; Garcia Del Caño et al. 2002). There is also evidence for cross-modal plasticity following sensory deprivation in a single sensory faculty (Van Brussel et al. 2011; Teichert, Isstas, Wieske, et al. 2018; Teichert, Isstas, Zhang, et al. 2018), indicating that plastic changes following this type of insult are far-reaching. However, physical injury is not necessarily required to observe such large-scale changes, as reorganization of cortical maps can also follow the mere disuse of peripheral structures (Allard et al. 1991; Kaneko et al. 2003).

1.3 Experience-dependent plasticity induced by passive exposure to white noise

1.3.1 Noise exposure induces CP plasticity in the adult rat auditory cortex

The first studies of noise exposure in adult animals asked whether masking sensory inputs from the acoustic environment could produce cortical plasticity similar to sensory deprivation. The random acoustic signal white noise is a sound produced by combining a continuum of audible frequencies at equal intensities. Noise is an efficient masker and reduces the ability of the auditory system to detect and identify other sounds when present (Arlinger and Gustafsson 1994; Phatak et

al. 2008), making it a powerful tool with which to test the influence of random stimulation on biological systems. Zhou and colleagues (2011, **Fig. 1.2**) observed a transition to CP-like plasticity in adult rats passively exposed to seven weeks of around-the-clock, moderate-intensity (65dB) broadband white noise. They found significant changes in A1 cortical organization and function that resembled the juvenile cortex, including absence of a typical tonotopic gradient, reduced tuning selectivity, and decreased spontaneous synchronization between neurons. To test the plastic capacity of noise-exposed rats, Zhou and colleagues performed a second exposure to tone pips for one week and witnessed an over-representation of this tone within the adult tonotopic map, directly in line with CP plasticity. These findings were later confirmed by Zheng (2012, **Fig. 1.2**), who observed map reorganization in adult rats exposed to noise for 30 days and by our lab (Kamal et al. 2013), which documented map reorganization, reduced tuning selectivity, and desynchronization in adult rats exposed to noise for eight weeks, although neither study submitted their exposed rats to a second tone pip exposure. Even so, the ability for passive sensory stimulation to induce cortical changes of this nature in the adult brain was unprecedented, leading noise-induced plasticity to be described as a "natural restoration of critical period plasticity" (Zhou et al. 2011).

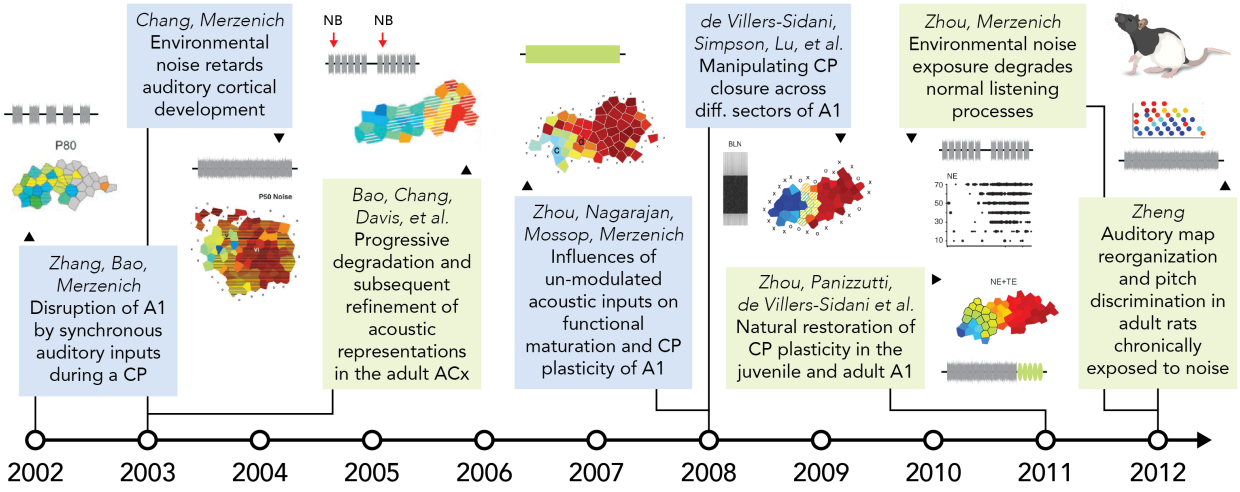

Figure 1.2: *Timeline of notable experiments of passive sound exposure*. Notable studies of passive sound exposure demonstrate the opposing effects of temporally modulated and unmodulated sound on tonotopic organization in the immature (blue boxes) and mature (green boxes) A1. Temporally modulated stimuli hasten CP closure in the immature cortex and degrade auditory processing in the adult cortex while unmodulated stimuli postpone CP closure in the developing brain and reinstate CP-like plasticity in the mature brain. From left to right: Zhang et al. 2002; Chang and Merzenich 2003; Bao et al. 2003; de Villers-Sidani et al. 2008; Zhou et al. 2008; Zhou et al. 2011; Zheng 2012; Zhou and Merzenich 2012.

1.3.2 Mechanisms of noise-induced plasticity

Studies of noise-induced plasticity also identified cellular and molecular changes that resembled the immature brain. Zhou and colleagues observed a decrease in the expression of specific GABA-A and NMDA receptor subunits, components that accelerate the CP (Berardi et al. 2003; Fagiolini et al. 2004). This was accompanied by a reduced expression of the trophic factor BDNF, which contributes to GABAergic innervation and inhibition during the CP (Huang et al. 1999). Our group further confirmed a decrease in inhibitory tone following noise exposure, reporting decreased GABA+ and PV+ cell counts, as well as reduced myelin staining in noise-exposed rats (Kamal et al. 2013). Accompanied by a return to baseline in functional plasticity, the above changes were all partially or completely reversed in rats that were returned to a normal acoustic environment for eight weeks after noise exposure (Zhou et al. 2011; Kamal et al. 2013) in good agreement with the notion that molecular brakes can be dynamically down- and upregulated throughout life. The above findings are limited, however, and a more complete account of the cellular and molecular mechanisms that contribute to noise-induced plasticity is lacking.

1.4 Neurotherapeutic potential of noise-induced plasticity

1.4.1 Sound-based neurotherapeutics

Given its ability to non-invasively and transiently drive youth-like plasticity in the adult auditory cortex, the question remains whether noise-induced plasticity could be harnessed as a therapeutic tool. Neurotherapeutics that focus on 'retuning' the cortical map as a primary means of altering perception are already being applied for human patients. For example, to treat phantom limb pain in amputees, some therapies utilize feedback-driven sensory discrimination training in an effort to change the localization of pain (Flor et al. 2001). In the auditory system, sound-based therapies are extremely common in the treatment of tinnitus, the uncomfortable sensation of ringing in the ears (For a review, see Pienkowski 2019). Strategies vary, although some therapies attempt to improve the perception of external sounds and mask tinnitus through hearing aids or special sound generators (Jastreboff and Jastreboff 2000) while others attempt to shrink the portion of the auditory cortical map dedicated to the tinnitus frequency by training individuals to better discriminate tones outside of that range (Flor et al. 2004). In a rat model of tinnitus, pairing vagus nerve stimulation, which enhances the release of neuromodulators that promote plasticity including serotonin and norepinephrine, with the presentation of pure tones outside of the tinnitus range was shown to improve behavioral and physiological symptoms of the condition (Engineer et al. 2011). As noise exposure has been shown to be an effective, passive, and non-invasive means of enhancing plasticity in selective regions of the adult tonotopic map, it could prove to be a beneficial addition to the currently available toolbox of neurotherapeutic methods. To date, however, only two studies have examined the behavioral performance of rodents pursuant to noise exposure (Zheng 2012, Zhou and Merzenich 2012) and none have attempted to use it to enhance auditory function. Moving forward, behavioral studies should evaluate the potential of noise-

induced plasticity in improving auditory learning and memory and treating pathological auditory functioning.

1.4.2 Tonotopic map expansion induced by noise resembles that of perceptual learning

The observation that greater cortical representation generally confers improved perception (Merzenich et al. 1984; Pantev et al. 1998; Rutkowski and Weinberger 2005; Wiestler and Diedrichsen 2013) provides additional encouragement for the concept of neurotherapeutics that target cortical plasticity. Perceptual learning has been extensively associated with cortical map expansions in the somatosensory and auditory domains across species (Feldman and Brecht 2005; McGann 2015). Somatosensory representations for the fingers are enhanced in string players (Elbert et al. 1995) and braille readers (Pascual-Leone and Torres 1993; Sterr et al. 1998), and classical conditioning can increase the functional representation of specific whiskers in the mouse barrel cortex (Siucinska and Kossut 1996). In the auditory cortex, both aversive and rewarding stimuli can induce frequency-specific map expansions (Pienkowski and Eggermont 2011; McGann 2015) that have even been shown to correlate with degree of learning (Recanzone et al. 1993). This body of work suggests that it might be possible to design passive sound exposures to elicit targeted plastic changes in the auditory cortex to improve or maintain perceptual abilities in adulthood. Despite these concordant findings, however, the perceptual significance of tonotopic map expansions remains debated. For one, inducing map expansions has not always been found to improve perceptual discrimination (Talwar and Gerstein 2001; Han et al. 2007). Nonetheless, white noise exposure provides a novel means of inducing map expansion in adult rodents that could be used to further investigate these questions.

1.5 Potential maladaptive consequences of noise exposure

1.5.1 Negative effects of chronic noise exposure

Despite the potential therapeutic applications of noise-induced plasticity, the effects of chronic noise exposure are more often described as maladaptive. Moderate intensity exposures to continuous or pulsed noise have been shown to degrade normal listening processes including tuning selectivity (Zhang et al. 2002; Zhou et al. 2011), neural gap detection (Jiang et al. 2015), fine pitch discrimination (Zheng 2012), and temporal rate discrimination (Zhou and Merzenich 2012). In animals and humans, precise spectral and temporal processing are crucial for the perception of complex signals including conspecific vocalizations and speech, especially in noisy listening environments (Shannon et al. 1995; Anderson et al. 2010; Shetake et al. 2011). Furthermore, sound exposure need not consist of noise to have profound effects, as demonstrated by a thorough investigation of chronic exposure to band-limited tone pip ensembles in adult cats by the Eggermont group. These studies demonstrate that over-stimulation triggers homeostatic plasticity mechanisms that reduce neural activity in the exposure frequency range, an effect that outlasts the exposure period by months (Noreña et al. 2006; Pienkowski and Eggermont 2010; Pienkowski et al. 2011). Altogether, these findings are especially concerning when taking into account the prevalence of human exposure to sound levels consistently higher than those used in the above studies. The experiments hitherto mentioned employed sound levels of 75dB or less, whereas the standard acceptable level of workplace noise in many countries is an average of 80dB for eight hours per day (Gourévitch et al. 2014). While these standards reflect the fact that hearing loss does not result from this duration of sound exposure, they do not take into account central changes that may take place in the absence of peripheral damage. Studies of noise exposure therefore have the potential to shed light on acceptable levels of noise in the world around us.

1.5.2 Tinnitus is associated with tonotopic map expansion

Two common central auditory disorders, tinnitus and hyperacusis, are thought to arise from maladaptive plastic processes. As mentioned earlier, subjective tinnitus is most commonly known as the uncomfortable sensation of ringing in the ears, although it can also manifest as a noisy, buzzing, or pulsing sound in one or both ears (McFadden 1982; Heller 2003). Hyperacusis is a hypersensitivity to sounds that would otherwise be acceptably loud to the general population (Baguley 2003). Together, tinnitus and hyperacusis affect between 6-15% of adults (Brozoski and Bauer 2016), usually emerging late in life comorbid with hearing loss. However, individuals with clinically normal audiograms can also report both conditions, and hyperacusis is present in other disorders including autism and Williams syndrome (Pienkowski 2019). Tinnitus and hyperacusis can also exist independent of each other, although the prevalence of hyperacusis in individuals with tinnitus is potentially 80% (Hayes et al. 2014). Following from their frequent co-occurrence with hearing loss, an agreed-upon trigger for these disorders is the loss of input from the inner ear (Eggermont and Roberts 2004; Moller 2007; Roberts et al. 2010; Kaltenbach 2012, **Fig. 1.3**) and a large portion of studies rely upon noise-induced acoustic trauma to induce chronic tinnitus or hyperacusis in animal models (Von Der Behrens 2014; Hayes et al. 2014; Brozoski and Bauer 2016). Accumulated non-traumatic sound experience has more recently been proposed as a possible risk factor for tinnitus and hyperacusis, in part because of the issues mentioned in the preceding section, however only a handful of studies have specifically examined this possibility (Attarha et al. 2018). Additional studies of white noise exposure could thus be pertinent in answering this question.

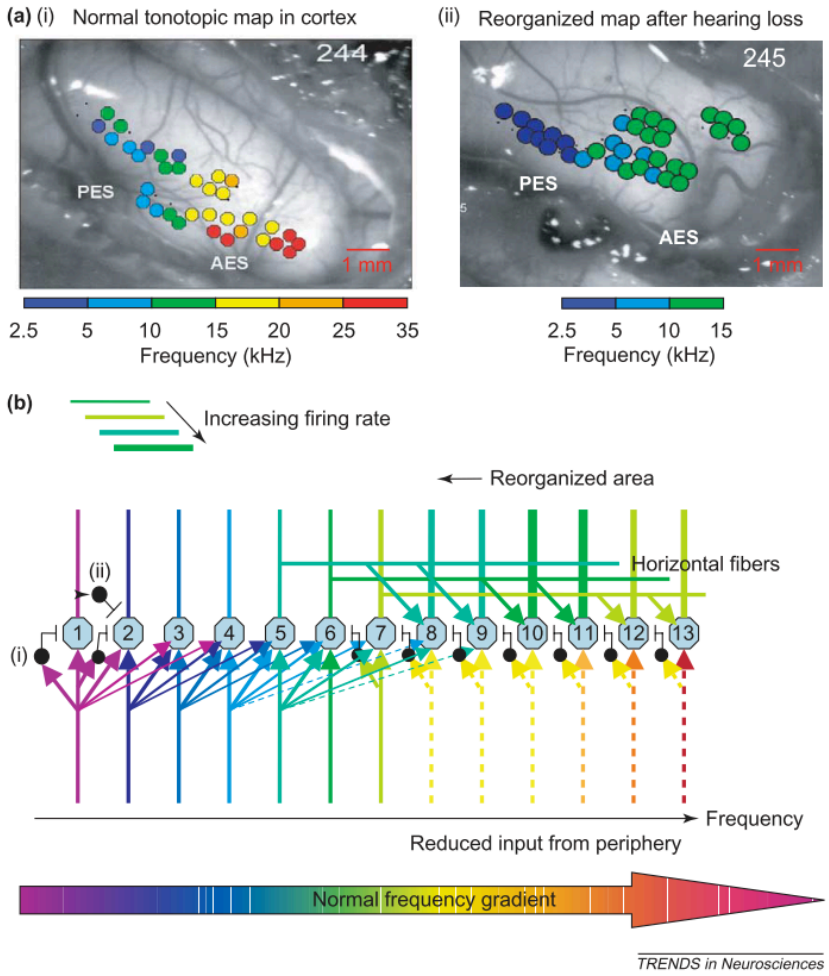

Figure 1.3: "*Normal and reorganized tonotopic maps in primary auditory cortex (AI)*. (a) The characteristic frequency at each recording site is color-coded and overlaid on a photograph of the cortical surface for a control cat (i) and a cat with a noise induced hearing loss (ii). The hearing loss was limited to frequencies >10 kHz and amounted to 3 dB at 12 kHz, 12 dB at 16 kHz, 22 dB at 24 kHz and 23 dB at 32 kHz. (244 and 245 are cat identification numbers.) (b) The effect of restricted high-frequency hearing loss on the input to pyramidal cells (numbered 1–13) in auditory cortex. The large colored arrow shows the normal frequency gradient of the inputs conveying the tonotopic mapping. The thin vertical lines leading to the cortical cells are color-coded to reflect their frequency-specific input from the thalamus… The assumption is that loss of input limits not only the excitation but also, even more strongly, the inhibitory feedforward activity. As a result, the diverging thalamic inputs from neighboring unaffected cells, and the inputs from cortical cells via horizontal fibers, face less competition from inhibition at those cortical cells deprived from thalamic input. Thus, these excitatory inputs are disinhibited or 'unmasked' and can impose their own frequency-selective inputs on cortical cells in the hearing loss range, which will ultimately result in a reorganization of the tonotopic map in the hearing-loss animal. Abbreviations: AES, anterior ectosylvian sulcus; PES, posterior ectosylvian sulcus." (Eggermont and Roberts 2004, page 678). *Figure reproduced from Eggermont and Roberts 2004 with permission*.

Cortical map reorganizations have been identified as a neural correlate of tinnitus in animal models. Along with hypersynchronization and increased spontaneous firing, map expansion has been observed in structures including the cochlear nucleus, inferior colliculus, and auditory cortex, indicating that frequency-specific tonotopic map plasticity may be a factor in generating the tinnitus percept (Eggermont and Roberts 2004; Roberts et al. 2010). Hyperacusis has similarly been associated with higher spontaneous firing rates in the central auditory pathway, but not map reorganization (Sun et al. 2012; Aazh et al. 2014; Hickox and Liberman 2014). Pienkowski and Eggermont (2012) recently pointed out that cats exposed to moderate-intensity band-limited noise or tone pip ensembles display all three putative correlates of tinnitus – map expansion, hypersynchronization, and increased spontaneous firing – and have proposed that this type of passive sound experience may indeed produce a tinnitus percept. Following from this, the tonotopic map expansion observed in noise-exposed rats may also be indicative of tinnitus. This question would be interesting to investigate, and could provide additional clarification about the perceptual consequences of CP-like plasticity following noise exposure.

1.6 The present investigation

Until relatively recently, the adult brain was believed to be impermeable to passive sensory experience. Studies including those of passive noise exposure have soundly refuted this theory, demonstrating an encouraging capacity for robust experience-dependent plasticity late in life, yet also revealing the brain's vulnerability to abnormal sensory environments. This newfound understanding strongly suggests that sensory environments should be understood and designed with care and demonstrates the need for research that elaborates both the sensory and neural properties that allow for such plasticity.

1.6.1 Rationales and objectives of the research

Passive exposure to moderate-intensity broadband white noise has been shown to drive robust experience-dependent plasticity in the adult rat auditory cortex. This plasticity differs both in quantity and quality from typical adult experience-dependent plasticity, implying that different mechanisms are likely at work. This phenomenon provides both the opportunity to study the neurotherapeutic applications of noise-induced plasticity and to better understand the neural consequences of environmental noise exposure, yet it remains relatively unresearched. The overarching goal of the present thesis is thus *to investigate the mechanisms and perceptual consequences of experience-dependent plasticity induced by passive exposure to white noise in the adult rat auditory cortex*. This aim will be addressed in three studies as outlined below.

1.6.2 Methods

The following studies each rely upon the model of the adult (2- to 6-month-old) female Long-Evans rat (Charles River Laboratories, Wilmington MA), which was chosen because of its previous use in studies of noise-induced plasticity by the de Villers-Sidani lab (Kamal et al. 2013). As a basis, each study employs passive exposure to white noise or a variation thereof to test the effects of chronic exposure. Following sound exposure, the persistent effects of noise-induced plasticity are investigated using four experimental approaches: in-vivo electrophysiological recordings of the auditory cortex under isoflurane anesthesia, post-mortem immunohistochemical staining of cortical sections, operant behavioral training, and auditory-mediated inhibition of the acoustic startle response.

1.6.3 The Studies

In sections 1.2-3 the importance of salient sensory experience was reviewed in the context of both developmental and mature cortical function. In Study 1 (Chapter 2), white noise exposures of varying degrees of amplitude modulation will be used to test the hypothesis that the masking of patterned auditory inputs with noise triggers CP-like plasticity. In addition, immunohistochemical staining will be performed to assess whether specific interneuron subtypes as described in section 1.1.2 and 1.3.2 are involved in the transition from experience-dependent to CP-like plasticity and will therefore be differently activated by unmodulated vs. modulated noise. The results from this study are expected to shed light on the statistics of the sensory environment that are necessary for maintaining mature cortical function.

In section 1.4.2, it was noted that noise-induced map expansion resembles the functional reorganization observed following perceptual learning. If map expansion implies improved perceptual abilities as some have put forward, then this observation suggests that it may be possible to design passive sound exposures to enhance specific perceptual abilities in adulthood. In Study 2 (Chapter 3), the hypothesis that noise exposure could be used to improve perceptual learning for a specific frequency will be tested. Pitch discrimination and learning will be assessed through progress on an adaptive tone discrimination task and electrophysiological recordings following the induction of map expansion with noise and tone pip exposure. The answer to this question will contribute to understanding both the perceptual significance of map expansion and the potential for passive sound experience to act as a neurotherapeutic.

In addition to phenotypes of perceptual learning, noise-induced map expansion also resembles symptoms of the plasticity-related disorder tinnitus as illustrated in section 1.5.2. Unlike the impact of traumatic noise exposure, the contribution of moderate-intensity sound exposures to

tinnitus and the related disorder hyperacusis are just beginning to be uncovered. In Study 3 (Chapter 4), the hypothesis that noise and tone pip exposure produce symptoms of tinnitus or hyperacusis will be tested. This will be done using both electrophysiological measures and behavioral assessments of inhibition of the acoustic startle response. Completing this study will likely demonstrate that windows of plasticity opened by chronic noise exposure should also be understood as windows of vulnerability to maladaptive plastic changes.

Chapter 2

The prolonged masking of temporal acoustic inputs with noise drives plasticity in the adult rat auditory cortex

Maryse E. Thomas, Nathan H.M. Friedman, J. Miguel Cisneros-Franco, Lydia Ouellet, Étienne de Villers-Sidani. *Cerebral Cortex,* 2019, 29(3):1032-1046. Reproduced in full by permission of Oxford University Press. The corresponding author of this publication is Étienne de Villers-Sidani, etienne.de-villers-sidani@mcgill.ca.

Preface

The first study of this thesis was designed to investigate the masking properties of white noise that lead to plasticity and link them with the activity of specific neuronal populations. After noting the importance of salient temporal patterns for both developmental and mature cortical function, it was proposed to show that noise exposure induces strong cortical plasticity in the adult auditory cortex by masking temporal inputs from the environment. This was accomplished by varying the signal-to-noise ratio of temporal modulation in the acoustic environment of adult rats with exposure to amplitude-modulated white noise of various modulation depths. Using immunohistochemical staining, the time course of changes in excitatory and inhibitory cellular activity during exposure to unmodulated and modulated noise was also documented, providing a comprehensive picture of the effect of temporal masking on cortical function.

2.1 Abstract

The prolonged masking of auditory inputs with white noise has been shown to reopen the critical period for spectral tuning in the adult rat auditory cortex. Here, we argue that the masking of salient temporal inputs in particular is responsible for changes in neuronal activity that lead to this experience dependent plasticity. We tested this hypothesis by passively exposing adult rats to two weeks of amplitude-modulated (AM) white noise with different modulation depths from 0% (no modulation) to 100% (strong modulation). All exposed rats displayed evidence of cortical plasticity as measured by receptive field bandwidths, tonotopic gradients, and synchronization during spontaneous activity. However, this plasticity was fundamentally different in nature for rats exposed to unmodulated noise, as a second passive exposure to pure tones elicited tonotopic reorganization in rats exposed to 0% AM noise only. Detection of c-FOS expression in excitatory and inhibitory cells through post-mortem immunohistochemistry also revealed different patterns of cellular activation depending on modulation depth. Together, these results indicate that the absence of temporal modulation promotes noise-induced plasticity in the adult auditory cortex and suggest an important and continuous role for temporally salient inputs in the maintenance of mature auditory circuits.

2.2 Introduction

The absence of normal sensory inputs, even after the closure of standard critical periods, can induce experience-dependent alterations within sensory cortices (Trachtenberg et al. 2002; Karmarkar and Dan 2006; Eggermont 2013). Manipulations such as digit amputation, lesions of the retina, or whisker trimming have long been known to drive functional reorganization of adult sensory cortices through deafferentation (Merzenich et al. 1984; Chino et al. 1992; Diamond et al. 1993) and visual deprivation has been shown to reactivate ocular dominance plasticity in the adult visual cortex (He et al. 2006). More recently, however, a growing body of evidence has demonstrated that even without peripheral injury or sensory deprivation, the masking of sensory inputs can lead to profound cortical reorganization. Specifically, masking auditory inputs with white noise has been shown to reopen the critical period for spectral tuning in the primary auditory cortex (A1). Exposing adult rats to continuous white noise for at least four weeks leads to broadening of auditory receptive field bandwidths, decreased neuronal synchronization, reduced inhibitory tone, and tonotopic reorganization in response to a second passive exposure to pure tones (Zhou et al. 2011; Zheng 2012; Kamal et al. 2013). While the ability to reopen critical periods during adulthood provides exciting potential for cognitive therapies based on neuroplasticity, the changes mentioned above are more often associated with a deterioration of auditory function (Zhou and Merzenich 2012; Gourévitch et al. 2014). For this reason, understanding how and why the masking of sensory information drives plasticity in the mature brain will be crucial both for harnessing plasticity for targeted therapeutic purposes and for preventing maladaptive changes due to environmental noise at the level of the cortex.

One aspect of understanding how sensory masking leads to plasticity is identifying which features of the sensory environment are important for maintaining mature auditory circuits.

Given that white noise is a random signal that contains all audible frequencies at equal intensities, it indiscriminately activates auditory neurons without spectral or temporal precision. However, local correlated activity in sensory cortices, whether spontaneous or stimulus-driven, is thought to be essential to maintaining existing cortical circuitry (Katz and Shatz 1996; Bao et al. 2003). Artificially reducing the correlation of local activity through long-term exposure to noise could thus deprive the cortex of spatiotemporally patterned activity and lead to cortex-wide plastic changes. As temporal signals have been shown to strongly modulate local activity (Bao et al. 2003; Ma et al. 2013) and critically influence cortical development (Zhang et al. 2002; Zhou et al. 2008; Insanally et al. 2010), this study aimed to test the notion that the presence of salient temporal inputs in particular are important for maintaining the stability of sensory representations in A1.

We investigated whether the absence of temporally structured inputs during noise exposure leads to the reopening of critical period plasticity in A1 by exposing adult rats to broadband white noise of different degrees of amplitude modulation from 0% (no modulation) to 100% (strong modulation) for two weeks. Through this manipulation, the amount of temporally structured information conveyed by each exposure was parametrically varied while salient temporal and spectral information from the environment were masked. Changes in cortical plasticity after noise exposure were assessed through electrophysiological recordings of A1 and immunohistochemical identification of specific excitatory and inhibitory neuronal populations including parvalbumin positive (PV+) and somatostatin positive (SST+) interneurons. It was found that all noise-exposed rats exhibited experience-dependent plasticity, however, this plasticity was fundamentally different in nature as a second passive exposure to pure tones elicited tonotopic reorganization in rats exposed to unmodulated noise only. These changes were

accompanied by opposing patterns of excitatory and inhibitory cellular activation depending on modulation depth. Our results suggest an important and continuous role for temporally salient auditory inputs in the maintenance of mature auditory circuits.

2.3 Materials & Methods

All experimental procedures were approved by the Montreal Neurological Institute Animal Care Committee and follow the guidelines of the Canadian Council on Animal Care.

Exposure conditions: Female three-month-old Long-Evans rats were housed in sound-attenuated chambers under a 12h light/dark cycle and given ad libitum access to food and water. One group of rats (Naïve, n = 5) had no acoustic manipulation of their environment (background sound level 40dB SPL). Four groups of rats were passively exposed to 70dB SPL continuous amplitude modulated (AM) white noise that had a modulation rate of 3Hz and depth of 0% (AM-0: n = 6), 25% (AM-25: n = 4), 50% (AM-50: n = 5), or 100% (AM-100: n = 4) for two weeks. Two additional groups of rats were exposed to 0% or 50% AM noise for two weeks immediately followed by a one-week exposure to trains of 7kHz tone pips (AM-0 + 7kHz, AM-50 + 7kHz: n = 4 for both groups). The noise and tone pips were generated using custom MATLAB scripts (The MathWorks, Inc., Natick, Massachusetts) and played through an Ultralite-mk3 Hybrid Interface (MOTU Inc., Cambridge, Massachusetts) with sampling at 192kHz. The noise stimuli were amplified to a free-field sound level calibrated so that the average intensity of each stimulus measured in the center of the chamber was 70dB SPL. The tone pip exposure was modeled after that used by Zhou and colleagues (2011). Tones were 50ms in duration (5ms onset and offset ramps) and delivered in trains of 5 pulses per second. To minimize adaptation effects, the interval between each train of tones was a random duration generated from a normal distribution

with a mean of 2.5 seconds. The tone pips were amplified to an intensity of 65dB SPL measured in the center of the chamber. All stimuli were played 24 hours per day for the duration of the exposure periods. To observe the time course of cellular activity after the onset of noise exposure, nine additional groups of female three-month-old rats were used for post-mortem immunohistochemical analyses only. One group was naïve and the other groups were exposed to 0% or 100% AM noise for one hour, 24 hours, one week, or two weeks under the same conditions as those described above (n = 3 for all groups). These rats did not undergo electrophysiological recordings.

Electrophysiological recordings: At the end of the exposure periods, electrophysiological recordings of the left auditory cortex were performed under isoflurane anesthesia in a shielded soundproof recording chamber. Rats were pre-medicated with dexamethasone (0.2mg/kg, i.m.) to minimize brain edema. Anesthesia was induced with ketamine/xylazine/acepromazine (63/13/1.5 mg/kg, i.p.) followed by continuous delivery of isoflurane 1% in oxygen via endotracheal intubation and mechanical ventilation. Heart rate and blood oxygen saturation were monitored with a pulse oximeter. Body temperature was monitored with a rectal probe and maintained at 37ºC with a homeothermic blanket system. Rats were held by the orbits in a custom designed head holder leaving the ears unobstructed. The cisterna magna was drained of cerebrospinal fluid to further minimize cerebral edema. To access the auditory cortex, the left temporalis muscle was reflected, the skull over the auditory cortex was removed, and the dura was resected. Once exposed, the cortex was maintained under a thin layer of silicone oil to prevent desiccation. Acoustic stimuli were delivered in a free field manner to the right ear through a calibrated speaker. Cortical responses were recorded with a high-impedance 64-channel tungsten microelectrode array (Tucker-Davis Technologies [TDT], Alachua, Florida)

lowered orthogonally into the cortex to a depth of 700-1000μm (layer 5). Electrode wire diameter was 33μm and electrodes were arranged in an 8x8 grid. To maximize recording density, neural responses were recorded from multiple electrode positions within each rat. The stereotaxic location of each position relative to the first was noted in order to accurately reconstruct auditory maps during offline analysis. Extracellular multi-unit responses were obtained, amplified, and filtered (0.3–5 kHz) using a TDT RZ2 processor. The TDT OpenEx software package was used to generate acoustic stimuli, monitor cortical activity online and store data for offline analysis.

Tonotopic map reconstruction: Frequency-intensity receptive fields were constructed using neuronal responses to a range of frequency-intensity combinations of pure tones. 66 frequencies (0.75-70kHz; 0.1 octave increments; 25ms duration; 5ms ramps) were presented at eight sound intensities (0-70dB SPL; 10dB increments) at a rate of one tone per second with three repetitions and in random presentation order. The characteristic frequency (CF) and threshold of a cortical site were defined, respectively, as the frequency and intensity at the tip of the V-shaped tuning curve derived from peri-stimulus time histograms (PSTHs). For flat-peaked tuning curves or tuning curves with multiple peaks, the CF was defined as the frequency with the lowest threshold and the strongest firing rate. Response bandwidths 20dB above the threshold of tuning curves (BW20) were measured for all sites. The onset latency, defined as the time in ms when the PSTH first exceeded mean baseline firing rate by 2.5 standard deviations, was also measured for each cortical site. The CF, threshold, BW20, and latencies were first determined by an automated custom MATLAB routine and then manually verified by an experimenter blind to the identity of the experimental groups. Cortical sites were identified as belonging to the primary auditory field (A1), anterior auditory field, ventral auditory field, or posterior auditory field based on published functional characteristics of each field (Polley et al. 2007). These were

reversal of tonotopic gradients, onset latencies, threshold, and PSTH morphologies. Only responses recorded from A1 sites were included in analyses. To generate A1 maps, Voronoi tessellation was performed using custom MATAB scripts to create tessellated polygons with electrode penetration sites at their centers. The color of each polygon represents the CF of the corresponding penetration site.

Tonotopic index: The tonotopic axis of each CF map was determined by drawing a line between the most anterior and posterior sites within A1, which were typically also the sites with the lowest and highest CFs. The maps were rotated to orient the tonotopic axis horizontally and the horizontal coordinates of each site were normalized to be within a range from 0 to 1 and plotted against CF after converting the logarithmic frequency range (0.75-70kHz) to a linear range (0-1). The tonotopic index was then determined by computing the average minimum distance from each data point on the scatterplot to the line describing the perfect tonotopic axis, which would be that connecting (0, 0) and (1, 1).

Neural synchrony: The degree of neuronal synchronization in the auditory cortex was computed from recordings of spontaneous neural activity that were at least five minutes long. First, offline spike sorting was performed using TDT OpenSorter software to isolate single unit activity based on an automated Bayesian sorting algorithm. The success of the spike sorting algorithm was assessed by inspecting the number of refractory period violations for all identified clusters (**Supplementary Fig. 2.1**). The fraction of spikes that fell within a 2ms refractory period was calculated and it was found that 34.0% of all clusters had zero refractory period violations and 94.3% of all clusters had 2 or fewer violations per 100 spikes (**Supp. Fig. 2.1A**). An average of 1.46 units was identified per electrode channel. Example histograms of the interspike interval and the autocorrelation of spike times for representative units are presented in **Supp. Fig. 2.1B,**

displaying a dearth of spikes occurring within the refractory period. In addition, the percentage of refractory period violations did not differ between experimental groups (**Supp. Fig. 2.1C**). These results indicate that there are a relatively small number of false positive classifications present in the data, which are unlikely to affect experimental outcomes.

Measures of synchronization were computed from binary spike events detected from A1 units in separate channels up to 2100 microns apart. Cross-correlation functions, R_{AB}, were computed by counting the number of spike coincidences for pairs of spike trains, denoted A and B, for time lags of −500 to 500ms with 1ms bin size and normalized by dividing each bin by the square root of the product of the number of total discharges in each spike train. The expectancy of the cross-correlation function, or the expected number of synchronous events at any time if the spike trains are not correlated, was estimated by $E = (N_A N_B \Delta)/T$, where N_A and N_B are the numbers of spikes, Δ (= 10ms) is the bin size, and T is the duration of the recording. A Z-score was computed for each bin using $Z(\tau) = (R_{AB}(\tau) - E)/(E)^{1/2}$ where τ is the time lag. Neuron pairs with a peak Z-score greater than 4 are considered to be synchronized with a statistical significance corresponding to $p < .0001$. Cross-correlation and Z-score analyses were based on Eggermont (1992) and Brosch and Schreiner (1999).

Immunohistochemistry: Post-mortem immunohistochemical analyses were performed on three rats from each exposure group, except those exposed to AM noise followed by pure tones. Immediately following the end of the exposure period or the end of electrophysiological recordings, rats were first anesthetized with ketamine/xylazine/acepromazine then perfused through the heart with 4% paraformaldehyde. Their brains were removed and preserved in the same fixative overnight then transferred to a sucrose solution until they were sectioned on a freezing microtome at a 40μm thickness in the coronal plane. Brain sections were co-stained

with markers for various cell types and c-FOS protein. The markers were neuron-specific NeuroTrace Nissl stain conjugated with AlexaFluor 488 (AF488) (Molecular Probes #N21480, 1:300, 30 min at room temperature), rabbit anti-GABA (Sigma #A2052, 1:5000), mouse anti-PV (Sigma #P3088, 1:10,000), and rat anti-Somatostatin (Chemicon, Temecula, CA #MAB354, 1:1000). These conditions were followed by rabbit anti-cFOS or goat anti-cFOS (Santa Cruz #sc-52 or #sc-52-G, 1:300, 48h at 4°C). All conditions except Nissl were also washed and incubated in secondary anti-sera 1:800 for 45 min at room temperature. The secondary antibodies were donkey anti-rabbit (conjugated to Cy3, Jackson ImmunoResearch, West Grove, PA), donkey anti-mouse (conjugated to AF488, Jackson), and donkey anti-rat (AF488, Jackson). Apart from GABA and PV, which were stained in the same sections, all conditions were performed in separate brain sections. A1 was located using stereotaxic coordinates and cortical layers were defined according to cell size, density, and depth. High-power field (HPF) images were taken with a confocal microscope at 40X magnification at random locations within both A1 hemispheres. To ensure uniform sampling, exactly seven pictures were taken in layers 2-3, 4, and 5-6 for each rat totaling 21 pictures per rat. The total number of cells expressing the markers above was counted in each picture. An observer blind to the experimental groups took all pictures and performed cell counts.

Pictures were also taken of the primary visual cortex (V1) in order to ensure that differences in c-FOS expression were specific to auditory processing and not other confounding factors brought on by the noise exposure or staining conditions. Brain sections previously stained for c-FOS containing both A1 and V1 in the same coronal slice were used for analysis. The three tested groups were Naïve, AM-0 (two week exposure), and AM-100 (two week exposure). V1 was located using stereotaxic coordinates, HPF images were taken from all layers as above, and

the total number of cells expressing c-FOS was counted. No difference in the number of c-FOS+ cells per HPF was found between groups (**Supplementary Fig. 2.2**, stats in legend).

For rats that were used for both electrophysiological recordings and immunohistochemical analysis, differences in the time outside of noise exposure and duration of auditory stimulation during recordings could have affected c-FOS results due to the rapid expression profile of this protein (Krukoff 1994). To ensure that the observed differences in c-FOS expression were due to exposure condition and not differences in experimental timing, total time outside of noise exposure and duration of auditory stimulation were compared across exposure groups. The five tested groups were Naïve, AM-0, AM-25, AM-50, and AM-100 (all two week exposures). Time under anesthesia was taken to approximate the time outside of noise exposure since animals were only removed from their cages once they were ready to be anesthetized. No group difference was found in total time under anesthesia (mean = 418.20 ± 18.15 min., one-way ANOVA $F(4,10) = 2.095$, $p = 0.156$, n = 15 rats) or duration of auditory stimulation (mean = 213.87 ± 7.02 min., one-way ANOVA $F(4,10) = 1.045$, $p = 0.432$, n = 15 rats).

A1 c-FOS expression was compared between rats that underwent electrophysiological recordings and those who did not (**Supplementary Fig. 2.3**, stats in legend). The three tested groups were Naïve, AM-0 (two week exposure), and AM-100 (two week exposure). A significant difference in c-FOS expression between recorded and unrecorded animals was only observed for naive rats, with unrecorded naive rats displaying a slightly greater number of c-FOS+ cells per HPF. This indicates that for naive animals, the conditions experienced shortly before perfusion elicited greater c-FOS expression than the effects of auditory stimulation during electrophysiological recording while these effects were indistinguishable for exposed animals.

For all analyses, conclusions were not different if either naïve group was used, however only comparisons made between recorded or unrecorded animals were reported.

Statistical analyses: For all statistical analyses, results are reported in parentheses as: (post-hoc comparison p value, test name and statistic, number of data points per level of nested data). Linear mixed-effects or generalized linear mixed (GLMM) models (Reed and Kaas 2010; Aarts et al. 2014) were used to analyze data collected through nested experimental designs (e.g. for synchronization analyses: neuron pair nested within recording position nested within rat). For these models, recording position nested within rat ID were included as random effects and for GLMM, family was specified as Poisson (log link) or Binomial (logit link) for count and binomial data respectively. Data normality was assessed using Q-Q plots, as was normality of residuals from each model. Analyses were conducted using MATLAB, JMP 11 (SAS Institute, Cary, NC), R (R Core Team, Vienna, Austria), and R packages lme4 (Bates et al. 2015), lmertest (Kuznetsova et al. 2016), lsmeans (Lenth 2016), and pbkrtest (Halekoh and Højsgaard 2014). The fixed effect test results are reported with the degrees of freedom denominator approximated for normal data using the Kenward-Roger adjustment and for count or binomial data using the Satterthwaite approximation from the equivalent linear mixed-effects test. Tukey's test evaluated at an alpha level of 0.05 was used for all post-hoc comparisons. Where applicable, back-transformed least squares means derived from statistical models were plotted in figures. Where results are not shown in figures, means ± 95% confidence intervals are reported in the text.

2.4 Results

The present study investigated whether a prolonged absence of temporally structured inputs is responsible for the transition to critical period-like plasticity following exposure to

continuous white noise. Adult rats were exposed to two weeks of broadband white noise with AM depths of 0% (AM-0), 25% (AM-25), 50% (AM-50) or 100% (AM-100) and amplitude modulation rate of 3Hz. A1 tonotopic maps were reconstructed following exposure and the tonotopic gradient and mean receptive field bandwidths were compared to those of age-matched naïve animals to determine whether noise exposure elicited map plasticity (**Fig. 2.1**). The tonotopic index was calculated to quantify the orderliness of the tonotopic gradient of each map; higher values are associated with greater map disorganization. We found that exposure to 0% AM noise significantly increased mean tonotopic index compared to naïve animals ($p = .008$) and AM-100 animals ($p = .018$), which were not significantly different from each other ($p = 0.99$). The tonotopic index for the AM-25 and AM-50 groups was slightly elevated as they were not different from naïve, AM-0, or AM-100 animals (all $p \geq .075$, one-way ANOVA $F(4,19) = 4.968$, $p = .007$ followed by Tukey's test, n = 24 rats, **Fig. 2.1B-D**). In all cases, the increase in tonotopic index appeared to be driven by a shift in tuning toward higher frequencies. To investigate this possibility, we attempted to quantify the directionality of the shift in tonotopic index by computing the distance of each point from the line describing the perfect tonotopic gradient in Fig. 2.1C. Points above and to the left of the line were given a positive value while points below and to the right of the line were given a negative value. We found that rats exposed to 0% AM noise had a significantly greater mean distance from the line than all other groups indicating greater positive dispersion of points (all $p \leq .012$, Welch's one-way ANOVA $F(4,299.83) = 3.724$, $p = .006$ followed by Tukey's test, n = 637 cortical sites within 51 positions and 24 rats). This was further confirmed by comparing the cumulative distribution functions of each exposure group (**Fig. 2.1E**) in which we observed a rightward shift for AM-0 rats indicating that the majority of cortical sites shifted their CFs to higher frequencies.

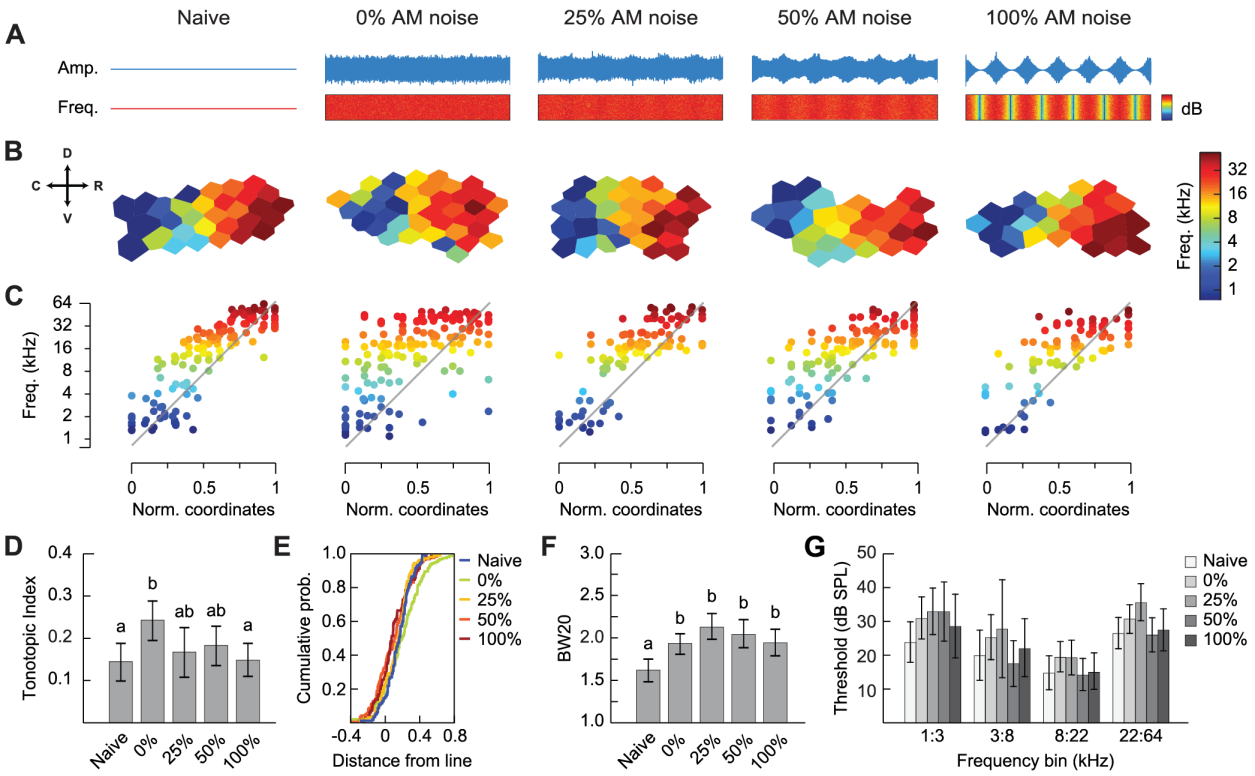

Figure 2.1: *Effect of AM noise exposure on A1 tonotopic maps and receptive field properties*. **A.** Example waveforms and spectrograms of 0%, 25%, 50%, and 100% AM noise used for exposure stimuli. **B.** Representative A1 characteristic frequency (CF) maps from each exposure group. **C.** CFs from all animals plotted against a normalized tonotopic axis. The gray line represents a perfect tonotopic gradient. **D.** Mean tonotopic index for each exposure group. **E.** Cumulative frequency distribution of dispersion from the line describing the perfect tonotopic gradient in panel C. The rightward shift for the 0% AM noise group indicates a greater proportion of points above the line. **F.** Mean BW20 for all cortical sites in each exposure group. **G.** Mean receptive field threshold for cortical sites with CFs in four frequency bins; within frequency bins, no comparisons are significant. Error bars represent 95% confidence intervals. Bars not connected by the same letter are significantly different with $p<.05$. Number of animals, recording positions, and cortical sites per group: Naïve 5,10,136, AM-0 6,13,165, AM-25 4,8,112, AM-50 5,10,131, AM-100 4,10,93.

We additionally compared the mean BW20 for each group to determine whether noise exposure altered the selectivity of frequency-intensity receptive fields. We found that all noise-exposed groups had significantly wider BW20s than naïve rats (all $p \leq .029$), however they did not differ from each other (all $p \geq .205$, mixed effects one-way ANOVA $F(4,33.28) = 4.135$, $p = .008$ followed by Tukey's test, n = 637 cortical sites within 51 positions and 24 rats, **Fig. 2.1F**).

Together, these results show that although all noise-exposed groups exhibited reduced tuning selectivity as evidenced by broadened BW20s, only AM-0 rats demonstrated global tonotopic reorganization as a function of this altered tuning. We also compared the average onset latency of neurons in each exposure group and found no group difference (mean = 9.05 ± 0.15 ms, mixed effects one-way ANOVA $F(4,36.49) = 2.079$, $p = .104$ followed by Tukey's test, n = 637 cortical sites within 51 positions and 24 rats). Finally, we compared the average receptive field threshold for each group to ensure that noise exposure did not elevate the sound intensity thresholds of exposed rats. As this measure can vary non-linearly with CF, it was calculated for four frequency bins spaced 1.25 octaves apart (bin limits approximately 1:3, 3:8, 8:22, and 22:64 kHz). The interaction between exposure group and CF bin was not significant ($F(12,596.40) = 0.87$, $p = .58$) nor was the group effect (mixed effects two-way ANOVA $F(4,49.21)= 2.32$, $p = .07$, n = 637 cortical sites within 51 positions and 24 rats, **Fig. 2.1G**) indicating that sound intensity thresholds were within normal range following exposure.

Neuronal correlation during spontaneous activity was assessed to determine whether noise exposure had a lasting effect on auditory cortical connectivity. As receptive field overlap is a strong predictor of neural synchrony, tonotopic reorganization and changes in connectivity are strongly interconnected (Brosch and Schreiner 1999; Eggermont 2007; Kilgard et al. 2007). First, the firing rate during spontaneous activity was not found to differ between exposure groups (mean = 3.66 ± 0.28 spikes/sec., mixed effects one-way ANOVA $F(4,24.11) = 1.389$, $p = 0.268$, n = 576 units within 30 positions and 19 rats). Next, the degree of spontaneous synchronization between A1 single units was measured by calculating cross-correlation functions for single-unit neuron pairs recorded in silence (**Fig. 2.2**). **Fig. 2.2A** shows the average cross-correlogram for all pairs < 0.5mm apart in each exposure group. The maximum value of the cross-correlogram (peak

correlation coefficient) tended to decrease with greater separation distance between units, r = -0.187, p < .0001, n = 5369 pairs, as well as greater ΔCF, r = -0.173, p < .0001, n = 5369 pairs. Distance and ΔCF were positively related, r = 0.290, p < .0001, n = 5369 pairs. A one-way ANCOVA with distance and ΔCF as covariates was conducted to determine whether peak correlation coefficient differed between exposure groups controlling for these two variables. We found that the mean peak for AM-0 rats was significantly lower than that of all other groups (all p ≤ .048). For AM noise-exposed rats, mean peak tended to be higher than naïve rats but no comparisons were significant (all p ≥ .0962, mixed effects one-way ANCOVA, F(4,23.98) = 8.715, p = 0.0002 followed by Tukey's test, n = 5369 pairs within 30 positions and 19 rats, **Fig. 2.2B**). **Fig. 2.2D** shows the peak of each pair plotted against their inter-unit distance. Since the distribution of peaks was positively skewed with the majority of single unit pairs displaying very low peaks, the data, originally on a 0-1 scale, was multiplied by 100 and transformed to a normal distribution using a natural logarithmic transform before statistical tests were applied. Back-transformed means are plotted in **Fig. 2.2A**, and **Fig. 2.2B** shows back-transformed means derived from the statistical models that control for distance and ΔCF, and **Fig. 2.2D** shows the raw peak values. The Z-score, a measure directly related to the cross-correlation function and also known as a detectability index for synchronization, was calculated to identify the proportion of synchronized units in each exposure condition. Pairs with a peak Z-score greater than 4 are considered to be synchronized with a statistical significance corresponding to p < .0001 (Eggermont, 1992). The proportion of synchronized vs. unsynchronized pairs in each group was compared with a binomial GLMM. It was found that rats in the AM-0 condition had a significantly lower proportion of synchronized pairs than the AM-25, AM-50, and AM-100 exposure groups (all p ≤ .022). No other comparisons were significant (all p ≥ .252, GLMM

binomial one-way ANCOVA, $F(4,3.157) = 6.139$, $p = .005$ followed by Tukey's test, n = 5369 pairs within 30 positions and 19 rats, **Fig. 2.2C**). Finally, we computed a one-way ANCOVA controlling for distance and ΔCF on the absolute lag of the cross-correlation function. A peak at or near zero is generally interpreted as an indication that the neurons in question share a common excitatory input, while a delayed peak suggests that one neuron is consistently activating the other through one or more synapses (Brosch and Schreiner 1999). We found that all modulated noise-exposed rats demonstrated a significantly shorter lag time than rats exposed to 0% AM noise (all $p \leq .031$). No other comparisons were significant (all $p \geq .271$, mixed effects one-way ANCOVA, $F(4,25.94) = 4.161$, $p = .01$ followed by Tukey's test, n = 5369 pairs within 30 positions and 19 rats. Group means: Naïve 43.54 ± 11.62ms, AM-0 60.87 ± 13.10ms, AM-25 18.03 ± 16.83ms, AM-50 22.88 ± 14.98, AM-100 28.75 ± 13.91ms). Along with cross-correlation peak and proportion of synchronized pairs, these results demonstrate that exposures to unmodulated and modulated noise have opposite effects on cortical synchronization. Specifically, rats exposed to unmodulated noise show a significant decrease in magnitude of correlated activity, a reduction in proportion of synchronized pairs, and a decreased influence of common excitatory inputs likely originating from the thalamus while rats exposed to modulated noise show no overall change in synchronization magnitude, a slight increase in proportion of synchronized pairs, and stronger common inputs from the thalamus.

The contribution of neuronal synchronization to tonotopic plasticity was investigated through nonlinear regression (**Fig. 2.2E**). It was hypothesized that exposure to unmodulated noise would increase plasticity by reducing the overall amount of correlated activity in the cortex and that exposure to modulated noise of perceptible depths would prevent this by maintaining or increasing correlated activity. This was supported by the results described above.

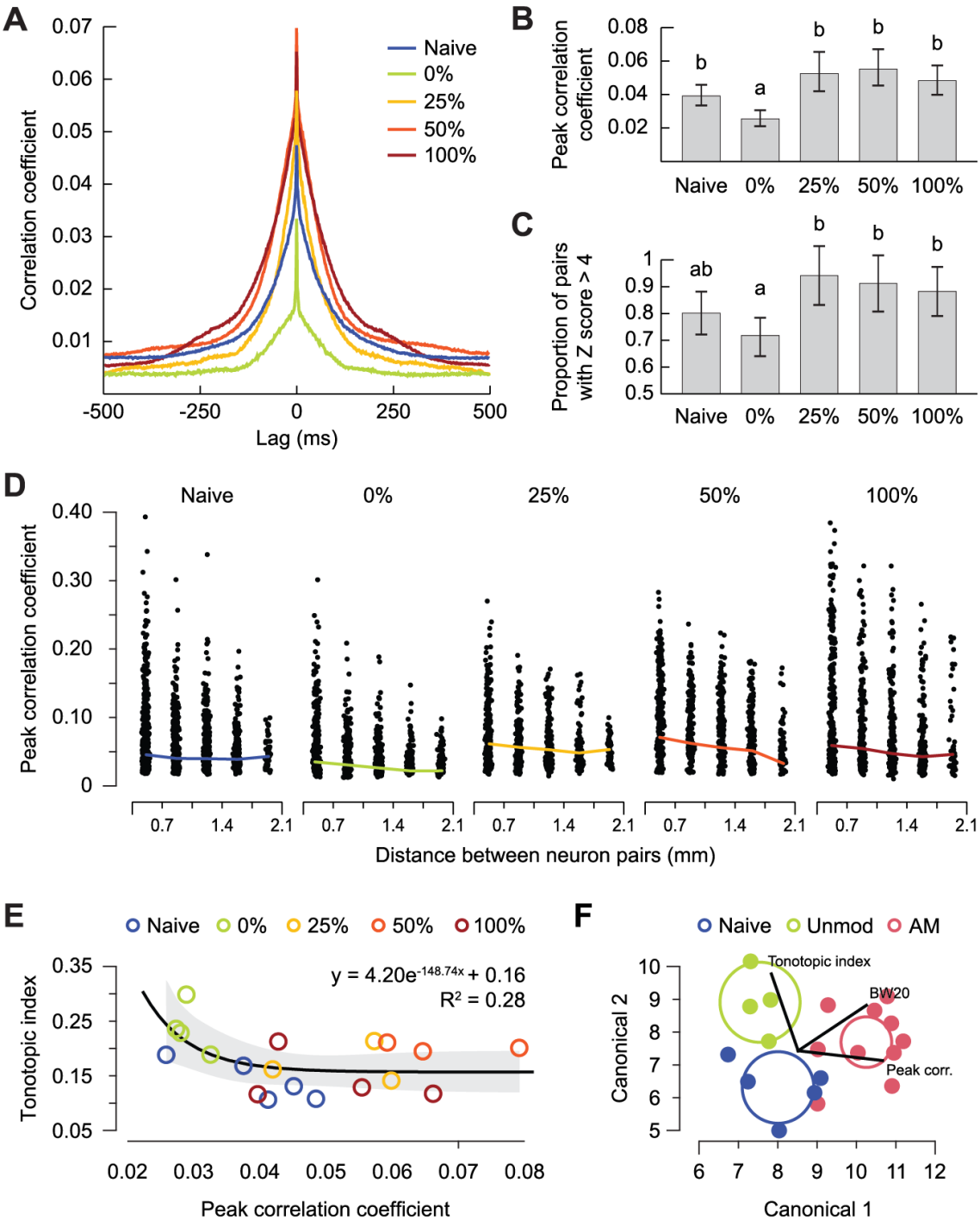

Figure 2.2: *Effect of AM noise exposure on A1 synchronization*. **A.** Mean cross-correlograms for all A1 unit pairs detected in separate channels between 0.35 and 0.5mm apart. **B.** Mean peak correlation coefficient ± 95% confidence intervals. **C.** Proportion of pairs with synchronization Z-score greater than 4 ± 95% binomial confidence intervals. **D.** Raw peak correlation coefficients vs. inter-unit distance bin. Bins are from 0.35 to 2.1mm, width = 0.35mm. Bold lines represent the mean correlation coefficient of each bin. **E.** Tonotopic index derived from characteristic frequency maps of each rat vs. their mean peak correlation coefficient. Bold line is exponential fit with equation $y = 4.20e^{-148.74x} + 0.16$. Shaded region represents confidence of fit. **F.** Canonical plot of the linear discriminant

analysis based on each rat's mean peak correlation coefficient, tonotopic index, and mean BW20. Rats were classified as either naïve, unmodulated noise-exposed (Unmod), or AM noise-exposed (AM). Ellipses represent the 95% confidence region for the true mean of each group. The Means in A-C, bold lines in D, and peak correlation coefficient means in E are back-transformed from the log or logit scale. Bars not connected by the same letter are significantly different with $p<.05$. Number of animals, recording positions, and unit pairs per group: Naïve 5,8,1609, AM-0 4,7,944, AM-25 3,4,836, AM-50 3,5,883, AM-100 4,6,1097.

However, increases in plasticity measured with either tonotopic index or BW20 were also observed in AM noise-exposed rats suggesting that the relationship between correlation and plasticity is not linear. An exponential function of the form $y = 4.20e^{-148.74x} + 0.16$ where x represents peak correlation coefficient and y represents tonotopic index was fit to the data, RMSE = 0.047, $R^2 = 0.28$, n = 19 rats (**Fig. 2.2E**). This function provides evidence that there is a complex relationship between peak correlation coefficient and tonotopic reorganization. Namely, below a certain threshold of synchronization (i.e. that possessed by naïve rats), degree of neuronal synchronization is negatively correlated with tonotopic index, or in other words, rats with low synchronization tend to have more disorganized tonotopic maps. Above this threshold, however, tonotopic organization does not appear to be affected by degree of synchronization.

Finally, we performed a linear discriminant analysis to test the hypothesis that rats would differ significantly on a linear combination of three of the variables explored above: peak correlation coefficient, tonotopic index, and BW20 (**Fig. 2.2F**). For this analysis, rats exposed to modulated noise of all depths (25%, 50% and 100%) were combined into one group, so the classifier tested whether naïve, unmodulated noise-exposed, and modulated noise-exposed rats could be discriminated on the basis of these three variables. The overall test was significant (Wilks' $\lambda = 0.142$, approx. $F(6,28) = 7.738$, $p < .0001$, n = 19 rats) as were the two extracted functions which explained 73.14% (canonical corr. = 0.85, $F(6,28) = 7.738$, $p < .0001$) and 26.86% (canonical corr. = 0.70, $F(2,15) = 7.186$, $p = .0065$) of the relative variance respectively.

The first canonical function was found to be positively correlated with both BW20 ($r = 0.634$, $p = .0035$, $n = 19$ rats) and peak correlation coefficient ($r = 0.888$, $p < .0001$, $n = 19$ rats), while the second canonical function was positively correlated with BW20 ($r = 0.634$, $p = 0.0036$, $n = 19$ rats) and tonotopic index ($r = 0.908$, $p < .0001$, $n = 19$ rats). Reclassification of the rats based on the new canonical variables was highly successful: all rats except for one (94.74%) were correctly reclassified into their exposure condition. The results of this classifier indicate that changes in neuronal synchronization, receptive field bandwidth, and tonotopic index can be used to confidently distinguish rats exposed to AM noise from those exposed to unmodulated noise.

To determine whether noise exposure facilitated plasticity past the duration of the exposures, tonotopic maps were also reconstructed from noise-exposed rats that underwent a second passive exposure to pure tones, a manipulation that typically only results in functional reorganization during the critical period for spectral tuning (Zhang et al. 2001). As it has been previously shown that mature rats exposed to this stimulus do not show tonotopic reorganization (Zhang et al. 2001; Zhou et al. 2011), naïve rats were used as controls and their A1 maps were compared to those of rats exposed to 0% or 50% AM noise for two weeks followed by a one-week exposure to 7kHz tone pips (**Fig. 2.3**). Data for rats exposed to other AM depths followed by tone pips was not acquired. We found no difference in the mean tonotopic index of these groups (one-way ANOVA $F(2,10) = 0.930$, $p = .426$, $n = 13$ rats, **Fig. 2.3C**). However, there was a clear effect of the 7kHz tone on rats exposed to 0% AM noise as determined by comparing the percentage of A1 sites with CFs in ten frequency bins (width = ½ octave, first and last bins = 1 ov) (**Fig. 2.3D**). The interaction between exposure group and frequency bin was significant (two-way ANOVA $F(18,100) = 2.174$, $p = .008$, $n = 13$ rats), as was the effect of group in the frequency bin centered at 7kHz (simple main effects test $F(2,100) = 9.235$, $p = .0002$).

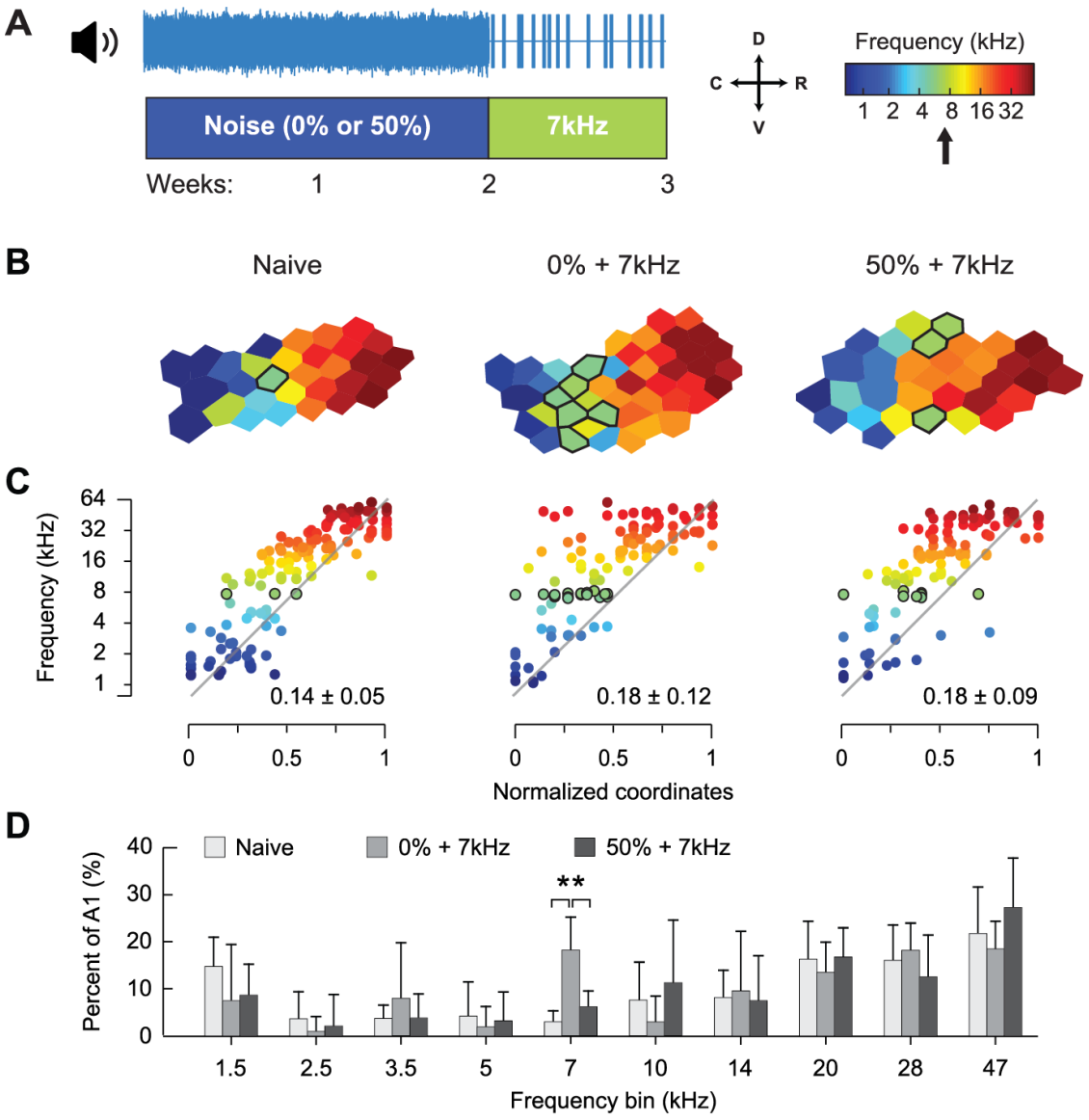

Figure 2.3: *Effect of 7kHz tone pip exposure following AM noise exposure on A1 tonotopic maps*. **A.** Experimental timeline. Rats were exposed to either 0% AM noise or 50% AM noise for two weeks followed by one week of exposure to 7kHz tone pips. **B.** Representative A1 CF maps. Outlined sites have CF of 7kHz ± 0.25 octaves. **C.** CFs from all rats plotted against a normalized tonotopic axis. Outlined points have CF of 7kHz ± 0.25 octaves. The average tonotopic indices ± 95% confidence intervals are written in the bottom right corner of each graph. **D.** Percentage of A1 area with CF in ten frequency bins (width = 1/2 octave). **p=.006. Error bars represent 95% confidence intervals. Number of animals, recording positions, and cortical sites per group: Naïve 5,10,136, AM-0 + 7kHz 4,7,96, AM-50 + 7kHz 4,9,122.

Post-hoc testing showed that rats exposed to 0% AM noise before the tone pips displayed a significantly greater percentage of sites with a CF of 7kHz ± 0.25 octaves than naïve rats and rats exposed to 50% AM noise (all $p \leq .0002$), which were not significantly different ($p = .510$). There was no difference in the representation of any other frequency bin between exposure groups (simple main effects tests, all $F(2,100) \leq 2.688$, all $p \geq .073$).

Until now, a comprehensive account of cell-specific changes in neural activity following noise exposure has not been undertaken. Here, excitatory neurons and GABA-expressing inhibitory interneurons were identified within A1 sections and co-stained with an antibody for c-FOS protein (**Fig. 2.4**). The expression of c-FOS, an immediate early gene expressed rapidly after a cell fires, was used as an indicator of recent neuronal activity and plasticity (Mello and Pinaud 2006; Terleph and Tremere 2006). To quantify plasticity, the number of c-FOS expressing and GABA-expressing cells were counted per A1 high-power field (HPF) in layers 2/3, 4, and 5/6. As cortical c-FOS is expressed almost invariably in neurons (Herdegen et al. 1995), every cell expressing c-FOS alone (c-FOS+/GABA-) was considered to be an excitatory neuron. For this cell type **(Fig. 2.4A-B)**, we found a significant interaction between layer and exposure group (GLMM two-way ANOVA $F(8,290) = 8.568$, $p < .0001$, $n = 315$ HPFs within 15 rats) and group differences in c-FOS expression in each layer (simple main effects tests, all $F(4,54.84) \geq 11.980$, $p < .0001$). Post-hoc testing indicated that in general, noise exposure tended to increase the number of c-FOS expressing excitatory cells per HPF, however this was the most pronounced in all layers for rats exposed to unmodulated noise and decreased with greater modulation depth. See **Fig. 2.4B** for pairwise statistics. Notably, AM-100 rats were not different from Naïve in layers 4 or 5/6 c-FOS expression, nor were AM-50 rats in layers 5/6. In the category of inhibitory neurons (c-FOS+/GABA+) (**Fig. 2.4C**), the interaction between layer and

exposure group was not significant (GLMM two-way ANOVA $F(8,290) = 0.764$, $p = .635$, n = 315 HPFs within 15 rats) so we analyzed the main effect of group only. The effect of group on c-FOS expression was significant ($F(4,10) = 9.901$, $p = .002$) and post-hoc testing showed that all exposure conditions had a greater number of c-FOS+/GABA+ co-stained cells per HPF than naive (all $p \leq .001$) with the degree of co-staining being inversely related to AM depth, however no further comparisons were significant (all $p \geq .16$, see **Fig. 2.4C** for pairwise statistics). We estimated the A1 excitatory-inhibitory (EI) balance following noise exposure by calculating the ratio of excitatory to inhibitory cells expressing c-FOS in each HPF (**Fig. 2.4D**). Only images with at least one c-FOS+ excitatory and inhibitory cell were included. We found a significant interaction between layer and exposure group for EI ratio (mixed effects two-way ANOVA $F(8,183.44) = 288.0$, $p < .0001$, n = 206 HPFs within 15 rats) and group differences in each layer (simple main effects tests, all $F(4,44.81) \geq 3.9342$, $p \leq .036$). Post-hoc testing revealed a significantly greater EI ratio per HPF in layers 2/3 for all noise-exposed rats compared to naïve rats. In layers 4 and 5/6, however, the depth of AM noise appeared to modulate the EI ratio, as only rats exposed to 0% AM noise had a significantly greater EI ratio than naïve rats. For all layers combined, rats exposed to 0% and 25% AM noise were significantly greater than Naïve, while rats exposed to 50% and 100% AM noise were not significantly different from the other conditions. See **Fig. 2.4D** for pairwise statistics. In sum, two weeks of noise exposure elevated c-FOS expression in both excitatory and inhibitory cells but was the most pronounced for rats exposed to noise with the smallest modulation depths. For rats exposed to 50% and 100% AM noise, increased activity in inhibitory cells balanced that of excitatory cells, as their total EI ratio was not different from naïve. For rats exposed to 0% and 25% AM noise, however, excess excitatory activity was not balanced by inhibition as the EI ratio was higher than naïve rats.

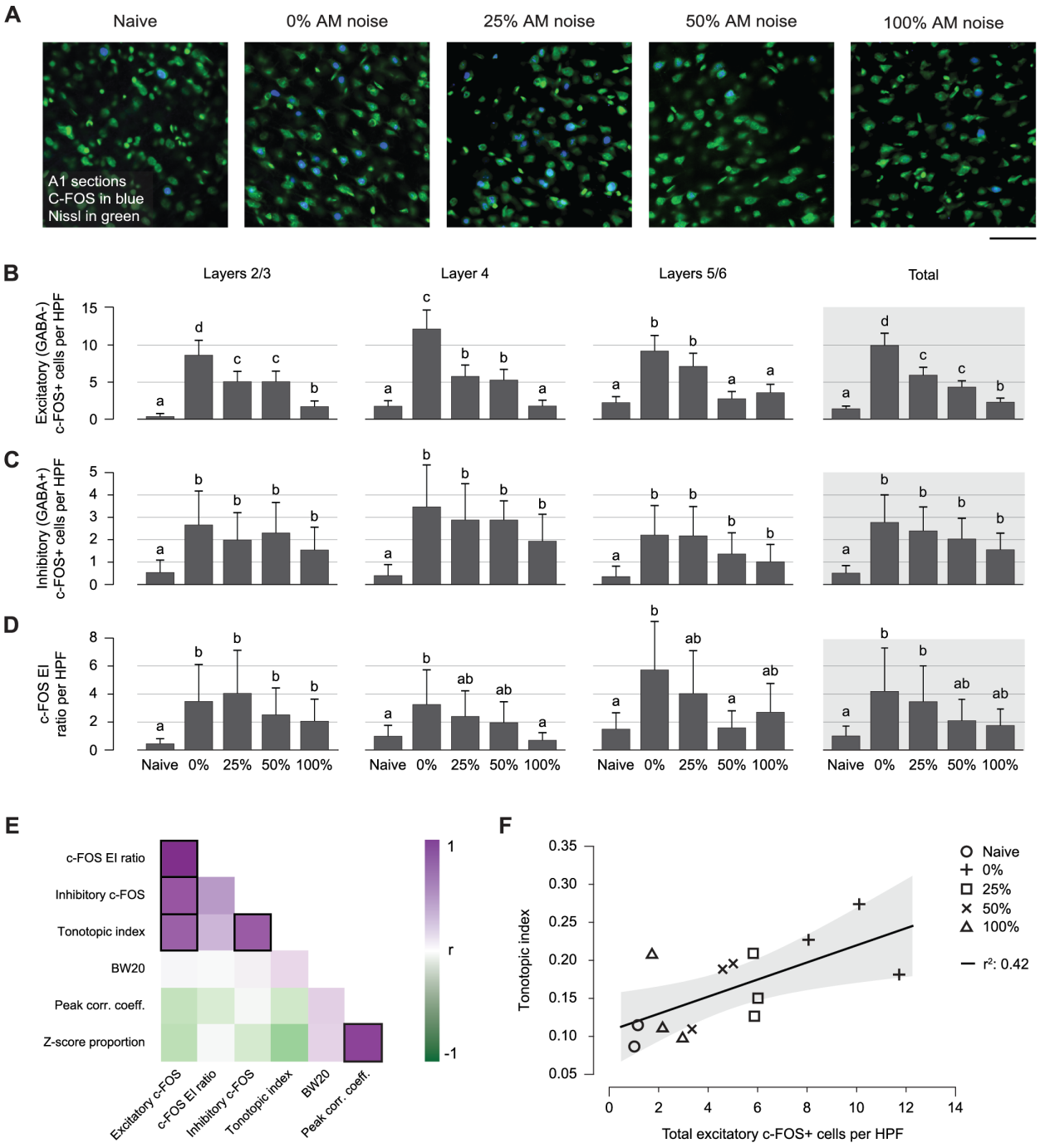

Figure 2.4: *C-FOS expression in A1 excitatory and inhibitory neurons following noise exposure*. **A.** High-power field (HPF) images of A1 sections at 40x magnification of naive rats and rats exposed to AM noise of 0%, 25%, 50%, or 100% depth for two weeks. Neuron-specific Nissl in green, c-FOS in blue. Scale bar represents 100μm. **B.** Mean number of excitatory (GABA-) cells expressing c-FOS per HPF. **C.** Mean number of inhibitory (GABA+) cells expressing c-FOS per HPF. **D.** Mean ratio of excitatory to inhibitory cells expressing c-FOS per HPF. **E.** Correlation matrix for c-FOS expression in all layers combined and electrophysiology measures. Color represents

Pearson R correlation coefficients. Boxes in bold are correlated with p<.05. **F.** One example of a correlation from E: tonotopic index derived from characteristic frequency maps of each rat vs. their mean number of c-FOS+ excitatory cells per HPF. Regression line is plotted for all exposure groups together, n = 14. Shaded region represents confidence of fit. Error bars represent 95% confidence intervals. Within layer division, bars not connected by the same letter are significantly different with p<.05. There were 3 rats per group and 21 HPF images per rat in the GABA- and GABA+ staining conditions. Number of HPF images per group in the EI ratio condition: Naïve 15 AM-0 50, AM-25 52, AM-50 52, AM-100 37.

To investigate potential electrophysiological correlates of c-FOS expression, the number of excitatory and inhibitory cells expressing c-FOS as well as the c-FOS EI ratio in all layers combined were correlated with the various electrophysiological measures acquired from rats in all exposure groups (**Fig. 2.4E**). These were tonotopic index, BW20, peak correlation coefficient, and proportion of synchronized neurons. Out of these measures, only tonotopic index was significantly related to c-FOS expression. Tonotopic index was significantly correlated with the number of c-FOS+ excitatory cells, r = .649, p = .012, n = 14 rats (**Fig. 2.4F**), and the number of c-FOS+ inhibitory cells, r = .684 p = .007, n = 14 rats, however not with the c-FOS EI ratio, r = 0.353, p = .216, n = 14 rats. See **Fig. 2.4E** for all pairwise correlations. These results indicate that absolute c-FOS expression in excitatory and inhibitory cells following two weeks of exposure to AM noise is linearly related to tonotopic organization. We also attempted to correlate the c-FOS EI ratio with two electrophysiological measures of EI ratio: spontaneous firing rate and onset latency for the response to pure tones. We found that c-FOS EI ratio was not significantly correlated with spontaneous firing rate, r = -0.050, p = .878, n = 14 rats, but was significantly negatively correlated with onset latency, r = -.534, p = .049, n = 14 rats, so rats with higher c-FOS EI ratios also had shorter onset latencies.

The time course of plasticity during noise exposure has previously not been investigated, as earlier studies examined rats chronically exposed to white noise for at least a month or longer

during adulthood (Zhou et al. 2011; Zheng 2012; Kamal et al. 2013). We performed a second set of c-FOS experiments to estimate the time course of plasticity in excitatory and inhibitory cells following the onset of noise exposure (**Fig. 2.5**). For these experiments, unexposed rats were compared to rats exposed to 0% or 100% AM noise for durations of one hour, 24 hours, one week, or two weeks. The AM depths 0% and 100% were chosen to compare the two most contrasting depths of noise exposure. None of the rats used in these analyses underwent electrophysiological recordings. C-FOS expression in excitatory (c-FOS+/GABA-) and inhibitory (c-FOS+/GABA+) neurons as well as c-FOS EI ratio was measured in layers 2/3, 4, and 5/6 and compared using three-way ANOVAs. Only the interactions that included AM exposure depth were analyzed. For excitatory neurons, the three-way interaction between AM depth, duration, and layer was not significant (GLMM three-way ANOVA $F(8,580.77) = 1.046$, $p = .40$, n = 567 HPFs within 27 rats), nor was the interaction between AM depth and layer ($F(2,580.77) = 1.977$, $p = .139$). However, the interaction between AM depth and duration was significant ($F(4,24.72) = 120.019$, $p < .0001$). The AM-0 and AM-100 groups were significantly different from each other at the one hour, one week, and two week time points (simple main effects tests, all $F(1,19.13) \geq 32.204$, $p < .0001$). While the number of c-FOS+ cells per HPF increased with exposure duration for AM-0 animals, for AM-100 animals it was greatest at one hour and returned to baseline at two weeks. See **Fig. 2.5A** for pairwise statistics. The three-way interaction was also not significant for inhibitory cells (GLMM three-way ANOVA $F(8,580.83) = 0.977$, $p = .453$, n = 567 HPFs within 27 rats), nor was the interaction between AM depth and layer ($F(2,580.77) = 0.275$, $p = .760$). The interaction between AM depth and duration was significant ($F(4,23.99) = 4.533$, $p = .007$). The AM-0 and AM-100 groups were significantly different from each other at the one hour and two week time points (simple main effects tests,

both F(1,18.98) ≥ 6.257, p ≤ .022). Similar to excitatory cells, the number of c-FOS-expressing inhibitory cells increased with exposure to 0% AM noise and initially increased after one hour of exposure to 100% AM noise but returned to baseline after two weeks. See **Fig. 2.5B** for pairwise statistics. Finally, the estimated EI ratio also did not yield a significant three-way interaction (Mixed effects three-way ANOVA F(8,268) = 0.712, p = .681, n = 288 HPFs within 27 rats) or two-way interaction between AM depth and layer (F(2,268.3) = 0.246, p = .782), but did reveal a significant interaction between AM depth and exposure duration (F(4,30.25) = 11.468, p < .0001). The EI ratio for AM-0 and AM-100 exposed rats were different at one hour, one week, and two weeks (simple main effects tests, all F(1,18.5) ≥ 9.337, p ≤ .007), following the trend of excitatory and inhibitory cells. See **Fig. 2.5C** for pairwise statistics.

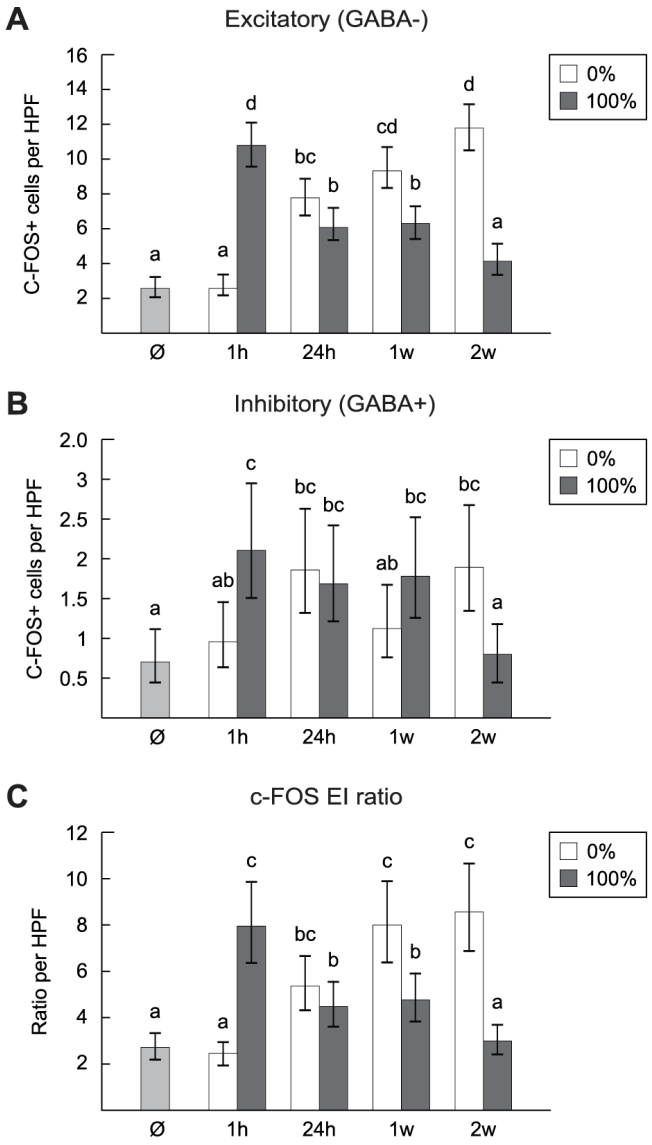

Figure 2.5: *Time course of C-FOS expression in A1 excitatory and inhibitory neurons during noise exposure*. The c-FOS expression of unexposed rats was compared to those of rats exposed to 0% or 100% AM noise for 1h, 24h, 1wk, or 2wk. **A.** Mean number of excitatory (GABA-) cells expressing c-FOS per high-power field (HPF). **B.** Mean number of inhibitory (GABA+) cells expressing c-FOS per HPF. **C.** Mean ratio of excitatory to inhibitory cells expressing c-FOS per HPF. Error bars represent 95% confidence intervals. Within layer division, bars not connected by the same letter are significantly different with p<.05. There were 3 rats per group and 21 HPF images per rat per staining condition. HPF images per group in the EI ratio condition: Unexposed 19; AM-0 1h 25, 24h 37, 1w 29, 2w 41; AM-100 1h 38, 24h 38, 1w 41, 2w 20.

Together, these results indicate a very different time course of c-FOS expression for rats exposed to unmodulated or modulated noise. While exposure to temporally modulated noise initially elicits strong c-FOS expression and a higher than normal EI ratio, inhibitory activity eventually appears to balance that of excitatory activity so that the EI ratio returns to baseline despite the persistence of noise during this time. Exposure to unmodulated noise, on the other hand, gradually increases cellular activity and EI ratio for the entire two-week duration. This important contrast between animals exposed to AM-0 and AM-100 noise at the two-week time point could account for our observed differences in measures of electrophysiological plasticity.

Two major non-overlapping subsets of GABA interneurons are those expressing the protein parvalbumin (PV+) and the neuromodulator somatostatin (SST+), representing approximately 40% and 30% of all interneurons respectively (Rudy et al. 2011). Changes in the activity of one or both of these subgroups are likely to play a role in noise-induced plasticity as both help shape spectral tuning in the auditory cortex (Wehr and Zador 2003; Zhang et al. 2011; Kato et al. 2017) and PV+ cells contribute to critical period regulation (Hensch 2005; Takesian and Hensch 2013) and have previously been linked to experience-dependent plasticity after moderate-level sound exposure (de Villers-Sidani et al. 2008; Zhou and Merzenich 2012; Kamal et al. 2013). To determine whether noise exposure affected the activity of these cell types, we examined the time course of c-FOS expression in PV+ and SST+ cells for rats exposed to 0% or 100% AM noise at the same time points as above (**Fig. 2.6**). We did so by calculating the percentage of PV+ and SST+ cells that expressed c-FOS per HPF at each of these time points and conducted three-way ANOVAs between AM depth, exposure duration, and cortical layer. For PV+ cells, we found that the three-way interaction was not significant (mixed-effects three-way ANOVA $F(8,477.5) = 0.585$, $p = .790$, $n = 539$ HPFs within 27 rats) and out of the two-way

interactions, only that between duration and layer was significant ($F(8,479) = 4.555$, $p < .0001$; AM Depth*Duration $F(4,33.74) = 2.177$, $p = .093$; AM Depth*Layer $F(2,477.3) = 0.175$, $p = .840$). For layers 2/3, there was a significant effect of duration on c-FOS expression ($F(4,151.9) = 6.984$, $p < .0001$), but not for any other layer (simple main effects tests, all $F(4,82.44) \leq 1.947$, $p \geq .109$). Post-hoc tests showed that animals exposed to noise for any duration expressed less c-FOS in layer 2/3 PV+ cells than unexposed animals (**Fig. 2.6A**). This difference was substantial as approximately 60% of layer 2/3 PV+ cells were c-FOS+ for unexposed animals and this number dropped to 10% for animals exposed to noise for two weeks. For SST+ interneurons, we found zero cells co-expressed with c-FOS in unexposed rats so this time point was not included in the model. The three-way interaction was also not significant (mixed-effects three-way ANOVA $F(6,405.8) = 1.809$, $p = .096$, n = 442 HPFs within 24 rats) and the only significant two-way interaction was that between AM depth and exposure duration ($F(3,16.12) = 5.358$, $p = .009$; Duration*Layer $F(6,405.8) = 1.622$, $p = .140$; AM Depth*Layer $F(2,406.1) = 1.311$, $p = .271$). The effect of duration was not significant for rats exposed to 0% AM noise (simple main effects test, $F(3,16.97) = 0.729$, $p = .549$), yet it was for rats exposed to 100% AM noise (simple main effects test, $F(3,15.23) = 6.094$, $p = .006$). For the durations of one hour and 24 hours, rats exposed to 100% AM noise expressed a greater percentage of c-FOS+/PV+ cells than rats exposed to 0% AM noise (simple main effects tests, both $F(1,13.81) \geq 8.545$, $p \leq .011$). The groups were not different from each other at the two other time points (simple main effects tests, both $F(1,16.27) \leq 1.701$, $p \geq .210$). In sum, noise exposure had diverse effects on c-FOS expression in PV+ and SST+ cells. Exposure to both unmodulated and modulated noise decreased c-FOS expression in layer 2/3 PV+ cells but did not affect c-FOS expression in layer 4 or layer 5/6 PV+ cells. The decrease in layer 2/3 cells occurred within one hour of the onset of

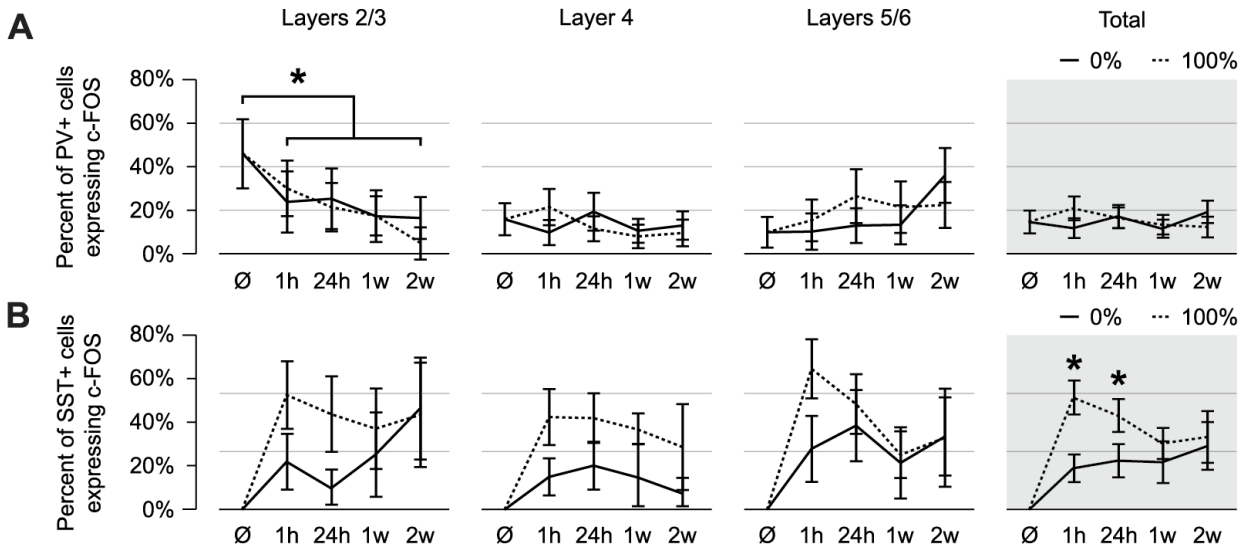

Figure 2.6: *Effects of AM noise exposure on percentage of A1 PV+ and SST+ inhibitory neurons expressing c-FOS*. The c-FOS expression of unexposed rats was compared to those of rats exposed to 0% or 100% AM noise for 1 hour, 24 hours, 1 week, or 2 weeks. **A.** Percentage of PV+ cells expressing c-FOS per high-power field (HPF). **B**. Percentage of SST+ cells expressing c-FOS per HPF. Error bars represent 95% confidence intervals. *p<.05. There were 3 rats per group. Number of HPF images per group in each staining condition: PV+ Unexposed 45; AM-0 1h 47, 24h 53, 1w 54, 2w 50; AM-100 1h 56, 24h 59, 1w 60, 2w 49; SST+ Unexposed 46; AM-0 1h 59, 24h 56, 1w 50, 2w 49; AM-100 1h 62, 24h 61, 1w 60, 2w 45.

noise exposure and lasted up to two weeks. Exposure to both types of noise elicited an increase in c-FOS expression in all layers for SST+ cells; this increase was compared to the zero co-stained cells found in naïve rats. However, for at least the first 24 hours of exposure, 100% AM noise elicited greater c-FOS expression than 0% AM noise in all layers combined. This greater early recruitment of SST activity could explain why rats exposed to 100% AM noise quickly regain a normal EI ratio following the onset of noise exposure while rats exposed to 0% AM noise do not.

2.5 Discussion

The absence of normal sensory inputs whether resulting from peripheral damage, sensory deprivation, or sensory masking drives experience-dependent plasticity in animals well beyond their sensory critical periods (Merzenich et al. 1984; Chino et al. 1992; Diamond et al. 1993; Trachtenberg et al. 2002; He et al. 2006; Karmarkar and Dan 2006; Zhou et al. 2011; Eggermont 2013). We compared rats exposed to unmodulated or amplitude-modulated white noise to show that masking salient temporal auditory inputs induces critical period-like plasticity in the adult auditory cortex. We first confirmed that two weeks of exposure to unmodulated noise was sufficient to produce experience-dependent plastic changes in A1 including broadening of receptive field bandwidths, disruption of the stereotypical tonotopic gradient, and desynchronization of neuronal activity. These observations replicated those reported in previous studies of noise exposure that used exposure durations of six weeks (Zhou et al., 2011; Kamal et al., 2013). We also confirmed that map disruption was driven by an invasion of high frequency representations into low frequency regions, previously observed after continuous unmodulated noise (Chang and Merzenich 2003; Zhou et al. 2011) and tone exposure (Zhou et al. 2008). Only the observations of Zheng (2012) did not match our findings, as they observed no change in receptive field bandwidth and a random dispersion of frequency representations instead of a shift towards high frequencies following 30 days of noise exposure. However, this difference might be explained by the fact that their study utilized best frequency instead of characteristic frequency. Rats exposed to AM noise of the tested modulation depths, which were 25%, 50%, and 100%, also showed experience dependent plasticity following noise exposure. AM depth appeared to modulate change in tonotopic gradient, as rats exposed to 100% AM noise showed no change in tonotopic index but rats exposed to 25% and 50% AM noise exhibited a slight

increase in map disorganization that was less than rats exposed to unmodulated noise. All AM noise-exposed rats exhibited broadened receptive field bandwidths, which has also been observed in rats exposed to pulsed noise during development (Zhang et al. 2002; Insanally et al. 2010) and adulthood (Zhou and Merzenich 2012) and indicates that frequency response selectivity is degraded following both unmodulated and modulated noise exposure. Broadening of receptive field bandwidth independent of changes in tonotopic organization could be explained by the slight neuronal hypersynchronization observed in rats exposed to AM noise as increased correlation between neurons could promote receptive field plasticity leading them to fire to the preferred tone of their neighbors while also maintaining their own preferred frequencies (Brosch and Schreiner 1999; Eggermont 2007). Hypersynchronization was likely driven by the rhythmic modulation rate of our exposure stimuli as a previous study also reported increased correlation during spontaneous activity following pulsed noise exposure even when the modulation rate was not constant (Zhou and Merzenich 2012). However, the effect appears to be limited to passive exposure of mature animals, as pulsed noise exposure during development (Zhang et al. 2002) and pulsed noise paired with nucleus basalis stimulation (Bao et al. 2003) were actually observed to decrease spontaneous synchronization. We aimed to describe the relationship between tonotopic map plasticity and synchrony with an exponential function and noted that only desynchronization below a certain threshold contributed to frequency instability. This supports the notion that spontaneous correlated activity plays an important role in maintaining global sensory representations in sensory cortices (Katz and Shatz 1996; Sur and Leamey 2001). Together, the three variables of tonotopic index, receptive field bandwidth, and synchronization were used to successfully classify rats as being unexposed, unmodulated noise-exposed, or modulated noise-exposed showing that these groups possess identifiable ‘profiles of

plasticity' that could explain their qualitative differences. Finally, we observed that only rats exposed to unmodulated noise showed additional tonotopic reorganization following a second passive exposure to pure tones, a phenomenon that is considered a hallmark of critical period plasticity (de Villers-Sidani et al. 2007). Since rats exposed to 50% AM noise did not display an over-representation of the exposure tone within A1 when submitted to the same tone pip exposure, we concluded that the masking of temporal auditory inputs in the unmodulated noise condition triggered critical-period like plasticity in the adult auditory cortex.

We chose to study tonotopic index, receptive field bandwidth, synchronization, and reorganization following passive tone pip exposure as electrophysiological measures of plasticity since they had been reported by previous studies of noise exposure. However, it is important to acknowledge that there could be much more subtle measures of plasticity affected by noise exposure that were not assessed here. For instance, we used extracellular mapping to measure large-scale tonotopic organization, which has been shown to be much more heterogeneous at smaller scales (Kanold et al. 2014). Furthermore we could not assess thalamocortical tuning or the excitatory/inhibitory balance of inputs onto individual cells, which would be necessary to establish the exact mechanisms of noise-induced plasticity. Future studies of noise exposure would undoubtedly benefit from these methods.

Using immunohistochemical techniques, unmodulated and modulated noise were found to elicit different patterns of c-FOS expression – used here as a measure of both cellular activity and plasticity – in populations of auditory neurons. We observed significant increases in excitatory and inhibitory c-FOS expression that depended on depth of AM noise exposure. Specifically, rats exposed to unmodulated noise showed the highest degree of c-FOS expression, which decreased for rats exposed to greater depths of AM noise. The trend was similar for all

cortical layers, but stood out the most in layers 4 and 5/6 where the c-FOS EI ratio was significantly greater than baseline for rats exposed to 0% AM noise only. In layers 2/3, on the other hand, the EI ratio was elevated for all noise-exposed rats regardless of AM depth. For all layers combined, rats exposed to 0% and 25% AM noise showed a significantly elevated EI ratio. These results suggest that while cellular activity increases during all forms of noise exposure, it is more disinhibited in the presence of unmodulated noise. This observation, coupled with the finding that rats exposed to unmodulated noise showed the greatest tonotopic disorganization, is consistent with the notion that transient imbalances in EI ratio contribute to receptive field and tonotopic map plasticity (Froemke and Martins 2011; Carcea and Froemke 2013). Accordingly, we hypothesized that a high EI ratio would be positively correlated with tonotopic index, however we found that only absolute excitatory and inhibitory c-FOS expression were significantly correlated with this measure. This finding likely reflects the fact that excitatory and inhibitory networks can be modified independently of each other, however since c-FOS is a relatively coarse measure of EI ratio, intracellular recordings would be required to conclusively determine whether an imbalance of excitatory and inhibitory inputs onto the same cell contribute to receptive field plasticity after noise exposure. To further investigate changes in excitatory and inhibitory function during noise exposure, we quantified c-FOS expression over time in rats exposed to 0% and 100% AM noise and observed opposite patterns of c-FOS expression. We found that the number of c-FOS+ excitatory cells and EI ratio were initially high for rats exposed to 100% AM noise but returned to baseline by two weeks of exposure whereas both measures gradually increased with exposure duration for rats exposed to 0% AM noise. Since c-FOS is an immediate early gene that regulates downstream gene transcription and reports changes in cellular activity occurring over very short time scales (West

et al. 2001; West et al. 2002), the observation that c-FOS expression continuously increases during exposure to 0% AM noise is significant. First, it indicates that A1 neurons do not adapt to unmodulated noise; instead, persistent activity increases the likelihood for activity-dependent plastic changes to take place, revealing a potential mechanism for noise-induced plasticity. Second, the gradual increase in c-FOS expression emphasizes that exposure duration is a key factor in experience dependent plasticity. Whereas passive sound experience can permanently modify the auditory receptive fields of immature animals in a matter of minutes (Dorrn et al. 2010), similar changes occur only after weeks or months of exposure in adult animals, and often do not persist (Eggermont 2013).

Finally, we also quantified c-FOS expression over time in PV+ and SST+ inhibitory interneurons. We observed a decrease in the proportion of layer 2/3 c-FOS+/PV+ cells for all exposure durations for both 0% and 100% AM noise; no differences were observed in other layers. In contrast, noise exposure increased the expression of c-FOS+/SST+ cells at all time points and in all layers, but this was the greatest for 100% AM noise during the first 24 hours of exposure. PV+ cells primarily target the basal dendrites of pyramidal cells in the same layer as themselves. As such, reduced PV activity in layer 2/3 could increase excitatory activity in these layers, where we observed a uniform increase in EI ratio for all noise-exposed rats. The finding that PV+ interneuron activity was not influenced by AM depth fits with previous studies that observed a decrease in PV+ cell count after exposure to both unmodulated (Kamal et al. 2013) and pulsed noise (Zhou and Merzenich 2012) in adult rats. The observation that AM depth modulated SST+ cell activity, on the other hand, suggests that SST+ interneurons may play a protective role in maintaining mature sensory representations during noise exposure. While much remains to be learned about the role of SST+ cells in auditory processing and plasticity, a recent

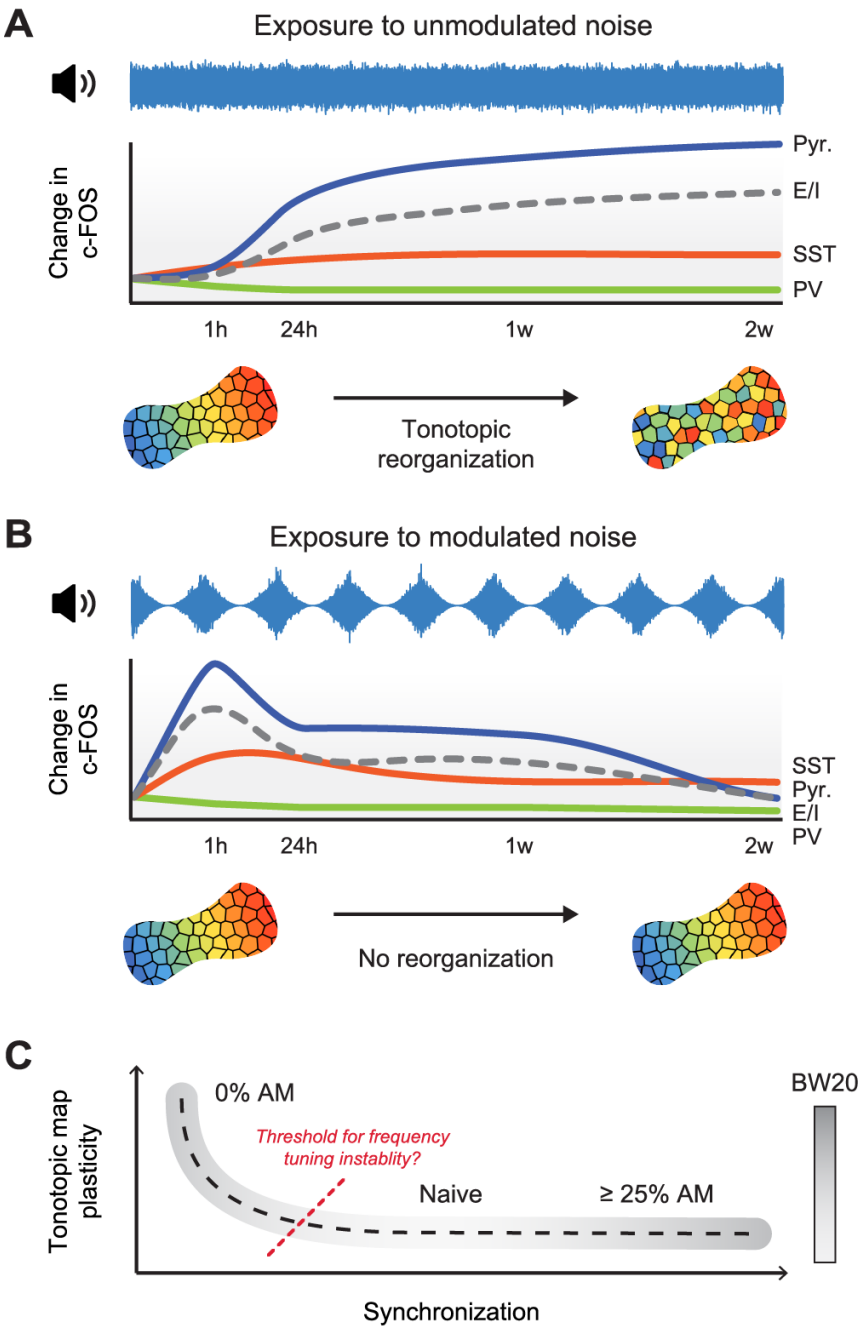

Figure 2.7: *How noise exposure contributes to tonotopic map plasticity*. Chronic exposure to moderate-intensity white noise induces experience-dependent plasticity based on the temporal modulation of the noise. Exposure to unmodulated noise (**A**) leads to disinhibition and A1 tonotopic reorganization while exposure to strongly modulated noise (**B**) only transiently increases disinhibition with no lasting effect on tonotopy. These changes are mediated by different patterns of cellular activation in cortical neuron populations as measured by c-FOS expression in excitatory neurons (primarily pyramidal - blue line) and inhibitory interneurons including SST+ cells (orange line), and PV+ cells (green line). The overall change in excitation is estimated by the c-FOS EI ratio (grey dashed line). The early

increase in SST+ cell activity during exposure to modulated noise suggests that these cells may play a role in maintaining stable sensory representations in the adult auditory cortex. Unmodulated and modulated noise also have opposite effects on cortical synchronization (**C**) that persist beyond the end of the exposure period. Desynchronization below a certain threshold could thus contribute to tonotopic frequency tuning instability during and after unmodulated noise exposure. Changes in receptive field bandwidth (BW20) are independent of map reorganization following noise exposure as both exposure to unmodulated and modulated noise decrease tuning selectivity. Together, measures of tonotopy, synchronization, and receptive field bandwidth can be used to describe and accurately distinguish animals exposed to unmodulated or modulated noise from naïve animals.

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report suggests that SST+ cells govern lateral inhibition in the auditory system (Kato et al. 2017) and the SST peptide, which has inhibitory action on the apical dendrites of pyramidal cells through G-coupled protein receptors, is known to be released in an activity-dependent manner (Lahlou et al. 2004; Tallent and Qiu 2008). Early SST+ interneuron activity triggered by temporal modulation could thus effectively reduce excitatory pyramidal drive during several days of noise exposure. In addition to other subtypes of inhibitory interneurons, there are also multiple other cellular and molecular inhibitory components that mediate cortical plasticity including elements of the extracellular matrix, myelin sheaths, inhibitory receptor subunits, and epigenetics (Hensch 2005; Bavelier et al. 2010). Many of these plasticity-regulators are also likely to be involved in the transition to noise-induced plasticity and could be investigated in future studies. Our findings regarding the time course of c-FOS activity and the relationship between c-FOS expression and tonotopic plasticity are summarized in **Fig. 2.7**.

It is important to note that while we attempted to mask all environmental sounds with our exposure stimuli, we cannot be sure that we achieved complete masking because the acoustic environment may have occasionally contained louder sounds that were not fully masked. We are confident, however, that neural responses to all spectrotemporal inputs were suppressed during the time of exposure since white noise shifts the threshold for sound-evoked responses to higher

intensities (Costalupes et al. 1984; Phillips 1985; Phillips and Cynader 1985). The exposures tested in this study also do not address the question of whether rats exposed to random temporal modulation, as opposed to the rhythmic 3Hz modulation used here, would exhibit experience-dependent plasticity similar to rats exposed to unmodulated noise. As we observed, the introduction of highly structured inputs with AM noise led to a slight hypersynchronization of neuronal activity, which could have had a specific effect on SST+ cells or played another role in preventing critical period-like plasticity. It is unclear whether a random temporal modulation would have had the same effect and answering this question would be necessary to state whether salient temporal modulation is sufficient to prevent noise-induced plasticity or whether rhythmic temporal modulation is necessary. In addition, rate of modulation is almost certain to play a role. Pure tones presented at an ethological rate – one which is behaviorally relevant – during the critical period lead to an over-representation of that tone within the tonotopic map while tones presented at a non-ethological rate do not (Kim and Bao 2009). The 3Hz rate used in this experiment could be considered to be within the ethological range for the rat, as rat calls are typically repeated at 3-10Hz (Kim and Bao 2009) and temporally modulated noise outside of this range might have elicited effects more closely resembling those of unmodulated noise.

Our findings contribute to a growing body of literature demonstrating that prolonged exposure to passive sounds, even at moderate volumes, can modify auditory function and behavior. These findings depart from the traditional view that the mature brain is resistant to changes from passive stimulation and have important consequences for auditory health during later stages of life. For example, chronic exposures to tone pip ensembles induce long-term suppression of neural activity within the central auditory system and auditory cortex of adult cats (Pienkowski and Eggermont 2009; Pienkowski and Eggermont 2010; Pienkowski et al. 2011).

This suppression is thought to arise from homeostatic mechanisms that decrease neural activity in the exposure frequency range and takes several days to build up. Adult rats exposed to pulsed noise bursts for two months show degraded neuronal responses to temporal and spectral stimuli and impaired temporal rate discrimination, which persist for at least six weeks after removal from noise exposure (Zhou and Merzenich 2012). Finally, young adult rats exposed to continuous white noise exhibit auditory responses similar to those of aging rats, suggesting that noisy sensory percepts have detrimental effects on mature cortical function (Kamal et al. 2013). The brain's ability to initiate plasticity in the absence of normal sensory inputs is likely an adaptive mechanism to facilitate cortical rewiring in case of deafferentation, neurological injury or trauma to peripheral sensory systems (Carmichael 2003; Dancause and Nudo 2011; Miltner and Witte 2016). However, this capacity could also lead to vulnerability in abnormal sensory situations brought about by endogenous or environmental factors such as chronic otitis-media, age-related hearing loss, or noisy working environments (Kotak et al. 2005; Kamal et al. 2013; Gourévitch et al. 2014; Caras and Sanes 2015), and if not regulated, could increase the risk of developing disorders of plasticity (Voss et al. 2017). Despite the risks of long-term exposure to noisy inputs on auditory function, the ability to modify mature neuronal circuits with passive sensory stimulation also poses an interesting option for plasticity-based therapeutics in a controlled clinical context. Having already been established as a robust and non-invasive means of driving plasticity, sound exposure could feasibly be used to enhance plasticity and cortical rewiring in specific regions of the auditory cortex. This technology could be applied to treat auditory disorders such as tinnitus (Moller 2007), facilitate adaptation to cochlear implants or hearing aids, or be paired with auditory training to enhance auditory learning and memory. For

these reasons, future studies should continue to investigate noise exposure to understand not only its risks, but also the potential therapeutic benefits of noise-induced plasticity.

2.6 Conclusion

We demonstrated that exposure to unmodulated white noise leads to a reopening of the critical period for spectral tuning in A1 while exposure to temporally modulated noise does not. Our findings show that changes in neuronal activity resulting from a lack of correlated temporal structure in white noise could account for the plastic changes previously observed in the auditory cortex after chronic noise exposure. These results suggest that salient temporal inputs from the environment are necessary for the maintenance of mature auditory circuits and stable sensory representations. Finally, SST+ interneurons may serve a protective function in maintaining this sensory stability. The results of this study underline the fact that plasticity in response to passive sensory stimulation is not restricted to early developmental stages emphasizing the continued importance of high fidelity, patterned sensory inputs throughout life for healthy auditory function yet also point to the potential use of passive sensory stimulation in rewiring adult neural circuits for therapeutic purposes.

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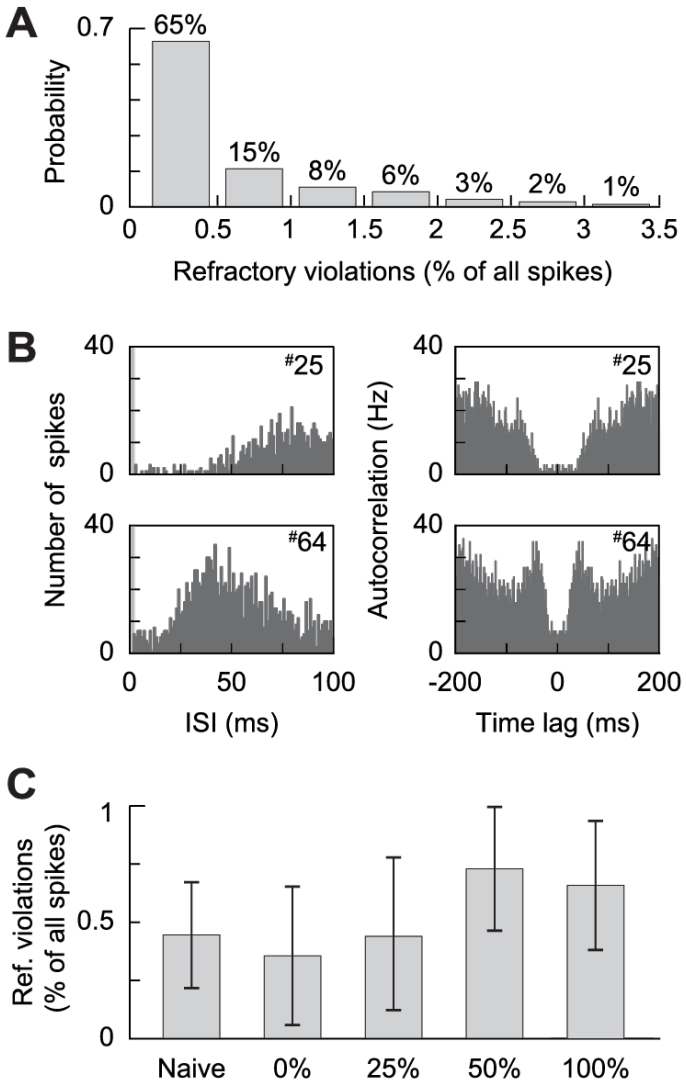
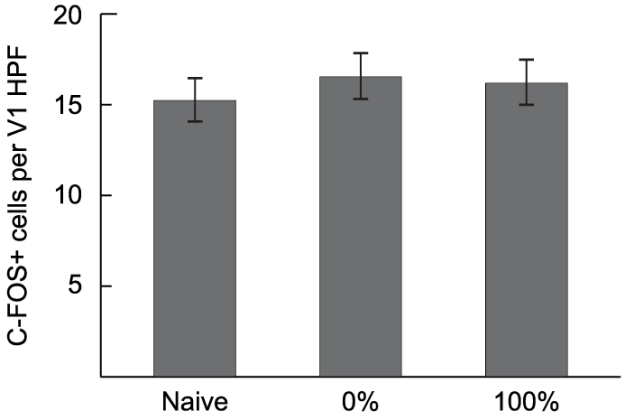
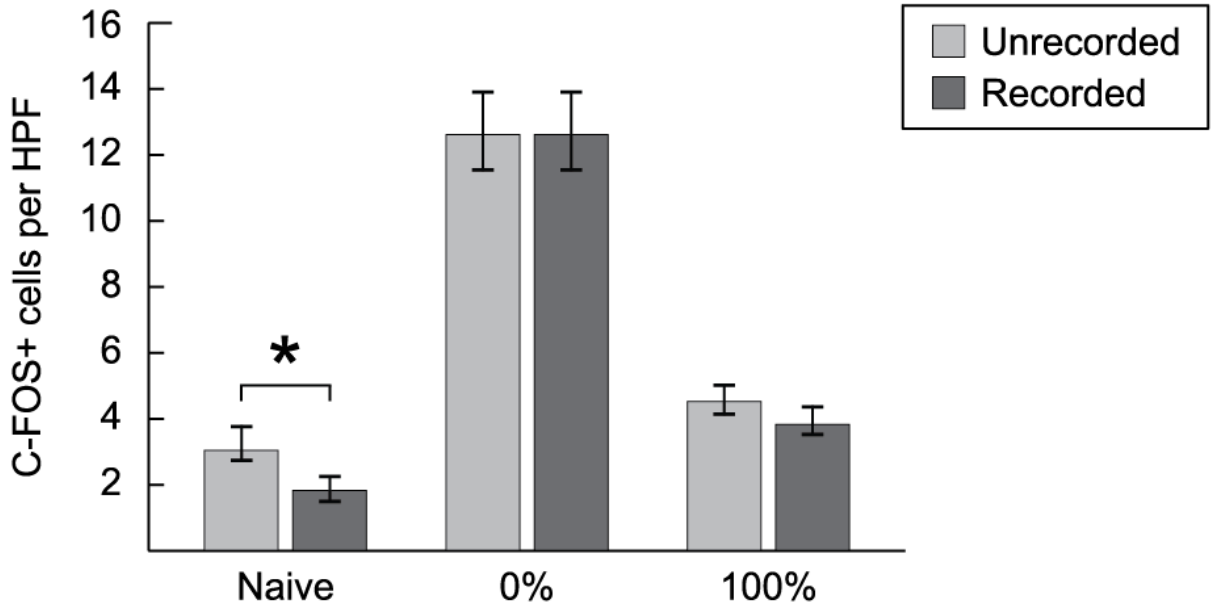
2.8 Supplementary figures



Supplementary figure 2.1: *Quality assessments for spike sorting*. **A.** Histogram of the fraction of refractory period violations out of all spikes for 576 identified clusters used for analyses. **B.** Representative histograms for two isolated units (#25 and #64) of inter-spike interval (ISI) times (left) and autocorrelation functions (right). Bin widths are 1ms and gray shaded line represents the 2ms refractory period bin. **C.** Mean fraction of refractory period violations out of all spikes for AM noise exposure groups. No difference was found between groups using a mixed-effects one-way ANOVA $F(4,23.2) = 1.922$, $p = .141$, $n = 576$ clusters within 30 positions and 19 rats. Error bars represent 95% confidence intervals. Number of animals, recording positions, and clusters per group: Naïve 5,8,176, AM-0 4,7,113, AM-25 3,4,84, AM-50 3,5,97, AM-100 4,6,106.

Supplementary figure 2.2: *C-FOS expression in the primary visual cortex (V1).* Mean number of cells expressing c-FOS per V1 HPF in Naïve rats, rats exposed to 0% AM noise for two weeks, and rats exposed to 100% AM noise for two weeks. These rats had undergone electrophysiological recordings. No difference in the number of cells per HPF was found between groups as determined by a one-way ANOVA $F(2,6) = 1.19$, $p = .41$, $n = 189$ HPFs within 9 rats. Error bars represent 95% confidence intervals. There were 3 rats per group and 21 HPF images per rat.





Supplementary figure 3: *Differences in c-FOS expression between recorded and unrecorded animals.* Mean number of cells expressing c-FOS per A1 HPF in Naïve rats, rats exposed to 0% AM noise for two weeks, and rats exposed to 100% AM noise for two weeks. Comparisons were made between rats that were either perfused immediately following the end of exposure (Unrecorded) or underwent electrophysiological recordings prior to perfusion (Recorded). Only Naïve rats differed in c-FOS expression as determined by a GLMM $F(1,4) = 2.573$, *$p=.01$, $n = 126$ HPFs within 6 rats. There was no difference between unrecorded and recorded AM-0 rats (GLMM $F(1,4) = 0.03$, $p=.979$, $n = 126$ HPFs within 6 rats) or AM-100 rats (GLMM $F(1,4) = 1.593$, $p=.111$, $n = 126$ HPFs within 6 rats). Error bars represent 95% confidence intervals. There were 3 rats per group and 21 HPF images per rat.

Chapter 3

Modifying the adult rat tonotopic map with sound exposure produces frequency discrimination deficits that are recovered with training

Maryse E. Thomas, Conor P. Lane, Yohann J.M. Chaudron, J. Miguel Cisneros-Franco, Étienne de Villers-Sidani.

Preface

The previous study revealed that only exposure to unmodulated noise leads to strong CP-like plasticity in the adult auditory cortex. The defining feature of this type of plasticity is the ability for a second passive exposure to pure tones to produce a functional over-representation of the frequency region corresponding to the exposure tone in the tonotopic map. Similar functional expansions have been observed following auditory perceptual learning, suggesting that greater cortical representations for a specific frequency may enhance its discrimination. In the current chapter, the ability for noise and tone pip exposure to improve perceptual learning and pitch discrimination by inducing map expansion prior to learning was tested. The results of this study are expected to inform future neuroplasticity-based therapies that take into account both the sensory statistics of our external environment and perceptual training strategies to improve learning and memory in the auditory system. This manuscript has been accepted for publication by *Journal of Neuroscience* (December 2019).

3.1 Abstract

Frequency discrimination learning is often accompanied by an expansion of the functional region corresponding to the target frequency within the auditory cortex. Although the perceptual significance of this plastic functional reorganization remains debated, greater cortical representation is generally thought to improve perception for a stimulus. Recently, the ability to expand functional representations through passive sound experience has been demonstrated in adult rats, suggesting that it may be possible to design passive sound exposures to enhance specific perceptual abilities in adulthood. To test this hypothesis, we exposed adult female Long-Evans rats to two weeks of moderate-intensity broadband white noise followed by one week of 7kHz tone pips, a paradigm that results in the functional over-representation of 7kHz within the adult tonotopic map. We then tested the ability of exposed rats to identify 7kHz amongst distractor tones on an adaptive tone discrimination task. Contrary to our expectations, we found that map expansion impaired frequency discrimination and delayed perceptual learning. Rats exposed to noise followed by 15kHz tone pips were not impaired at the same task. Exposed rats also exhibited changes in auditory cortical responses consistent with reduced discriminability of the exposure tone. Encouragingly, these deficits were completely recovered with training. Our results provide strong evidence that map expansion alone does not imply improved perception. Rather, plastic changes in frequency representation induced by bottom-up processes can worsen perceptual faculties, but because of the very nature of plasticity these changes are inherently reversible.

3.2 Introduction

Statistical variations in the sensory environment can have profound effects on cortical sensory representations long after traditional developmental windows have closed. In the acoustic domain, prolonged exposure to moderate-intensity sounds may elicit experience-dependent plasticity in the mature auditory cortex under conditions such as environmental enrichment with varied stimuli (Engineer et al. 2004), or persistent exposure to uninformative (Noreña et al. 2006; Zhou et al. 2008; Pienkowski and Eggermont 2009) or disruptive (Zheng 2012; Zhou and Merzenich 2012; Kamal et al. 2013) sounds. The resulting effects on the adult tonotopic map can be broad or precise, as illustrated by passive exposure to broadband white noise, which disrupts frequency-tuning in a nonspecific manner but can induce the highly specific expansion of a selected iso-frequency band if followed by a subsequent exposure to pure tones (Zhou et al. 2011; Thomas et al. 2019a). These findings reveal the possibility that passive sound exposures could be designed to elicit targeted plastic changes in the auditory cortex in adulthood.

In frequency discrimination training, learning is often associated with an increase in the cortical representation of the frequency region corresponding to the rewarded sound (Polley et al. 2006; Keuroghlian and Knudsen 2007; McGann 2015; Voss et al. 2016). And although greater representation is generally thought to confer improved perception (Merzenich et al. 1984; Pantev et al. 1998; Rutkowski and Weinberger 2005; Wiestler and Diedrichsen 2013), artificially inducing tonotopic map expansion for a specific frequency has not always been found to improve discrimination for that frequency. While map expansion induced by pairing passive tone exposure with nucleus basalis stimulation enhanced discrimination for the paired tone in adult rats (Reed et al. 2011; Froemke et al. 2013), expansion by direct cortical microstimulation with a weak electric current did not alter frequency-discrimination performance (Talwar and Gerstein 2001), and rats

with expanded frequency representation due to tone exposure during the critical period were worse at discriminating those frequencies as adults (Han et al. 2007). The diverging results of these studies suggest that while map expansion may be a shared phenotype, its mechanism of induction determines whether or not it will carry perceptual significance.

Here, we attempted to improve frequency discrimination learning by enhancing the representation of a specific frequency in the primary auditory cortex (A1) of adult rats using passive sound exposure. We exposed young-adult Long-Evans rats to two weeks of moderate-intensity white noise followed by one week of 7kHz tone pip clouds to increase the functional representation of 7kHz within A1 similar to Zhou and colleagues (2011) and our previous work (Thomas et al., 2019a). We then trained exposed and non-exposed rats on an adaptive tone discrimination task in which the target tone was 7kHz. Although we had hypothesized that the induced early overrepresentation of the target tone would improve the behavioral performance of exposed animals, we found that it actually impaired discrimination for the exposure frequency and delayed perceptual learning. Rats exposed to noise followed by 15kHz tone pip clouds were not impaired at the same task. We also investigated the electrophysiological response properties of A1 neurons of exposed animals before and after training and found evidence of reduced neural discriminability for the target tone that was completely recovered with training. Our results confirm that map expansion alone does not imply improved perception. Rather, changes in frequency representation induced by passive sound experience can worsen perceptual faculties, but because of the very nature of plasticity these changes are inherently reversible.

3.3 Materials & Methods

Experimental groups: Six groups of female young adult Long-Evans rats (3.5-6 months) were used for this experiment: three untrained (UT), and three trained (T). Of the untrained groups, one was housed in a standard acoustic environment (Naïve-UT, N = 10). The other two were passively exposed to white noise for two weeks followed by 7kHz (7kHz-UT, N = 6) or 15kHz (15kHz-UT, N = 5) tone pip clouds for one week. The three trained groups underwent the same exposures prior to starting training. The groups were Naïve-T (N = 10), 7kHz-T (N = 10), and 15kHz-T (N = 8). A seventh group of rats was used to examine whether the effects of passive sound exposure persisted for the duration of training (7kHz-UT+12wks, N = 4). This group was exposed to white noise for two weeks followed by 7kHz tone pip clouds for one week and then returned to a standard acoustic environment for 12 weeks. All experimental procedures were approved by the Montreal Neurological Institute Animal Care Committee and follow the guidelines of the Canadian Council on Animal Care.

Exposure conditions: Rats were housed in pairs in cages within sound-attenuated chambers (background sound level 40dB SPL) under a 12h light/dark cycle with unlimited access to water. Those undergoing training were lightly food restricted. The weights of all rats were monitored to ensure that training and noise exposure conditions did not result in greater than 20% body-weight reduction. Sound-exposed rats were passively exposed 24 hours per day to moderate intensity (70dB SPL) broadband white noise for two weeks followed by one week of tone pip clouds. The tone pip clouds consisted of 50ms tones (5ms onset and offset ramps) of random frequencies within a 0.25 octaves range centered on either 7.6kHz (ranging from 7-8.3kHz) or 15kHz (ranging from 13.8-16.4kHz) and delivered in trains of 5 pulses per second. The interval between each train of tones was a random duration generated from a normal distribution with a mean of 2.5 seconds.

The stimuli were generated using custom MATLAB scripts (The MathWorks, Inc., Natick, Massachusetts) and played through an Ultralite-mk3 Hybrid Interface (MOTU Inc., Cambridge, Massachusetts) with sampling at 192kHz. The stimuli were amplified to a free-field sound level calibrated so that the intensity of the white noise measured in the center of the chamber was 70dB SPL (decibels sound pressure level, RMS) and the intensity of the tone pips was 65dB SPL.

Training procedure: Rats were trained in wire cages within sound-attenuated chambers. Behavior was shaped in two parts: pre-training and training. During pre-training, rats learned to poke their noise in a nose-poke (Lafayette Instrument, Lafayette, Indiana) to obtain a food reward of chocolate flavored sucrose pellets (45mg, BioServ, Flemington, New Jersey). Rats received a pellet if they poked within 5s of stimulus presentation, which was a 7kHz tone. Rats graduated from pre-training after three consecutive sessions in which they poked within 5s of the tone at a rate significantly greater than chance. This stage lasted approximately 2 weeks. Following pre-training, rats were either housed in a standard acoustic environment or exposed to sound for three weeks. After this period, the training program of interest began (see **Supplementary Figure 3.1**). Rats were trained on an adaptive go/no-go tone discrimination task in which the 7kHz pure tone target stimulus was presented in 20% of trials. The non-target tone started as 7kHz + ½ octave at the beginning of each session and became 0.025 octaves closer to the target tone as the task level increased. For a given trial, the rat's behavior was scored according to the combination of behavioral state (go or no-go) and stimulus property (target or non-target). Go responses within 5s of a target were scored as a hit; a failure to respond was scored as a miss. A go response within 5s of a non-target stimulus was scored as a false positive; the absence of a response was scored as a withhold. Go responses outside of these time windows were scored as false alarms and initiated a 5s time-out period during which no stimuli were presented. A hit triggered the delivery of a

chocolate pellet and an increase in task level. A miss or false positive initiated a decrease in task level and a 5s time-out. A withhold did not produce any consequences. The stimulus target recognition index, d-prime (Macmillan and Creelman 1990), was calculated for each training session from the hit rate (hits/hits + misses) and the false positive rate (false positives/false positives + withholds). Tones were 50ms in duration (5ms ramps) with 4-6 seconds between presentations and delivered in a free-field manner through a calibrated speaker at 60dB SPL. Sound presentation and response recording were performed with custom MATLAB scripts and Arduino hardware (Arduino LLC).

Training evaluation: Out of a total of 32 trained rats, four (one Naïve-T, two 7kHz-T, and one 15kHz-T) did not successfully relearn how to poke in response to the tone after this period. This was not found to be related to exposure group (chi square = 0.32, p = .8508, df = 2). These rats were not included in analyses. The rest of the animals were trained for approximately 12 weeks and completed between 60 and 71 training sessions. For analyses, we focused on the first 60 sessions for which we had an equal number of data points per rat, although most rats completed more than 60 sessions in total. We did not include a small number of training sessions in which rats were considered to be unmotivated. Unmotivated sessions were identified as having a hit rate less than 60% after a rat had obtained a d' ≥ 1 at least once (typically after 4-5 weeks of training). At this point in the training, rats were very familiar with the task and a low hit rate indicated that there was either a technical error with the hardware (e.g. pellets were not being released) or they were not hungry enough to be motivated by the food reward. The number of unmotivated sessions was not different between the three groups (combined mean = 2.0 ± 2.0 sessions, one-way ANOVA $F(2, 25) = 1.36$, $p = .2747$). For both pre-training and training, rats were trained for one hour per day approximately six times per week. The rate of training also did not differ between the three

groups (combined mean = 5.47 ± 0.46 sessions/week, one-way ANOVA $F(2,25) = 0.701$, $p = .5057$).

Electrophysiological recordings: Rats underwent electrophysiological recordings the day after exposure or training ended. Untrained rats were 3.5 months old at the time of recordings, trained rats were 2 months old at the beginning of training and 6 months old at the time of recordings, and 7kHz-UT+12wks rats were 6 months old at the time of recordings. Electrophysiological recordings of the left auditory cortex were performed in a shielded soundproof recording chamber. Rats were pre-medicated with dexamethasone (0.2mg/kg, i.m.) to minimize brain edema. Anesthesia was induced with ketamine/xylazine/acepromazine (63/13/1.5 mg/kg, i.p.) followed by continuous delivery of isoflurane 1% in oxygen via endotracheal intubation and mechanical ventilation. Heart rate and blood oxygen saturation were monitored with a pulse oximeter. Body temperature was monitored with a rectal probe and maintained at 37ºC with a homeothermic blanket system. Rats were held by the orbits in a custom designed head holder leaving the ears unobstructed. The cisterna magna was drained of cerebrospinal fluid to further minimize cerebral edema. To access the auditory cortex, the left temporalis muscle was reflected, the skull over the auditory cortex was removed, and the dura was resected. Once exposed, the cortex was maintained under a thin layer of silicone oil to prevent desiccation. Acoustic stimuli were delivered in a free field manner to the right ear through a calibrated speaker. Cortical responses were recorded with a 64-channel tungsten microelectrode array (Tucker-Davis Technologies [TDT], Alachua, Florida) lowered orthogonally into the cortex to a depth of 600-900μm (layers 4/5). The electrode wires (33μm diameter) were arranged in an 8x8 grid orthogonal to the cortex spaced 375μm apart with row separation of 500μm. To maximize recording density, neural responses were consecutively recorded from multiple overlapping electrode positions

within each rat. The stereotaxic location of each position relative to the first was noted in order to accurately reconstruct auditory maps during offline analysis. Extracellular multi-unit responses were obtained, amplified, and filtered (0.3–5 kHz) using a TDT RZ2 processor. The TDT OpenEx software package was used to generate acoustic stimuli, monitor cortical activity online and store data for offline analysis.

Tonotopic map reconstruction: Frequency-intensity receptive fields were constructed using neural responses to frequency-intensity combinations of pure tones. 66 frequencies (0.75-70kHz; 0.1 octave increments; 25ms duration; 5ms ramps) were presented at eight sound intensities (0-70dB SPL; 10dB increments) at a rate of one tone per second with three repetitions and in pseudo-random presentation order. The onset latency for each cortical site was defined as the time in ms when the peri-stimulus time histogram (PSTH) first exceeded the mean baseline firing rate by 2.5 standard deviations. The period of time between the onset latency and the time when the PSTH returned to less than 2.5 standard deviations of the mean baseline firing rate was defined as the response duration. Receptive fields were generated from the average firing rate at each frequency and intensity combination over the response duration. The characteristic frequency (CF) and threshold of a cortical site were defined, respectively, as the frequency and intensity at the tip of the V-shaped tuning curve. For flat-peaked tuning curves or tuning curves with multiple peaks, the CF was defined as the frequency that elicited the strongest firing rate at the lowest threshold. The best frequency (BF) was defined as the frequency that elicited the strongest firing rate over the response duration when presented at 60dB. The latency, response duration, CF, BF, and threshold were first determined by an automated custom MATLAB routine and then manually verified by an experimenter blind to the identity of the experimental groups. The receptive field bandwidth (BW) at each intensity was computed by estimating 2σ from the Gaussian fit to the

tuning curve using a standard 50-ms response window starting 8ms after stimulus onset (Han et al. 2007; Montgomery and Wehr 2010). We determined goodness of fit with corrected r^2 and, for BW analyses, only retained sites where r^2 was greater than 0.25 and the mean of the function, μ, was within the range of presented frequencies. Cortical sites were identified as belonging to the primary auditory field (A1) based on published functional characteristics of each field (Polley et al. 2007). These were reversal of CF tonotopic gradients, onset latencies, threshold, and PSTH morphologies. Only responses recorded from full A1 maps were included in analyses. A full A1 map was defined by having low, medium, and high frequency regions and by the detection of a reversal of the tonotopic gradient on the rostral border of A1 and the detection of non-auditory sites on the caudal and medial borders of A1. To generate A1 maps, Voronoi tessellation was performed using custom MATAB scripts to create tessellated polygons with electrode penetration sites at their centers. To verify that an individual iso-frequency region was not oversampled for any experimental group, we investigated the distribution of distances between each penetration site and its nearest neighbor. Out of all sites, 92.70% (1550/1672 sites) had a nearest neighbor distance of 312.5μm. We confirmed that this proportion did not differ between groups for frequency bins centered on 1.25, 2.5, 3.5, 5, 7, 10, 14, 20, 28, and 48kHz by performing Kruskal-Wallis tests for each bin and correcting for multiple comparisons by evaluating at the Bonferroni adjusted alpha level of 0.005. No test was significant for any CF (chi square $\leq$ 11.71, $p \geq .0687$, df = 6, n = 50 rats. 7kHz bin: chi square = 3.18, p = .7859. 14kHz bin: chi square = 2.23, p = .8979) or BF bin (chi square $\leq$ 12.65, $p \geq .0489$, df = 6, n = 50 rats. 7kHz bin: chi square = 4.26, p = .6417. 14kHz bin: chi square = 4.64, p = .5904).

Spatial overlap analysis: To estimate the number of cortical sites that robustly responded to a given frequency, we first smoothed and normalized the frequency-intensity receptive field for

each site by applying a median filter and dividing by the maximum response. We then selected a response threshold of greater than 0.5 to indicate whether a site was robustly responsive at a given frequency-intensity combination. A strict response threshold was necessary to separate neural responses to the target and non-target frequencies, which are relatively close tonotopically. Finally, the amount of spatial overlap between A1 sites that responded to the target and non-target frequencies was computed by dividing the number of A1 sites that robustly responded to both frequencies by the total number of A1 sites.

Statistical analyses: Statistical results appear in parentheses with test name, statistic, and number of data points per level of nested data. Where data are not shown in figures, means ± standard deviation are reported in the text. Linear mixed-effects models (Reed and Kaas 2010; Aarts et al. 2014) were used to analyze data collected through nested experimental designs. For these models, recording position nested within rat ID were included as random effects. Analyses were conducted using MATLAB and JMP Pro 13 (SAS Institute, Cary, NC). The fixed effect test results are reported with the degrees of freedom denominator approximated for normal data using the Kenward-Roger adjustment. All tests were evaluated at an alpha level of 0.05 unless otherwise noted.

3.4 Results

We attempted to improve frequency discrimination learning in adult rats by enhancing the functional representation of 7kHz in A1 with passive sound exposure. Two-month-old female Long-Evans rats were exposed to two weeks of moderate-intensity (70dB SPL) white noise followed by one week of 7kHz tone pip clouds. We then trained exposed and non-exposed rats on an adaptive go/no-go tone discrimination task that tested their ability to identify a 7kHz target tone

amongst distractor (non-target) tones. The non-target tones were ½ octave higher than the target at the beginning of each training session and became progressively closer to the target tone in 0.025 octave increments as task level increased, up to a maximum of 20 levels, following a one-up/one-down staircase procedure (**Supplementary Figure 3.1**).

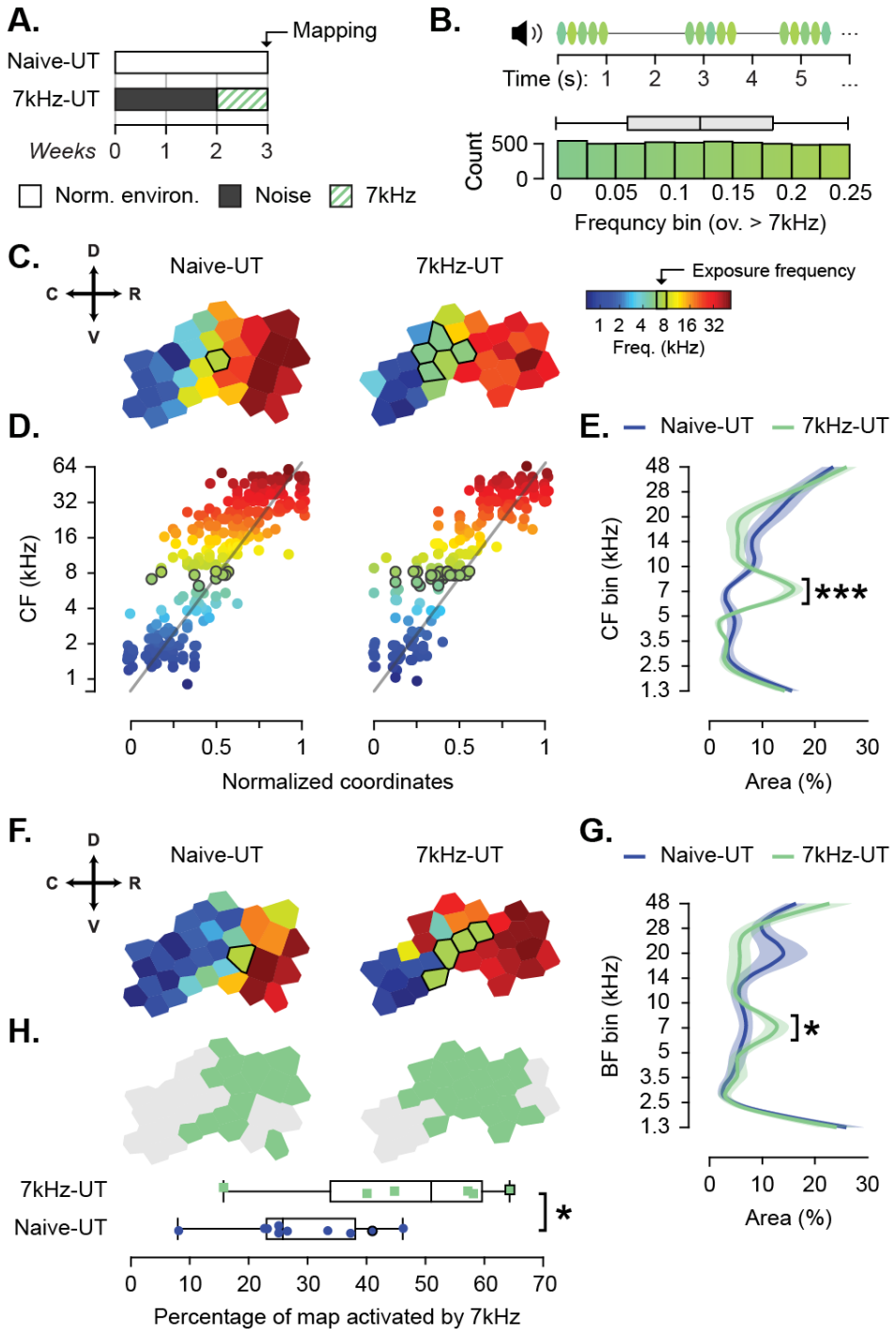


Figure 3.1: *Passive sound exposure increases cortical representation of the 7kHz frequency region*. **A.** Experimental timelines for each group. **B.** Depiction of the 7kHz tone pip cloud exposure stimulus. The clouds consisted of 50ms tones of random frequencies between 7kHz and 7kHz + 0.25 octaves and delivered in trains of 5 pulses per second. The histogram shows the even distribution of tones within this range for a sample of approximately 60min. **C.** Representative A1 characteristic frequency (CF) maps from each exposure group. **D.** CFs from all animals plotted against a normalized tonotopic axis. The gray line represents a perfect tonotopic gradient. **E.** Percentage of A1 area with CFs in 10 frequency bins. **F.** A1 best frequency (BF) at 60dB maps from the same animals as in C. **G.** Percentage of A1 area with BFs in 10 frequency bins. **H.** Top: A1 maps from the same animals as in C and F showing sites that robustly respond (green) or not (gray) to 7kHz at 60dB SPL. Bottom: Box plot of the percentage of A1 area that robustly responds to 7kHz at 60dB SPL. Outlined points indicate the animals from the representative maps in C, F, and H. Outlined sites in C, D, and F have CFs of 7kHz ± 0.25 octaves. Shading in E and G represents SEM. *** = $p<.0001$, * = $p<.05$. Number of animals, recording positions, and cortical sites per group: Naïve-UT 10,21,277; 7kHz-UT 6,14,228.

3.4.1 Passive sound exposure induces map expansion of the 7kHz frequency region

We first verified that sound exposure led to expansion of the 7kHz frequency region in the adult tonotopic map prior to behavioral training. Tone pip clouds that included an even distribution of frequencies between 7kHz and 7kHz + 0.25 octaves were chosen in order to increase the representation of the target tone and nearby frequencies corresponding to levels 11-20 of training (**Fig. 3.1A-B**). In untrained rats housed in a standard acoustic environment (Naïve-UT, N = 10) and in untrained rats that underwent sound exposure (7kHz-UT, N = 6), we reconstructed characteristic frequency (CF) and best frequency (BF) tonotopic maps using in-vivo extracellular responses to presentations of tone pips of various frequencies and intensities under isoflurane anesthesia. We were interested in assessing both CF and BF as CF describes tuning at threshold intensities, which vary per neuron, and BF describes tuning at 60dB, which was the intensity of the training stimuli. After binning CF and BF values into 10 frequency bins with centers at approximately 1.25, 2.5, 3.5, 5, 7, 10, 14, 20, 28, and 48kHz, we observed a significantly greater percentage of map area in the 7kHz bin for the 7kHz-UT group in both CF (unpaired t-test $t(14) =$

7.44, p < .0001, n = 16 rats, Fig. 3.1**C-E**) and BF maps (unpaired t-test t(14) = 2.47, p = .0272, n = 16 rats, **Fig. 3.1F-G**). The percentage of map area robustly activated (see Spatial Overlap Analysis in methods) by 7kHz at 60dB SPL was also significantly greater for the 7kHz-UT group (unpaired t-test t(14) = 2.52, p = .0246, n = 16 rats, **Fig. 3.1H**).

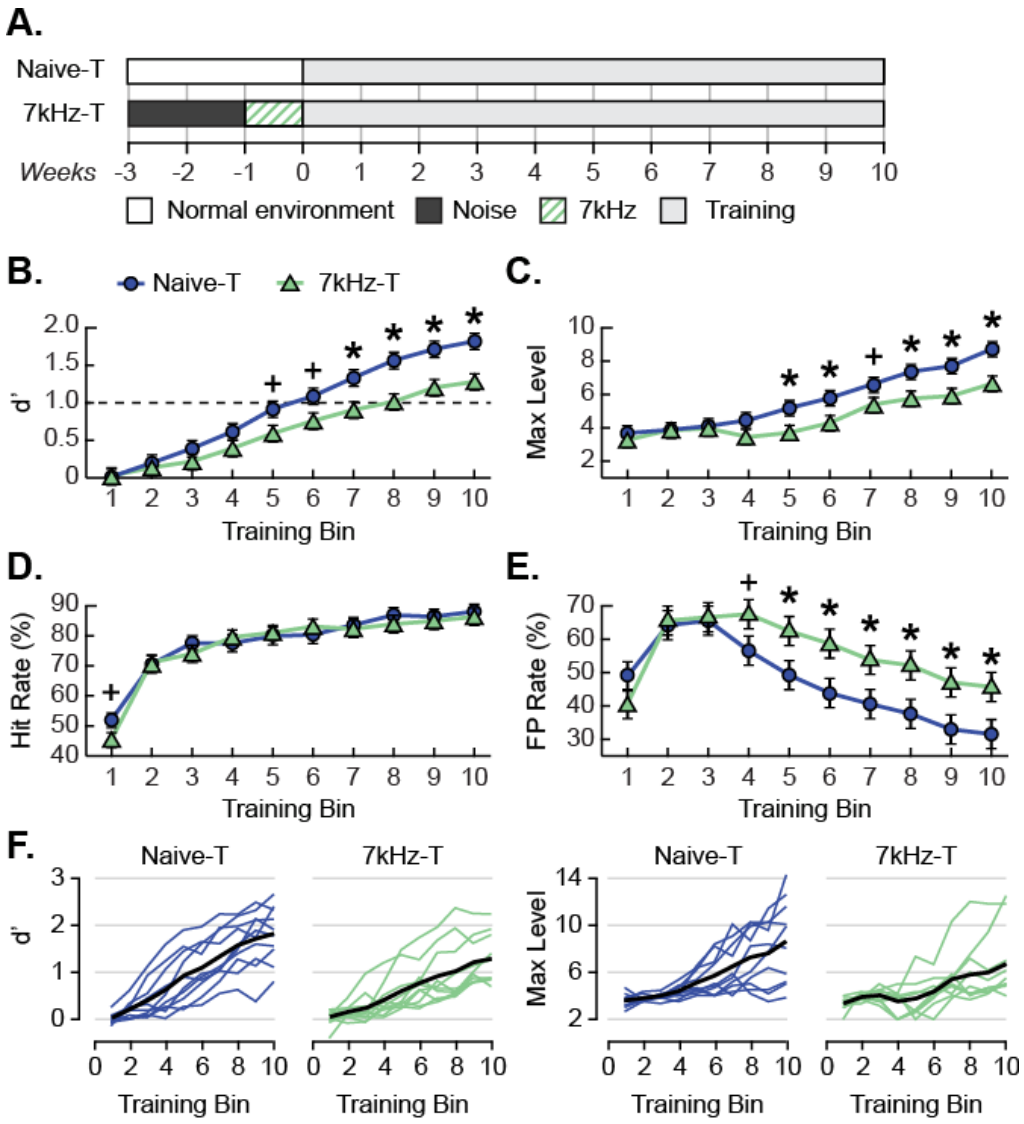


Figure 3.2: *Sound-exposed animals exhibit impaired perceptual learning*. **A.** Experimental timelines for each group. **B.** Mean *d'* reached over 10 training bins (6 sessions per bin) in an adaptive tone discrimination task where the frequency of the target tone was 7kHz. **C.** Mean maximum level reached over 10 training bins. **D.** Mean hit rate over 10 training bins. **E.** Mean false positive (FP) rate over 10 training bins. **F.** Individual rats' performance for *d'* and maximum level over 10 training bins. The bold line represents the group mean as plotted in B and C. * = p < .05, + = p < .09. Error bars represent SEM. Number of animals and training sessions per group: 10,600.

3.4.2 Sound-exposed animals demonstrate impaired perceptual learning

Next, we evaluated the behavioral performance of non-exposed (Naïve-T, N = 10) and exposed (7kHz-T, N = 10) rats on the adaptive tone discrimination task (**Fig. 3.2A**). Contrary to our initial hypothesis, we found that the 7kHz-T group was worse than Naïve-T on several measures of behavioral performance. The sensitivity index *d'* was used to estimate detection accuracy for the target tone during each session, and a threshold of $d' \geq 1$ was used to indicate successful detection. We calculated the average *d'* for training bins of 6 sessions per bin, representing approximately one week of training each. Over 10 bins comprising 60 one-hour training sessions, the discrimination performance of both groups improved steadily. However, Naïve-T rats obtained a bin with $d' \geq 1$ on average 2.66 bins before 7kHz-T rats, corresponding to approximately 16 training sessions (Naïve-T mean = 5.78 ± 1.79 bins, 7kHz-T mean = 8.44 ± 2.60 bins, unpaired t-test $t(16) = 2.53$, $p = 0.0221$, n = 18 rats, one rat from each group was not included because they did not have any bin where average $d' \geq 1$). When comparing performance between the two groups, we found that *d'* was significantly higher for the Naïve-T group toward the end of training, from bins 7-10 (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1162) = 8.48$, $p < .0001$. Bins 7-10: all $F(1,23.57) \geq 5.44$, all $p \leq .0285$, n = 20 rats, **Fig. 3.2B**). The Naïve-T group also reached a higher maximum level per session for bins 5, 6, 8, 9, and 10 of training (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1162) = 4.73$, $p < .0001$. Bins 5,6,8-10: all $F(1,30.86) \geq 5.38$, all $p \leq .0271$, n = 20 rats, **Fig. 3.2C**). *D'* is calculated from the hit rate and false-positive (FP) rate from each training session. We found that differences in *d'* between the two groups were driven entirely by FP rate, since hit rate was not significantly different for any bin (Two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1162) = 1.92$, $p = .0462$. Simple main effects tests did not yield a significant difference for any bin, all $F(1,37.34) \leq 3.49$, $p \geq .0697$, n = 20 rats, **Fig.**

3.2D). The average FP rate of the 7kHz-T group was significantly higher than that of Naïve-T for bins 5-10 (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1162) = 10.67$, $p < .0001$. Bins 5-10: all $F(1,24.19) \geq 4.44$, all $p \leq .0457$, n = 20 rats, **Fig. 3.2E)**. Despite responding to the target tone at the same rate as Naïve-T rats, these findings reveal that 7kHz-T rats were unable to suppress their response to the non-target tones, demonstrating a deficit in their ability to properly discriminate these sounds from 7kHz.

3.4.3 Training recovers electrophysiological measures of reduced discriminability

We reconstructed A1 maps from Naïve-T and 7kHz-T rats at the end of behavioral training and compared these to the maps of untrained animals (**Fig. 3.3A**). In line with previous studies, we observed an increase in CF area dedicated to the 7kHz target frequency for trained rats (**Fig. 3.3B,E top**). For the Naïve-T group, this percent area was significantly greater than that of Naïve-UT ($p = .0016$), while for 7kHz-T it was greater but the difference approached significance ($p = .0677$). The increase for both groups was less than that of 7kHz-UT, which remained significantly higher than Naïve-UT ($p < .0001$) (one-way ANOVA $F(3,29) = 10.71$, $p < .0001$, followed by Dunnett's test, n = 33 rats). The percent area dedicated to the CF bin containing the non-target frequency for training level 1 did not significantly change with either exposure or training (one-way ANOVA $F(3,29) = 2.53$, $p = .0763$, n = 33 rats). On the other hand, we did not observe a training effect on BF area for either the target (one-way ANOVA $F(3,29) = 1.52$, $p = .2290$, n = 33 rats) or non-target frequency (one-way ANOVA $F(3,29) = 1.23$, $p = .3168$, n = 33 rats, **Fig. 3.3C,E bottom**). These results show that perceptual learning during training led to an overrepresentation of the target tone within A1 when measured with CF, but not BF.

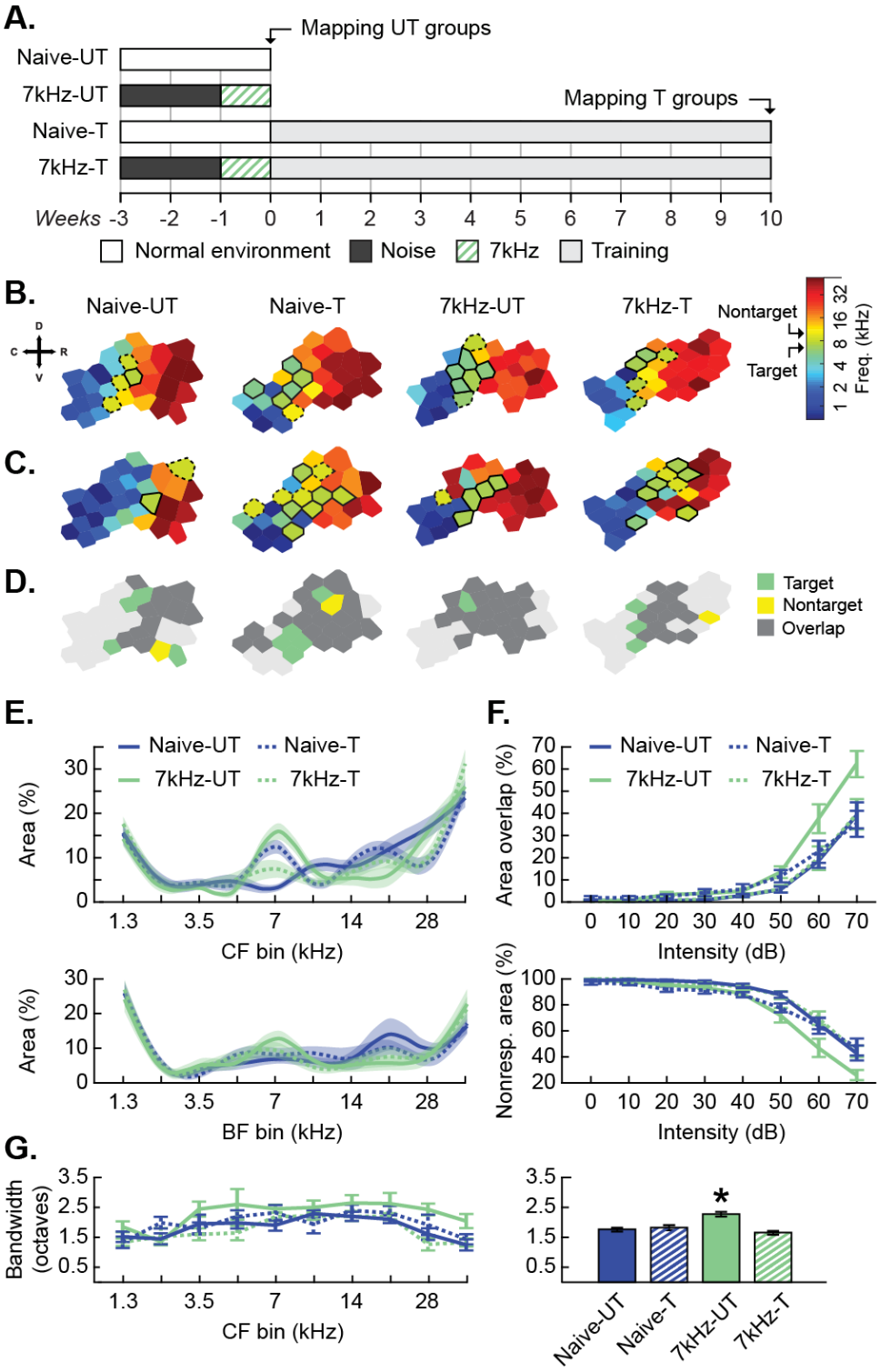


Figure 3.3: *Training recovers typical neuronal responses to the exposure frequency*. **A.** Experimental timelines for each group. **B.** Representative A1 characteristic frequency (CF) maps from each exposure group. Bold outlined sites have CFs of 7kHz ± 1/4 octave representing the target/exposure frequency, dotted outlined sites have CFs of 9.9 ± ¼ octave representing the non-target frequency. **C.** A1 best frequency (BF) at 60dB maps from the same animals as in

B. Bold and dotted outlined sites have BFs corresponding to the target or non-target frequencies, respectively. **D.** A1 maps showing sites that robustly respond to the target frequency (green), non-target frequency (yellow), or both (dark gray) at 60dB. **E.** Percentage of A1 area with CFs (top) or BFs (bottom) in 10 frequency bins. **F.** Top: Percent overlap of A1 area that robustly responds to both the target and non-target frequency at all sampled intensities. Bottom: Percentage of A1 area that does not respond to either the target or non-target frequency. **G.** Comparison of tuning curve bandwidths at 60dB. Left: Average bandwidth per CF bin. Right: Average bandwidth for all sites. Error bars and shading represent SEM. Number of animals, recording positions, and cortical sites per group: Naïve-UT 10,21,277; Naïve-T 8,18,265; 7kHz-UT 6,14,228; 7kHz-T 9,22,319.

We also noted earlier that sound exposure led to a greater proportion of A1 robustly responding to the target frequency at 60dB regardless of CF. We next decided to investigate the proportion of map area that responded to either the target or non-target frequency at the full range of stimulus intensities in trained rats (**Fig. 3.3D,F top**). We computed the amount of spatial overlap between these two regions at all intensities for each group and compared them to the spatial overlap exhibited by the 7kHz-UT group. We found that the average overlap for the 7kHz-UT group was significantly greater or approaching significance for all comparisons at 60 and 70dB than all of the other groups ($p \leq .0580$) except Naïve-T ($p = .1306$) (two-way repeated measures ANOVA with Group and Intensity as factors. Interaction: $F(21,203) = 2.51$, $p = .0005$. Intensities 60 and 70dB: both $F(3,195.2) \geq 7.16$, both $p \leq .0001$, followed by Dunnett's Test, n = 33 rats, **Fig. 3.3F top**). The percent map area that was not responsive to either tone also differed between groups at high intensities. 7kHz-UT rats had less cortical area that did not respond to either the target or non-target tone at 50, 60, and 70dB. This was significant or approaching significance ($p \leq .0725$) for all comparisons except Naïve-T at 50dB and 60dB (both $p \geq .1263$) (two-way repeated measures ANOVA with Group and Intensity as factors. Interaction: $F(21,203) = 2.38$, $p = .0010$. Intensities 50-70dB: all $F(3,171.5) \geq 5.45$, all $p \leq .0013$, followed by Dunnett's Test, n = 33 rats, **Fig. 3.3F bottom**).

In addition to population measures, the tuning bandwidth of individual neurons can provide an estimate of A1 response specificity, with more broadly tuned neurons indicating a less specific response. We extracted the tuning bandwidth at 60dB from each cortical site and compared the average bandwidth in ten BF bins. We determined that the relationship between bandwidth and frequency bin did not differ between groups, however the 7kHz-UT group exhibited significantly broader overall bandwidths than all other groups (all $p \leq .0469$) (mixed effects two-way ANOVA with Group and BF Bin as factors. Interaction: $F(27,672) = 0.69$, $p = .8779$. Main effect of Group: $F(3,672) = 10.61$, $p < .0001$. Followed by Tukey's test, n = 712 observations within 33 rats, **Fig. 3.3G**). This non-frequency-specific broadening of receptive fields was likely a consequence of white noise exposure, and not 7kHz tone pip exposure, since rats exposed to broadband white noise have been shown to exhibit wider receptive field bandwidths for at least two weeks following noise exposure (Zhou et al. 2011).

3.4.4 Reduced neural discriminability persists for at least 12 weeks following sound exposure

The above results show that immediately after sound exposure, the 7kHz-UT group exhibited a considerable increase in map area robustly responding to both the target and non-target tones that could contribute to reduced discriminability. This effect did not persist in the 7kHz-T group, however, which suggests that it was reversed with either training or time. To investigate this further, we exposed a third group of rats (7kHz-UT+12wks, N = 4) to the same stimuli as above but waited 12 weeks, the average duration of training, before performing electrophysiological recordings (**Fig. 3.4A**). After this period, 7kHz map expansion persisted in both CF (unpaired t-test $t(12) = 3.54$, $p = .0041$, n = 14 rats, **Fig. 3.4B,E left**) and BF maps (unpaired t-test $t(12) = 2.52$, $p = .0267$, n = 14 rats, **Fig. 3.4C,E right**) compared to Naïve-UT.

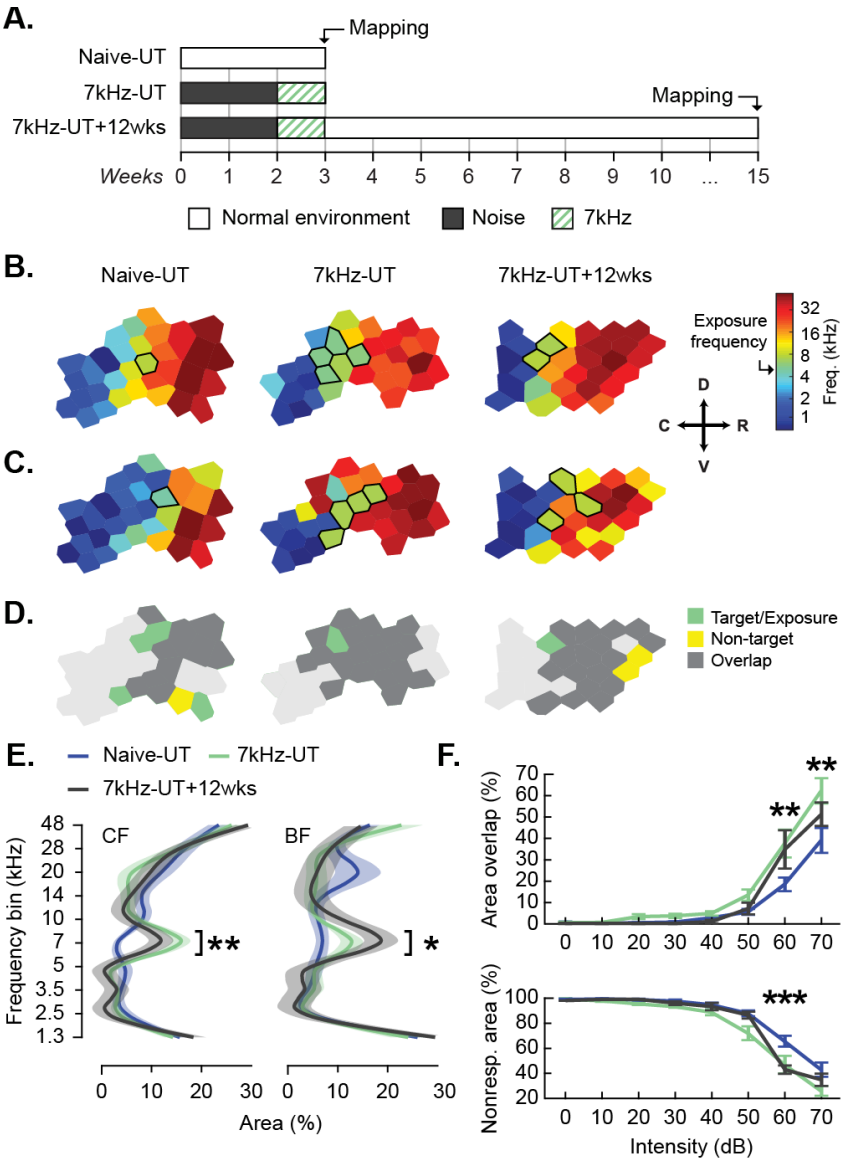


Figure 3.4: *Increased cortical representation and reduced discriminability of the 7kHz frequency region persists for at least 12 weeks*. **A.** Experimental timelines for each group. 7kHz-UT+12wks rats were recorded 12 weeks after exposure ended, which was the average duration of training. **B.** Representative A1 characteristic frequency (CF) maps from each exposure group. **C.** A1 best frequency (BF) maps from the same animals as in B. **D.** A1 maps showing sites that robustly respond to the target/exposure frequency (green), non-target frequency (yellow), or both (dark gray) at 60dB. **E.** Percentage of A1 area with CFs (left) or BFs (right) in 10 frequency bins. **F.** Top: Percent overlap of A1 area that robustly responds to both the target and non-target frequency at all sampled intensities. Bottom: Percentage of A1 area that did not respond to either the target or non-target frequency. Outlined sites in B and C have CFs or BFs of 7kHz ± ¼ octave. Shading in E represents SEM. Data from Naïve-UT and 7kHz-UT is the same as in Fig. 3.1. *** $p < .0001$, ** = $p < .01$, * $p < .05$ for the comparison between Naïve-UT and 7kHz-UT+12wks. Number of animals, recording positions, and cortical sites per group: Naïve-UT 10,21,277; 7kHz-UT 6,14,228; 7kHz-UT+12wks 4,8,108.

The 7kHz-UT+12wks group also exhibited spatial overlap that was significantly greater than that of Naïve-UT animals at 60 and 70dB (two-way repeated measures ANOVA with Group and Intensity as factors. Interaction: $F(7,84) = 2.62$, $p = .0169$. Intensities 60 and 70dB: both $F(1,87.57) \geq 7.46$, both $p \leq .0076$, $n = 14$ rats, **Fig. 3.4D,F top**), and reduced map area that did not respond to either the target or non-target frequency at 60dB (two-way repeated measures ANOVA with Group and Intensity as factors. Interaction: $F(7,84) = 3.37$, $p = .0032$. Intensity 60dB: $F(1,69.96) = 21.20$, $p < .0001$, $n = 14$ rats, **Fig. 3.4F bottom**). Finally, the 7kHz-UT+12wks group showed incomplete recovery of typical receptive field bandwidths as their average bandwidths were not significantly different from the Naïve-UT or 7kHz-UT group (both $p \geq 0.2695$, mixed effects two-way ANOVA with Group and BF Bin as factors. Interaction: $F(18,360.1) = 1.29$, $p = .1916$. Main effect of Group: $F(2,48.89) = 4.21$, $p = .0206$. Followed by Tukey's test, $n = 409$ observations within 20 rats). We concluded that sound exposure resulted in a long-lasting reduction in population discriminability of the training frequencies that was slightly diminished with time, but reversed through training.

3.4.5 Impaired perceptual learning is not due to noise exposure

Although not known to elevate hearing thresholds, moderate intensity exposures to continuous or pulsed noise have been shown to degrade listening processes including cortical tuning selectivity (Kamal et al. 2013; Thomas et al, 2018), gap detection (Jiang et al. 2015), fine pitch discrimination (Zheng 2012), and temporal rate discrimination (Zhou and Merzenich 2012). Some of the changes we observed in the 7kHz-UT group above are consistent with these established measures of degraded listening processes. In order to test the possibility that perceptual learning deficits in the 7kHz-T group were driven by noise exposure instead of 7kHz map expansion we exposed a group of rats to white noise for two weeks followed by 15kHz tone pip

clouds for one week (15kHz-T, n = 8). The frequencies in the tone pip clouds were evenly distributed between 15kHz ± ¼ octave. We then tested this group on the same adaptive tone discrimination task with 7kHz as the target tone (**Fig. 3.5A**). The frequencies contained in the 15kHz exposure stimulus were outside the range of trained frequencies, so we did not expect 15kHz map expansion to have any effect on task performance.

We first verified that two weeks of noise followed by one week of 15kHz tone pip clouds would result in increased cortical representation of the 15kHz frequency region (**Fig. 3.5B,C left)**. There was a clear expansion of the 15kHz frequency region in the CF maps of untrained rats exposed to this stimulus (15kHz-UT, n = 5) compared to Naïve-UT ($p < .0001$). Map expansion persisted throughout training as the 15kHz-T group also had a greater percentage of map area in the bin containing 15kHz than Naïve-UT ($p = .0107$, one-way ANOVA $F(3,27) = 10.97$, $p < .0001$, followed by Dunnett's test, n = 31 rats). Next, we investigated the effects of exposure and training on representation of the target and non-target frequencies (Also **Fig. 3.5B,C left)**. The 15kHz-UT group did not differ from Naïve-UT in percentage of CF map area corresponding to the target frequency ($p = .9973$), although training significantly increased its representation in the 15kHz-T group compared to Naïve-UT ($p = .0006$) (one-way ANOVA $F(3,27) = 10.74$, $p < .0001$, followed by Dunnett's test, n = 31 rats). Of note, both the 15kHz-UT and 15kHz-T groups exhibited reduced CF map area dedicated to the non-target frequency (both $p \leq .0075$) (one-way ANOVA $F(3,27) = 5.70$, $p = .0037$, followed by Dunnett's test, n = 31 rats). There was no difference between the BF representation of 15kHz, the target frequency, or the non-target frequency in these groups, on the other hand (one-way ANOVAs all $F(3,27) \geq 0.35$, all $p \leq 0.7894$, n = 31 rats, **Fig. 3.5C right**).

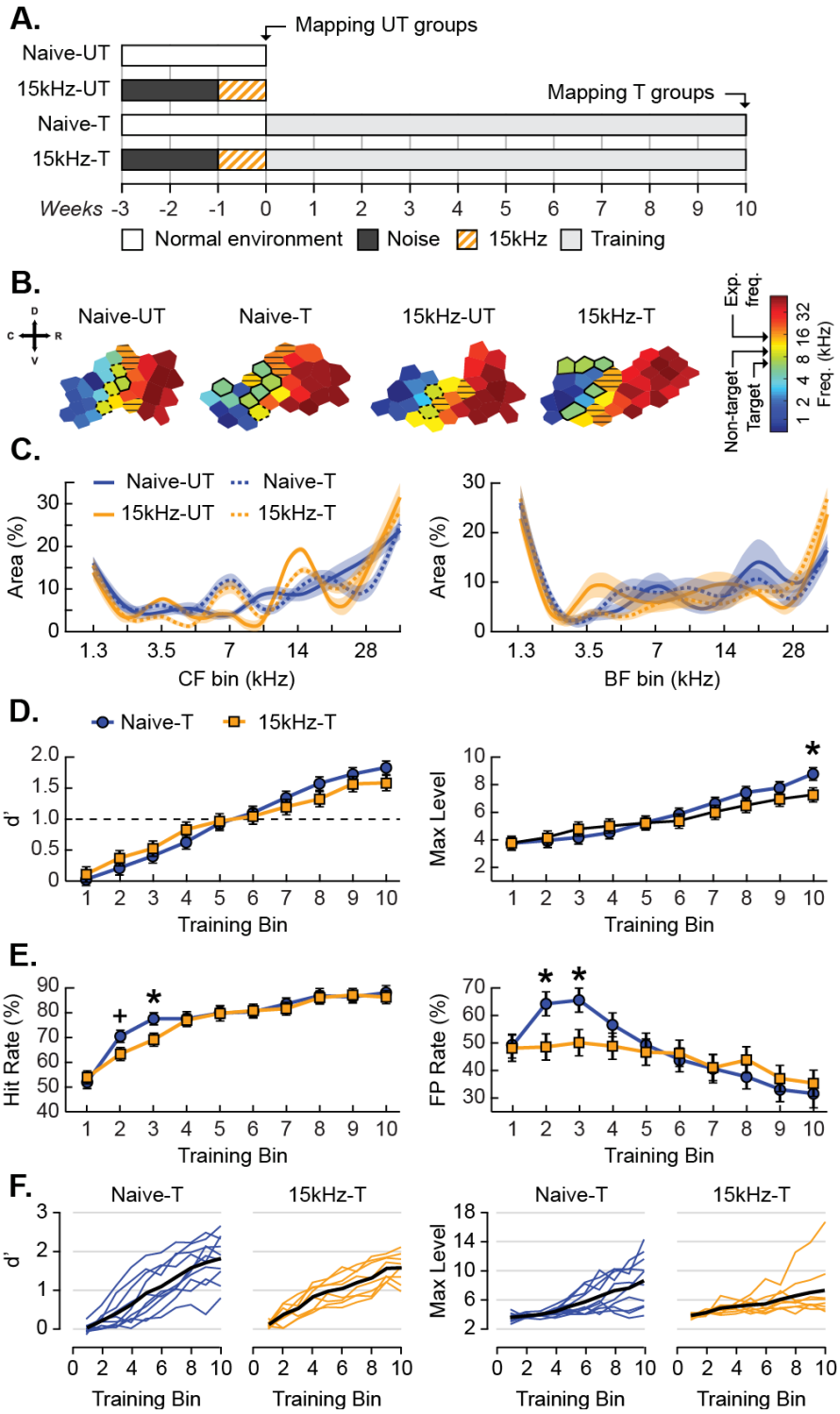


Figure 3.5: *Passive sound exposure increases cortical representation of 15kHz without impairing 7kHz tone discrimination*. **A.** Experimental timelines for each group. **B.** Representative A1 characteristic frequency (CF) maps from each exposure group. Bold outlined sites have CFs of 7kHz ± 1/4 octave representing the target frequency, dotted outlined sites have CFs of 9.9 ± 1/4 octave representing the non-target frequency, and striped

sites have CFs of 15kHz ± ¼ octave representing the exposure frequency. **C.** Percentage of A1 area with CFs (left) and BFs (right) in 10 frequency bins. **D.** Mean d' (left) and maximum level (right) reached over 10 training bins (6 sessions per bin) in the same adaptive tone discrimination task as *Fig. 3.3*. **E.** Mean hit rate (left) and false positive (FP) rate (right) for the first 10 training bins. **F.** Individual rats' performance for *d'* and maximum level over 10 training bins. The bold line represents the group mean as plotted in B and C. Error bars and shading represent SEM. Number of animals, recording positions, and cortical sites per group for electrophysiological data: Naïve-UT 10,21,277; Naïve-T 8,18,265; 15kHz-UT 5,14,172; 15kHz-T 8,20,332. Number of animals and training sessions per group for behavioral data: Naïve-T 10,600; 15kHz-T 8,480.

Next, we examined the effect of 15kHz map expansion on training performance (**Fig. 3.5D-F)**. The average *d'* learning curve of the 15kHz-T group was not different from Naïve-T (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1044) = 6.46$, $p < .0001$. Simple main effects tests did not yield a significant difference for any bin, all $F(1,20.62) \leq 1.93$, all $p \geq .1791$, n = 18 rats, **Fig. 3.5D left**), although the Naïve-T group reached higher maximum levels during training bin 10 (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1044) = 3.66$, $p = .0002$. Bin 10: $F(1,26.40) = 4.50$, $p = .0435$ n = 18 rats, **Fig. 3.5D right**). Both groups reached an average $d' > 1$ around bin 6 (Naive-T mean = 5.78 ± 1.79 bins, 15kHz-T mean = 6.38 ± 2.39 bins, unpaired t-test $t(15) = 0.59$, $p = 0.5650$, n = 17 rats, one rat from the Naïve-UT group was not included because it did not have any bin where $d' > 1$). The two groups differed in hit rate and FP rate during the early weeks of training. The 15kHz-T group had a lower hit rate during bin 3 of training (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1044) = 3.06$, $p = .0012$. Bin 3: $F(1,28.80) = 5.42$, $p = .0271$, n = 18 rats, **Fig. 3.5E left**) and a lower FP rate during bins 2 and 3 (two-way repeated measures ANOVA with Group and Bin as factors. Interaction: $F(9,1044) = 11.36$, $p < .0001$. Bins 2-3: both $F(1,20.73) \geq 5.75$, both $p \leq .0260$, n = 18 rats, **Fig. 3.5E right**). Because hit rate and FP rate decreased proportionally, *d'* and maximum level reached during bins 2-3 were not affected.

Finally, we directly compared the performance of all three trained groups over coarse training bins of 20 sessions each corresponding to the early, mid, and late thirds of training (**Fig. 3.6**). This comparison revealed that the 15kHz-T group had performance equivalent to the Naïve-T group and superior to the 7kHz-T group in all stages of training except for maximum level reached during the late stage, in which the 15kHz-T and 7kHz-T were not significantly different (statistics are reported in figure legend). Since noise exposure is known to affect fine but not coarse frequency discrimination (Zheng 2012), this could be a result of the rats encountering more fine pitch discriminations during the late stage of training. Taken together, the above results led us to conclude that noise may have had a negative effect on fine frequency discrimination during late sessions of the adaptive tone discrimination task, but that this could not account entirely for the deficits in perceptual learning demonstrated by the 7kHz-T group.

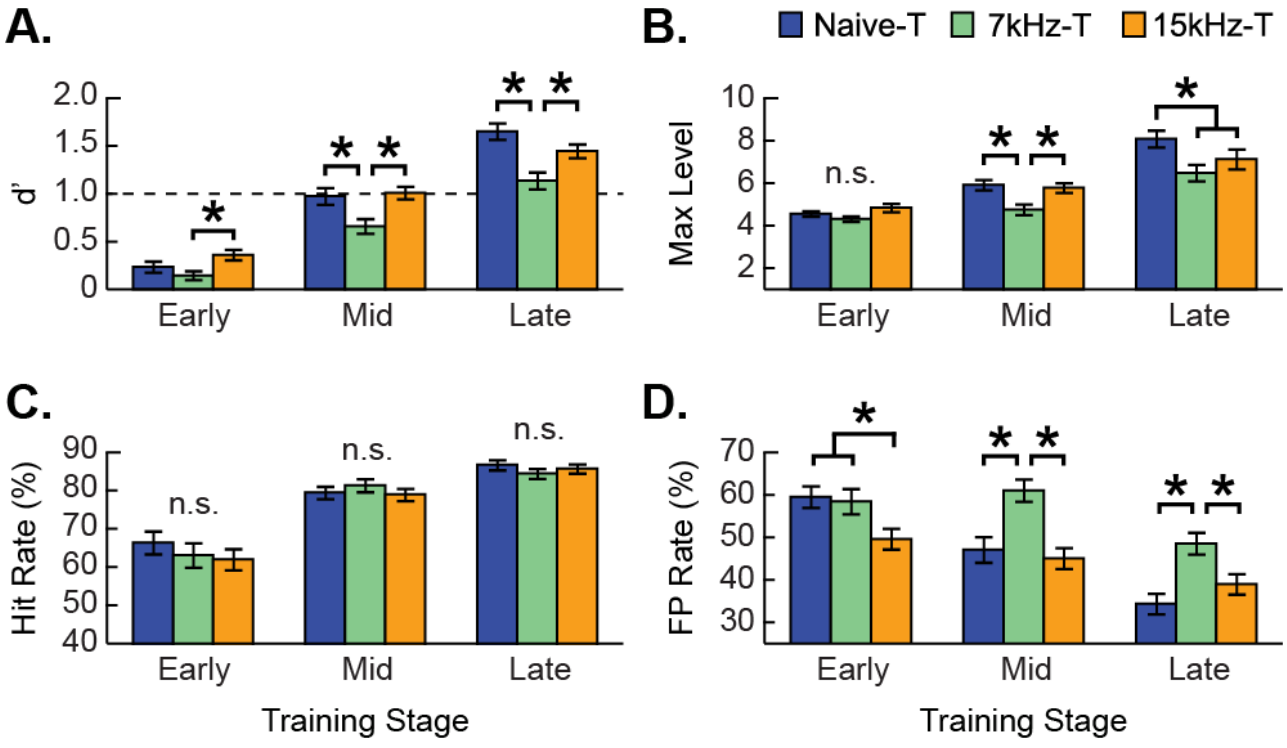


Figure 3.6: *Behavioral performance over coarse training bins*. Behavioral performance during early, mid, and late training stages of the adaptive tone discrimination task corresponding to sessions 1-20, 21-40, and 41-60 respectively. All measures were evaluated by two-way ANOVAs with Group and Training Stage as factors followed by simple main effects tests and Tukey's post-hoc test. **A.** Mean d'. Interaction: $F(4,1671) = 10.85$, $p < .0001$. Stages: all $F(2,1671) \geq 7.97$, all $p \leq .0004$. **B.** Max level. Interaction: $F(4,1671) = 7.86$, $p < .0001$. Stages: all $F(2,1671) \geq 3.01$, all $p \leq .0494$. **C.** Hit rate. Interaction: $F(4,1671) = 2.28$, $p = .0585$. **D.** FP rate. Interaction: $F(4,1671) = 13.95$, $p < .0001$. Stages: all $F(2,1671) \geq 15.10$, all $p < .0001$. * = $p < .05$, n.s. = not significant. Error bars represent SEM. Number of animals and training sessions per group: Naïve-T 10,600, 7kHz-T 10,600, 15kHz-T 8,480.

3.4.6 Map expansion is related to task performance for high-performing rats only

We investigated the possibility that cortical representation of the target tone was related to behavioral performance by correlating the percentage of A1 sites possessing a CF of 7kHz ± ¼ octave with measures of task performance for all trained animals. Since some animals completed more than 60 training sessions, we used average performance from the last 6 sessions rather than training bin 10 for correlations. We considered all trained animals together because the percentage of map area dedicated to the target tone was not found to differ between the Naïve-T, 7kHz-T and 15kHz-T groups. We did not find a significant relationship between map area and average *d'*, $r = 0.28$, $p = .1798$, $n = 25$ rats, FP rate, $r = -0.17$, $p = .4123$, $n = 25$ rats, or hit rate, $r = 0.11$, $p = .5878$, $n = 25$ rats, for the last 6 training sessions for all trained groups combined. However, we observed a noticeable divide between rats who were able to reach higher levels during training and those whose performance plateaued at lower levels. We decided to investigate the difference between 'high performers' and 'low performers' by dividing them on the basis of average maximum level reached during the last six training sessions (**Fig. 3.7A**). The criteria for high performers was an average maximum level greater than 6, which was chosen to split the rats ($n = 28$) into two equal-sized groups. High performers (HP) had a significantly greater average *d'* during the last six training sessions than low performers (LP) (HP mean = 2.05 ± 0.35, LP mean = 1.29 ± 0.41, unpaired t-test $t(26) = 5.25$, $p < .0001$, $n = 28$ rats), as well as a significantly lower average FP rate

during the same time frame (HP mean = 23.06 ± 8.67%, LP mean = 51.89 ± 8.92%, unpaired t-test $t(26) = 8.67$, $p < .0001$, n = 28 rats), but did not differ in hit rate (HP mean = 87.87 ± 6.46%, LP mean = 88.67 ± 6.20%, unpaired t-test $t(26) = 0.33$, $p = .7417$, n = 28 rats). Next, we explored whether the percentage of A1 sites dedicated to the target tone would be related to task performance in the high-performing and low-performing groups (**Fig. 3.7B**). We found evidence of a weak relationship between map expansion and behavioral performance for high performers only, as determined by comparing the p values obtained from linear correlations. For high performers, the relationship between map expansion and average *d'* during the last 6 training sessions approached significance ($p = .0515$) and was not significant ($p = .0905$) for average FP rate during the last 6 training sessions. Low performers exhibited much higher p values for *d'* and FP rate (both $p \geq .5452$). Hit rate was not related to map expansion for either low performers or high performers (both $p \geq .5709$). Interestingly, the average percentage of A1 area with a CF of 7kHz ± ¼ octave did not differ between the two groups (HP mean = 10.19 ± 5.32%, LP mean = 9.67 ± 5.63%, unpaired t-test $t(23) = 0.24$, $p = .8157$, n = 25 rats), suggesting that high-performing and low-performing rats may have employed different cognitive strategies to advance in training.

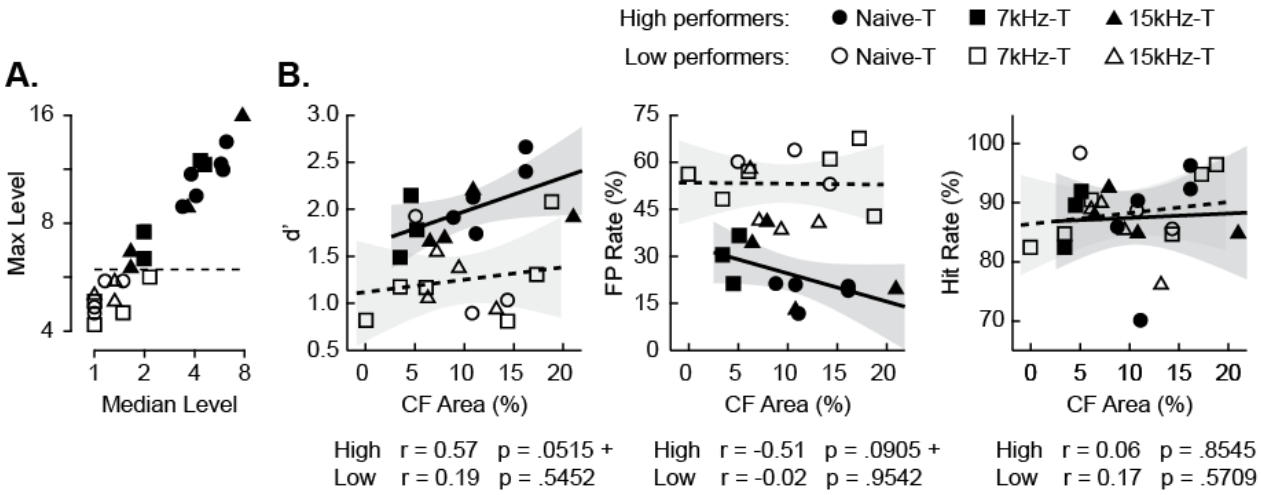


Figure 3.7: *Map expansion is related to training performance for high performing rats only*. **A.** Average maximum level vs. average median level during the last six training sessions. Both axes are on a logarithmic scale. Rats were divided into an equal number of 'High performers' and 'Low performers' based on the criteria of maximum level > 6 (dashed line). **B.** Behavioral performance measures *d'*, false positive (FP) rate, and hit rate vs. percent A1 area with a characteristic frequency (CF) of 7kHz ± 0.25 octaves for High and Low performers. Bold and dashed lines represent the linear fit for High and Low performers respectively while shaded areas represent the confidence of fit. Pearson's r, and uncorrected p values are below each graph, n = 12 High and 13 Low performers. Number of animals per group for panel A (including all trained rats) and panel B (including only rats for which full A1 maps were obtained): Naïve-T 10,8; 7kHz-T 10,9; 15kHz-T 8,8.

3.5 Discussion

Perceptual learning has been extensively associated with cortical map expansions in the somatosensory and auditory domains across species (Recanzone et al. 1992; Recanzone et al. 1993; Feldman and Brecht 2005; McGann 2015). Although these findings strongly suggest that map expansion provides some perceptual advantage to the organism, the exact nature of that advantage has remained elusive. Here we induced a similar phenotype to perceptual learning with three weeks of passive sound exposure; however, this did not confer a perceptual advantage for discriminating the over-represented frequency. Over more than 60 training sessions, exposed animals displayed a deficit in frequency discrimination and a marked delay in perceptual learning. When comparing the map expansion phenotype between exposed and trained animals, we found that both exposure and training led to CF map expansion but only exposure resulted in BF map expansion. This asymmetry could be indicative of a fundamental difference in the nature of these two types of expansion, possibly explaining why the early over-representation of the target frequency did not confer a task advantage in exposed rats. Map expansion was also accompanied by a greater overlap in population responses to the target and non-target frequencies at training intensity in exposed animals, very likely contributing to the impaired discrimination of these stimuli. The uncoupling of CF and BF plasticity in trained rats was unexpected, as increased functional representation of

the target frequency is well-described in both CF and BF maps (Weinberger 2015). Although shifts in cortical representation for both CF and BF are attributable to thalamocortical plasticity, excitatory-inhibitory balance is more variable at threshold levels (Zhao et al. 2015), suggesting that there is stronger natural variability at the CF than the BF, which could have been highlighted here.

The map expansion-renormalization hypothesis suggests that map expansion improves learning but is not necessary for the maintenance of learned information since representations can renormalize while task performance remains stable (Reed et al. 2011). In line with this, we employed an adaptive training paradigm in order to explicitly target the learning phase of discrimination training as opposed to basic discrimination abilities. Our adaptive task was difficult; even naïve rats required an average of 5.78 training bins (~35 sessions) to achieve a $d' > 1$ and no rats had plateaued in performance before the end of the experiment resulting in an extremely long learning phase. Our results therefore show that map expansion induced by passive sound exposure is not sufficient to improve perceptual learning. Rather than implying that all map expansions that accompany learning are epiphenomenon, however, our findings support the view that the mode of induction determines whether map expansion will have perceptual significance (Pienkowski and Eggermont 2011). Based on a small number of studies performed in rodent auditory cortex, techniques that invoke top-down changes through the recruitment of neuromodulatory systems produce map expansion that results in perceptual enhancement (Reed et al. 2011; Froemke et al. 2013; Blundon et al. 2017), while bottom-up changes resulting from electrical stimulation (Talwar and Gerstein 2001) or passive sound exposure during development (Han et al. 2007) either do not enhance or impair discrimination for the expanded frequency. In this respect, our methods and findings most closely resemble those of the lattermost study. Han

and colleagues observed that 7.1kHz map expansion impaired two-month-old rats' ability to discriminate the over-represented frequency from tones 0.1 (but not 0.5) octaves apart from it. Interestingly, discrimination for frequencies exactly ¼ octave above and below 7.1kHz was improved, possibly because of a greater number of neurons with tuning curve slopes falling within these frequency bins. This might suggest that the sound-exposed rats in our study had improved discrimination capabilities for frequencies neighboring 7kHz, including those falling between the target and non-target frequency. However, the performance of the 7kHz-T group began to significantly differ from the Naïve-T group in training bin 5, when Naïve-T animals reached average maximum levels of 4 or greater corresponding to a 0.425 octave difference from the target frequency. This suggests that map expansion interfered with tone discrimination for relatively coarse frequency comparisons, outweighing any perceptual advantage the rats could have had at higher training levels.

By itself, noise exposure has been shown to have a profoundly disruptive effect on both spectral and temporal auditory cortical responses in the adult brain, which has even led to calls for eliminating white noise therapy as a treatment for tinnitus (Attarha et al. 2018). Adult rats exposed to moderate-intensity broadband white noise for 30 days show impairments in fine frequency discrimination (Zheng 2012). For this reason, it was important for us to rule out the possibility that deficits in task performance were noise-related. We observed only limited impairment in the performance of 15kHz-T rats that underwent the same noise exposure and training as 7kHz-T rats. This led us to conclude that map expansion caused frequency-specific perceptual deficits separate from any deficits introduced by noise alone. We did not test the possibility that 7kHz tone exposure on its own could have led to perceptual deficits, as this type of exposure has not been shown to produce map expansion when not paired with a plasticity-inducing treatment (Zhou et al. 2011).

Tonotopic map expansion has become a household tool for auditory neuroscientists to validate strategies of enhancing cortical plasticity (such as in Bieszczad et al. 2015; Blundon et al. 2017). However, a concern is that map expansion may simply be an indicator that plasticity has taken place without giving specific clues as to which mechanism produced it or what consequences it may have for perception. Although we found that noise-induced map expansion impaired frequency discrimination, given the near-ubiquitous and highly reproducible nature of this outcome, it would be strange if it was not adaptive in at least some respect. We found preliminary evidence that at least the highest-performing rats may have used a successful learning strategy that relied on degree of map expansion, illustrating that different learning strategies may exist with respect to this phenomenon. Related to this, variations in parameters such as methodology, species used, duration of training, and training paradigm, are also likely to influence the relevance of map expansion to training outcomes (Irvine 2007; Pienkowski and Eggermont 2011). Map expansion could also improve perceptual acuity for other, yet untested sound features such as detection of the exposure frequency at near-threshold intensities. We recently demonstrated that noise- and tone pip-exposed rats exhibit enhanced sensorimotor gating for the over-represented frequency (Thomas et al. 2019b). However, this is accompanied by electrophysiological evidence of hyperexcitability associated with hyperacusis, including increased spontaneous and tone-evoked firing rates, leading us to conclude that heightened sensorimotor gating was related to maladaptive plastic mechanisms.

The enduring effects of passive sound exposure are another reason to pay attention to the maladaptive aspects of this form of plasticity. Here, we observed that map expansion and accompanying measures of reduced neural discriminability persisted for at least 12 weeks following sound exposure. This is in line with previous studies that showed incomplete recovery

of tonotopic reorganization in rats and cats at least 7 (Zhou et al. 2011), 8 (Kamal et al. 2013), and 12 (Pienkowski and Eggermont 2009) weeks after passive sound exposure. However, Reed and colleagues (2011) found that map expansion reversed at some point between 20 and 100 days (14.3 weeks) after paired nucleus basalis and tone pip stimulation.

Perhaps the most encouraging aspect of our findings is that despite an early impairment in perceptual learning, training was able to recover physiological and performance deficits in sound-exposed animals. Compared to the 7kHz-UT+12wks group, trained animals completely recovered both population and neural measures of selectivity for the exposure tone. Furthermore, it is possible that exposed rats could reach identical performance metrics as non-exposed rats given enough time, as they were still improving at the end of training. Auditory training has similarly been shown to enhance recovery from abnormal sensory experiences during development (Merzenich et al. 1996; Guo et al. 2012; Kang et al. 2014) and improve auditory response properties in aged rodents and humans (de Villers-Sidani et al. 2010; Anderson and Kraus 2013; Mishra et al. 2014). If properly harnessed, the ability to drive plastic changes in a specific and non-invasive manner through passive sound exposure and targeted training programs therefore has potential neurotherapeutic value. We expect that our results will further inform non-invasive training strategies that focus on 'retuning' the cortical map as a primary means of altering perception, such as those already used in the treatment of tinnitus (Flor et al. 2004; Pienkowski 2019). Our findings underline the need for future neuroplasticity-based treatments that take advantage of both the sensory statistics of our environment and the brain's innate capacity to change.

3.6 References

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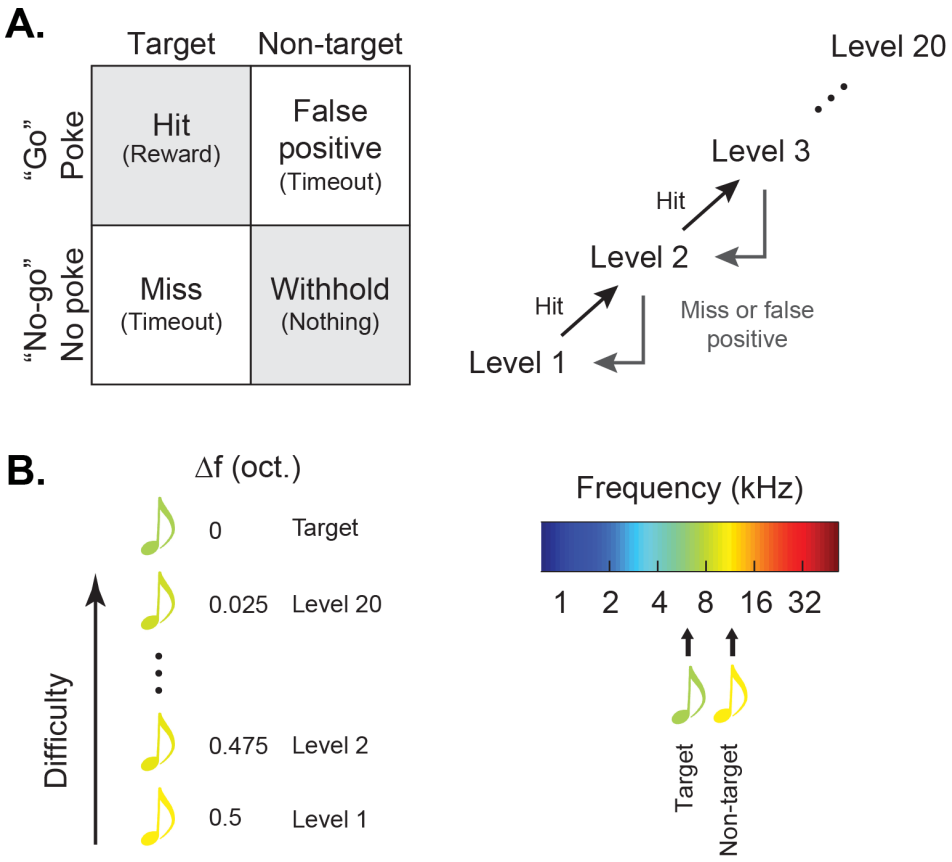
3.7 Supplementary figures



Supplementary Figure 3.1: *Behavioral paradigm*. A. Classification of behavioral responses. For a given trial, rats could have performed one of four responses according to the combination of behavioral state (go or no-go) and stimulus property (target or non-target). Go responses within 5s of a target were scored as a *hit*; a failure to respond within this time window was scored as a *miss*. A go response within 5s of a non-target stimulus was scored as a *false positive*; the absence of a response was scored as a *withhold*. A hit triggered the delivery of a chocolate pellet and an increase in task level. A miss or false positive initiated a decrease in task level and a 5s time-out. A withhold did not produce any consequences. The number of levels was 20. B. Task stimuli. The target stimulus was a 7kHz pure tone. At the beginning of each training session, the non-target tone would start at 7kHz + 0.5 octaves and become 0.025 octaves closer to the target tone as the task level increased.

Chapter 4

Evidence of hyperacusis in adult rats following non-traumatic sound exposure

Maryse E. Thomas, Gerson D. Guercio, Kristina M. Drudik, Étienne de Villers-Sidani. *Frontiers in Systems Neuroscience,* 2019, 13:1-17.

Preface

The previous study demonstrated that tonotopic map reorganizations may be maladaptive, since map expansion facilitated by noise exposure impaired frequency discrimination. Map expansion has also been identified as a potential maladaptive component of the central auditory disorder tinnitus. In the following study, the electrophysiological and behavioral correlates of 7kHz map expansion in sound-exposed animals are assessed with respect to the symptomology of tinnitus and the closely related condition, hyperacusis. Importantly, this chapter documents electrophysiological changes in multiple auditory fields, extending previous findings of noise-induced plasticity beyond A1. Through this work, the potentially harmful effects of reopening critical periods with noise are revealed.

4.1 Abstract

Manipulations that enhance neuroplasticity may inadvertently create opportunities for maladaptation. We have previously used passive exposures to non-traumatic white noise to open windows of plasticity in the adult rat auditory cortex and induce frequency-specific functional reorganizations of the tonotopic map. However, similar reorganizations in the central auditory pathway are thought to contribute to the generation of hearing disorders such as tinnitus and hyperacusis. Here, we investigate whether noise-induced reorganizations are accompanied by electrophysiological or behavioral evidence of tinnitus or hyperacusis in adult Long-Evans rats. We used a two-week passive exposure to moderate-intensity (70dB SPL) broadband white noise to reopen a critical period for spectral tuning such that a second one-week exposure to 7kHz tone pips produced an expansion of the 7kHz frequency region in the primary auditory cortex (A1). We demonstrate for the first time that this expansion also takes place in the ventral auditory field (VAF). Sound exposure also led to spontaneous and sound-evoked hyperactivity in the anterior auditory field (AAF). Rats were assessed for behavioral evidence of tinnitus or hyperacusis using gap and tone prepulse inhibition of the acoustic startle response. We found that sound exposure did not affect gap-prepulse inhibition. However, sound exposure led to an improvement in prepulse inhibition when the prepulse was a 7kHz tone, showing that exposed rats had enhanced sensorimotor gating for the exposure frequency. Together, our electrophysiological and behavioral results provide evidence of hyperacusis but not tinnitus in sound-exposed animals. Our findings demonstrate that periods of prolonged noise exposure may open windows of plasticity that can also be understood as windows of vulnerability, potentially increasing the likelihood for maladaptive plasticity to take place.

4.2 Introduction

As recent decades of neuroscience research have revealed the brain's lifelong capacity for plastic change (Hofer et al. 2006; de Villers-Sidani and Merzenich 2011), the goal of reopening critical periods (CPs) in order to stimulate learning and recovery in adulthood has become an important area of study. Researchers have already demonstrated the ability to reopen CPs in the auditory (Blundon et al., 2017; Reed et al., 2011; Zhou et al., 2011), visual (Pizzorusso et al. 2002; He et al. 2006; Harauzov et al. 2010), and somatosensory domains (Chung et al. 2017) in animal models. And steps have even been taken in humans, as the histone-deacetylase inhibitor, valproate, was found to reopen a CP for absolute pitch in adult non-musicians (Gervain et al. 2013). The inevitable quest for lifelong adaptability, however, should not be undertaken without considering the potential risks of opening windows of vulnerability on the brain (Hensch and Bilimoria 2012).

One such vulnerability is the opportunity for maladaptive plasticity, which refers to structural or functional nervous system changes that disrupt normal function. Dysplastic symptoms such as hyperexcitation, altered neural connectivity, and topographic reorganizations can interfere with perceptual discrimination (O'Reilly et al. 2019), cause hypersensitivities or phantom percepts (Flor et al. 2001; Costigan et al. 2009; De Ridder et al. 2011), and contribute to chronic pain (Kuner and Flor 2016). In the central auditory system, maladaptive plasticity is thought to underlie the generation of auditory disorders including chronic tinnitus and hyperacusis, the uncomfortable sensations of ringing in the ears and sound hypersensitivity. These potentially debilitating conditions usually emerge late in life comorbid with hearing loss and affect between 6-15% of the general population (Brozoski and Bauer 2016). Although the exact neural underpinnings of tinnitus and hyperacusis remain elusive, their frequent co-

occurrence with hearing loss points to the reduction of auditory inputs as a potential trigger for plasticity in spatially-defined regions of the auditory pathway (Eggermont and Roberts 2004; Roberts et al. 2010; Langers et al. 2012). In animal models, tinnitus has primarily been associated with expanded representations of mid-to-high frequency regions, hypersynchronization, increased spontaneous firing, and increased burst firing in structures including the cochlear nucleus, inferior colliculus, and auditory cortex (Eggermont and Roberts 2004; Roberts et al. 2010). Hyperacusis has been related to increased gain in the central auditory pathway in animal models detectable via higher spontaneous firing rates and sound-evoked potentials (Sun et al. 2012; Aazh et al. 2014; Hickox and Liberman 2014). At present, some evidence links spontaneous and sound-evoked hyperactivity to tinnitus or hyperacusis in humans (Adjamian et al. 2009; Gu et al. 2010), but neuroimaging studies have yet to demonstrate macroscopic tonotopic reorganization in patients with tinnitus (Langers et al. 2012; Elgoyhen et al. 2015), illustrating that much remains to be understood in the etiology of both conditions.

Tinnitus has been tentatively linked to lifetime environmental noise exposure (Holgers and Pettersson 2005; Guest et al. 2017; Moore et al. 2017). In adult rats and cats, prolonged moderate-intensity sound exposures have been shown to produce strong experience-dependent plasticity altering tonotopic organization and auditory excitability (Pienkowski and Eggermont 2009; Pienkowski et al. 2011; Zhou et al. 2011; Zheng 2012). We have previously demonstrated that two weeks of passive exposure to moderate-intensity white noise can reopen windows of CP-like plasticity in the adult rat auditory cortex (Thomas et al. 2019). We confirmed CP plasticity with a second passive exposure to pure tones that led to the expansion of the corresponding frequency region in the primary auditory cortex. The perceptual consequences of this map expansion are incompletely understood and differ based on the mode of induction, with

primarily sound-driven – as opposed to neuromodulatory-driven – expansions impairing discrimination for the exposure frequency (Han et al. 2007; Eggermont 2013; Froemke et al. 2013). Based on the common phenotype of map expansion in both sound-exposed animals and animals with tinnitus, we wondered if the sound exposure used in our previous study could have imparted our rats with tinnitus or another auditory disorder.

In the present study, we investigated the possibility that cortical map expansion could be indicative of maladaptive plasticity in sound-exposed animals. To this end, we induced 7kHz map expansion in female adult Long Evans rats using continuous exposure to moderate-intensity (70dB SPL) broadband white noise for two weeks followed by 7kHz tone pips for one week. We hypothesized that this exposure would lead to specific maladaptive plasticity in cortical regions that preferentially respond to 7kHz accompanied by behavioral evidence of hyperacusis or tinnitus as measured by prepulse inhibition (PPI) and gap-prepulse inhibition (GPIAS) of the acoustic startle reflex, respectively. We documented the effects of exposure on electrophysiological response properties in the primary auditory cortex (A1), anterior auditory field (AAF), and ventral auditory field (VAF). We found evidence of hyperactivity in the AAF of exposed animals consistent with hyperacusis, which was supported by an improvement in PPI when the prepulse was a 7kHz pure tone. We did not find electrophysiological or behavioral evidence of tinnitus. Our findings indicate that although non-traumatic white noise exposure can open windows of plasticity on the brain, these can also be understood as windows of vulnerability that may increase the likelihood for maladaptive plasticity to occur.

4.3 Materials & Methods

The experimental procedures used in this study were approved by the Montreal Neurological Institute Animal Care Committee and follow the guidelines of the Canadian Council on Animal Care.

Sound exposure: Female 3- to 4-month-old Long-Evans rats were housed in sound-attenuated chambers under a 12h light/dark cycle and given ad libitum access to food and water. Rats were assigned to either the naive or sound exposure condition. Naive rats (N = 23) had no acoustic manipulation of their environment (background sound level 40dB SPL). Exposed rats (N = 25) were passively exposed to 70dB SPL (decibels sound pressure level, RMS) continuous white noise for two weeks immediately followed by a one-week exposure to trains of 7kHz tone pips. From each group, 12 rats were used for behavioral testing (12 Naïve-BEH and 12 Exposed-BEH) while the remaining rats (11 Naive and 13 Exposed) were used for electrophysiological recordings. To reduce the number of animals sacrificed for this study, the electrophysiological data for the Exposed group came from combining two groups of noise + 7kHz-exposed animals that underwent slightly different 7kHz exposures. Four rats came from Thomas et al. (2019) and were exposed to 7kHz pure tones. The other nine rats were exposed to 7kHz tone pip clouds consisting of pure tones of random frequencies within a ¼ octave range centered on 7.6kHz (ranging between 7-8.3kHz). Other than tone frequencies, all other properties of the tone exposures were the same. The noise and tone pips were generated using custom MATLAB scripts (The MathWorks, Inc., Natick, Massachusetts) and played through an Ultralite-mk3 Hybrid Interface (MOTU Inc., Cambridge, Massachusetts) with sampling at 192kHz. The noise stimuli were amplified to a free-field sound level calibrated so that the average stimulus intensity measured in the center of the chamber was 70dB SPL. Tones were 50ms in duration (5ms onset

and offset ramps) and delivered in trains of 5 pips per second. The interval between each train of tones was a random duration generated from a normal distribution with a mean of 2.5 seconds. The tone pips were amplified to an intensity of 65dB SPL measured in the center of the chamber. All stimuli were played 24 hours per day for the duration of the exposure periods.

Electrophysiological recordings: Electrophysiological recordings of the left auditory cortex were performed under isoflurane anesthesia in a shielded soundproof recording chamber. Rats were pre-medicated with dexamethasone (0.2mg/kg, i.m.) to minimize brain edema. Anesthesia was induced with ketamine/xylazine/acepromazine (63/13/1.5 mg/kg, i.p.) followed by continuous delivery of isoflurane 1% in oxygen via endotracheal intubation and mechanical ventilation. Heart rate and blood oxygen saturation were monitored with a pulse oximeter. Body temperature was monitored with a rectal probe and maintained at 37ºC with a homeothermic blanket system. Rats were held by the orbits in a custom designed head holder leaving the ears unobstructed. The cisterna magna was drained of cerebrospinal fluid to further minimize cerebral edema. To access the auditory cortex, the left temporalis muscle was reflected, the skull over the auditory cortex was removed, and the dura was resected. Once exposed, the cortex was maintained under a thin layer of silicone oil to prevent desiccation. Acoustic stimuli were delivered in a free field manner to the right ear through a calibrated speaker. Cortical responses were recorded with a high-impedance 64-channel tungsten microelectrode array (Tucker-Davis Technologies [TDT], Alachua, Florida) lowered to a depth of 600-900μm (layers 4/5). The electrode wires (33μm diameter) were arranged in an 8x8 grid orthogonal to the cortex spaced 375μm apart with row separation of 500μm. To maximize recording density, neural responses were consecutively recorded from multiple positions within each rat. The stereotaxic location of each position relative to the first was noted in order to accurately reconstruct auditory maps

during offline analysis. Extracellular multi-unit responses were obtained, amplified, and filtered (0.3–5 kHz) using a TDT RZ2 processor. The TDT OpenEx software package was used to generate acoustic stimuli, monitor cortical activity online, and store data for offline analysis.

Acoustic Stimulation: Frequency-intensity receptive fields were constructed using neuronal responses to a range of frequency-intensity combinations of pure tones. 66 frequencies (0.75-70kHz; 0.1 octave increments; 25ms duration; 5ms ramps) were presented at eight sound intensities (0-70dB SPL; 10dB increments) at a rate of one tone per second with three repetitions and in random presentation order. The characteristic frequency (CF) and threshold of a cortical site were defined, respectively, as the frequency and intensity at the tip of the V-shaped tuning curve derived from peri-stimulus time histograms (PSTHs). For flat-peaked tuning curves or tuning curves with multiple peaks, the CF was defined as the frequency with the lowest threshold and the strongest firing rate. Response bandwidths 20dB above the threshold of tuning curves (BW20) were measured for all sites. The onset latency, defined as the time in ms when the PSTH first exceeded mean baseline firing rate by 2.5 standard deviations, was also measured for each cortical site. The CF, threshold, BW20, and latencies were first determined by an automated custom MATLAB routine and then manually verified by an experimenter blind to the identity of the experimental groups. Cortical sites were identified as belonging to A1, AAF, VAF, or posterior auditory field (PAF) based on published functional characteristics of each field (Polley et al. 2007). These were reversal of tonotopic gradients, onset latencies, threshold, and PSTH morphologies (**Supplementary Fig. 4.1**). To generate tonotopic maps, Voronoi tessellation was performed using custom MATLAB scripts to create tessellated polygons with electrode penetration sites at their centers.

Neural synchrony: The degree of neural synchronization in the auditory cortex was computed from recordings of spontaneous neural activity that were at least five minutes long. Recordings with apparent burst suppression were not included in analyses. Burst suppression was characterized by periods of high spontaneous firing alternating with periods of no activity determined through visual inspection of the raster plots and continuous average firing rate. If a portion of any recording was deemed to have burst suppression, the recording was rejected. The average coefficient of variation (CV) of the inter-spike interval – a measure of burstiness – corresponded well with our classification of burst suppression, as the mean CV was significantly higher for recordings identified as having burst suppression (mean = 3.45, SD = 0.15) than those that were not (mean = 1.86, SD = 0.07) (mixed effects one-way ANOVA $F(1,48.13) = 93.34$, $p < .0001$, n = 2070 units within 53 positions and 24 rats). Offline spike sorting was performed using TDT OpenSorter software to isolate single unit activity based on an automated Bayesian sorting algorithm. The success of the spike sorting algorithm was assessed by inspecting the number of refractory period violations for all identified clusters (**Supplementary Fig. 4.2**). The fraction of spikes that fell within a 2ms refractory period was calculated and it was found that 36.1% of all clusters had zero refractory period violations and 96.9% of all clusters had 2 or fewer violations per 100 spikes (**Supp. Fig. 4.2A**). An average of 1.63 units was identified per electrode channel. Example histograms of the interspike interval and the autocorrelation of spike times for representative units are presented in **Supp. Fig. 4.2B,** displaying a dearth of spikes occurring within the refractory period. In addition, the percentage of refractory period violations did not differ between experimental groups (**Supp. Fig. 4.2C**). These results indicate that there are a relatively small number of false positive classifications present in the data, which are unlikely to affect experimental outcomes. Measures of

synchronization were computed from binary spike events detected from A1 units in separate channels. Cross-correlograms were computed by counting the number of spike coincidences for pairs of spike trains for time lags of −500 to 500ms with 1ms bin size and normalized by dividing each bin by the square root of the product of the number of total discharges in each spike train (Eggermont 1992).

Behavioral testing: Behavioral testing took place during the day at the Glen Site of the McGill University Health Centre. At the end of the exposure period, each Naïve-BEH animal was randomly assigned to a pair with one Exposed-BEH animal. Once paired, the rats were transported in their original cages to a loading area by cart. There, they were transferred to a vehicle and driven to the Glen Site, approximately 25 minutes away. The rats were again transported to a holding area adjoining the behavioral testing facility by cart where they were acclimatized for a minimum of two hours. Rats remained covered for all of the steps above until they reached the holding area. The paired rats then underwent behavioral testing simultaneously in order of pairing (two rats were tested at a time). This procedure took place twice, with six animals from each group tested on each day. All behavioral data were collected in sound-attenuating chambers. Sounds were delivered from a free-field speaker and rats were free to roam the chamber. The acoustic startle response was measured using the LE 118-8 Startle and Fear Interface (Panlab, Barcelona, Spain). The startle pulse was a white noise burst (120dB SPL, 40 ms) for both GPIAS and PPI. For GPIAS, rats were acclimatized for three minutes in a pure tone background that was either 3.5kHz or 7kHz (65 dB SPL), followed by four randomly interleaved no-gap and gap (30 ms) trials (intertrial interval 12-30s). During the gap trials, the gap preceded the pulse by 60ms. This procedure was performed three times, and startle activity for the no-gap and gap trials were averaged across a total of 12 trials each. For PPI, rats were

acclimatized for three minutes in a white noise background (65dB SPL). The subsequent experimental protocol consisted of 10 trials each of no stimulus, the startle pulse alone, and two prepulse frequencies (3.5 or 7kHz, 20 ms, 75dB SPL) presented 60 ms before the startle pulse, in pseudorandom order (intertrial interval 12-30s). The startle activity for the no stimulus, startle pulse, 3.5kHz prepulse, and 7kHz prepulse trials were averaged across the 10 trials. We calculated prepulse inhibition of the startle response using the formula: %PPI = 100 – (startle response for prepulse trials/startle response for startle pulse alone trials) × 100. We calculated gap-prepulse inhibition of the startle response with the formula: %GPIAS = 100 - (startle response for gap trials/startle response for no-gap trials) x 100.

Statistical analyses: For all statistical analyses, results are reported in parentheses including test name and statistic and number of data points per level of nested data. Linear mixed-effects models (Reed and Kaas 2010; Aarts et al. 2014) were used to analyze data collected through nested experimental designs (e.g. for synchronization analyses: neuron pair nested within recording position nested within rat). For these models, recording position nested within rat ID were included as random effects. A matched pairs design using paired t-tests was employed to analyze behavioral data in order to control for potential confounding effects of transport, handling, waiting, and testing times on the acoustic startle response (Geyer and Swerdlow 1998; Longenecker and Galazyuk 2012). Accordingly, the effect size calculated by Cohen's d_{av} is reported for behavioral results (Lakens 2013). Analyses were conducted using MATLAB and JMP 13 (SAS Institute, Cary, NC). The mixed effect test results are reported with the degrees of freedom denominator approximated for normal data using the Kenward-Roger adjustment. Unless otherwise stated, Tukey's test evaluated at an alpha level of 0.05 was used for all post-hoc comparisons. Where applicable, back-transformed means derived from statistical

models were plotted in figures. Where results are not shown in figures, means ± standard error are reported in the text.

4.4 Results

4.4.1 Electrophysiological correlates of sound exposure

We documented the effects of two weeks of passive exposure to white noise followed by one week of 7kHz tone pip exposure on electrophysiological response properties in 13 rats (Exposed Group) and compared them to 11 rats that were housed in a standard acoustic environment (Naïve Group) (**Fig. 4.1A**). Characteristic frequency (CF) tonotopic maps were reconstructed from the left auditory cortex under isoflurane anesthesia using in-vivo multiunit responses to presentations of tone pips of various frequencies and intensities (**Fig. 4.1B**). Responsive sites were classified as belonging to A1, AAF, VAF, or posterior auditory field (PAF) based on the published functional characteristics of each field (Polley et al. 2007; Profant et al. 2013), specifically reversal of tonotopic gradients, onset latencies, threshold, and peri-stimulus time histogram (PSTH) morphologies (**Supp. Fig. 4.1**). Using functional properties alone, we were not able to distinguish VAF from the fifth rat auditory field, suprarhinal auditory field (SRAF), so any presumed VAF or SRAF site was classified under the common label of VAF. In addition, we did not conduct analyses on the data we obtained from PAF due to the difficulty of assigning a CF to most PAF units, which have broad and noisy tuning curves. For each animal, we determined whether we obtained full or partial A1, AAF, and VAF maps. A full map was defined by having low, medium, and high frequency regions as well as a reversal of the tonotopic gradient on one border and non-auditory sites on the opposite border. In Fig. 1B, the representative CF maps from each group were selected for having full maps of each field. **Table**

4.1 lists the number of cortical sites obtained for each auditory field and experimental group for both full and partial maps.

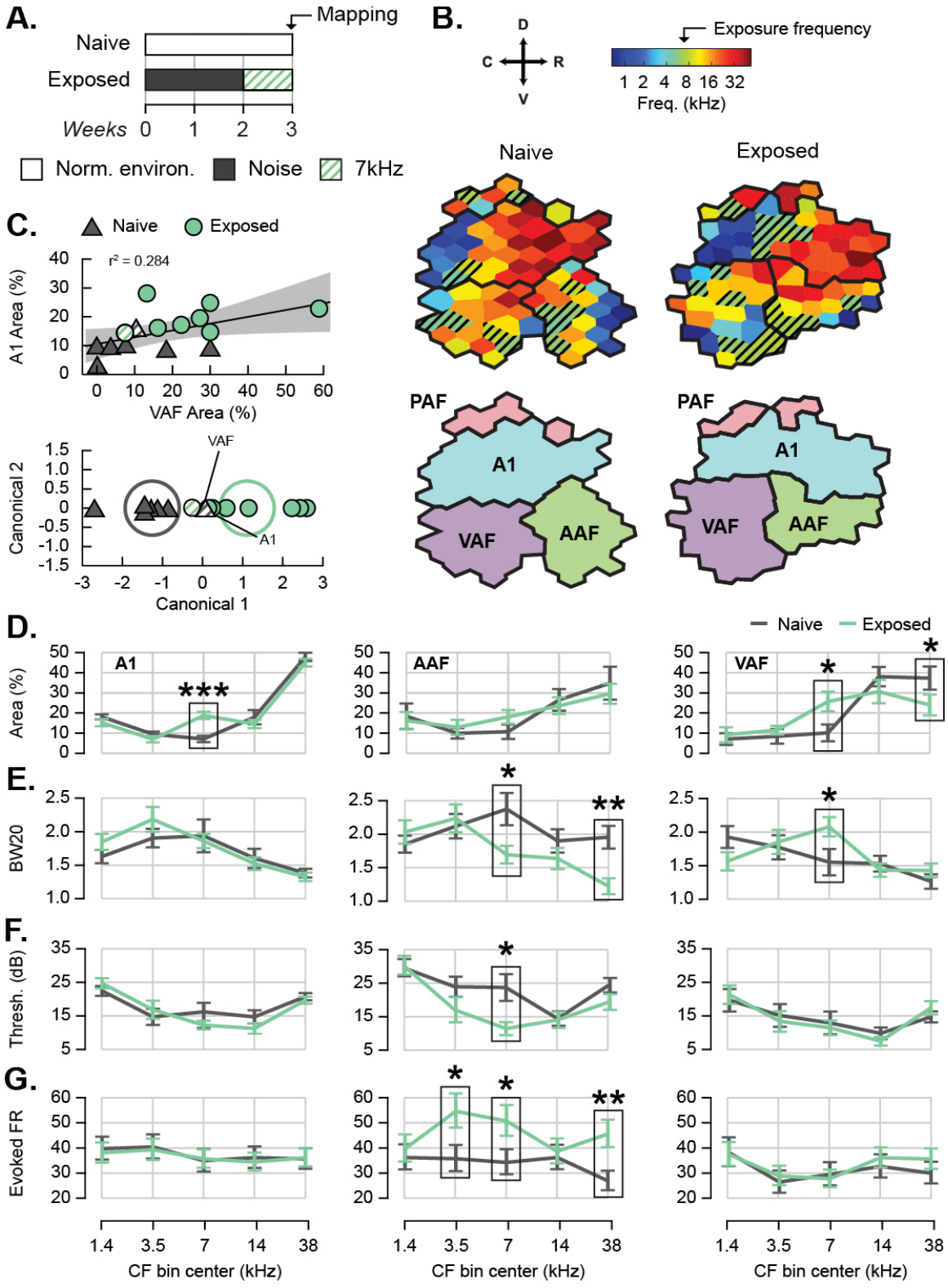


Figure 4.1: *Effect of sound exposure on cortical tuning and tone-evoked activity*. **A.** Sound exposure protocol. Naïve rats were housed in a normal acoustic environment while exposed rats were passively exposed to two weeks of moderate-intensity broadband white noise followed by one week of 7kHz tone pips. **B.** An example characteristic frequency (CF) map from each experimental group containing all auditory fields. Hatched sites represent those with a CF of 7kHz ± 1/2 octave. **C.** Top: Correlation between the percent A1 area and percent VAF area with a CF of 7kHz ± ½ octave. Shaded region represents 95% confidence of fit for the regression. Bottom: Canonical plot of the linear discriminant analysis based on the percent of A1 and VAF area with a CF of 7kHz ± ½ octave. Rats were automatically classified as either naïve or exposed; hatched points identify rats that were misclassified. Ellipses represent the 95% confidence region for the true mean of each group. **D.** Average map area with CF in five frequency bins. Only full auditory fields were used for map percentages. **E.** Average BW20 for receptive fields with CF in five frequency bins. **F.** Average cortical threshold for receptive fields with CF in five frequency bins. **G.** Average tone-evoked firing rate for units with CF in five frequency bins. * $p < .05$, ** $p < .01$, *** $p < .001$. Error bars represent SEM. A1–primary auditory field, AAF–anterior auditory field, VAF–ventral auditory field, PAF–posterior auditory field. See Table 1 for number of rats, recording positions, and cortical sites per group.

We first compared the degree of 7kHz map expansion between exposed and naïve animals (**Fig. 4.1B-D**). Using full field maps only, we calculated the percentage of map area with CFs in five frequency bins with centers at approximately 1.4, 3.5, 7, 14, and 38kHz. The range of each bin was 1 octave except for the first and last bin, which were 1.7 and 1.8 octaves respectively. The bins were defined in relation to 7kHz in order to maximize specificity for the middle bins while covering the full range of recorded CFs. In A1, as expected, we observed a significantly greater percentage of map area tuned to 7kHz for the Exposed group (two-way ANOVA with Group and Bin as factors. Interaction $F(4,4) = 5.30$, $p = .0007$ followed by simple main effects for 7kHz $F(1,85) = 16.94$, $p < .0001$, n = 19 rats). This over-representation was not compensated by a consistent under-representation in another frequency bin as no other simple main effect was significant ($F(1,85) \leq 1.41$, all $p \geq .2387$). In AAF, we detected no difference in map area for any frequency bin (two-way ANOVA with Group and Bin as factors. Interaction: $F(4,4) = 0.55$, $p = .6965$, n = 15 rats), whereas in VAF we observed a significant over-representation of the 7kHz frequency bin for exposed animals, as well as a significant decrease in

map area in the highest frequency bin (two-way ANOVA with Group and Bin as factors. Interaction $F(4,4) = 2.96$, $p = .0257$ followed by simple main effects for 7kHz $F(1,70) = 5.85$, $p = .0182$ and 38kHz $F(1,70) = 4.30$, $p = .0418$, n = 16 rats). No other frequency bin was significantly changed (simple main effects $F(1,70) \leq 1.33$, all $p \geq .2521$). To ensure that we did not oversample the 7kHz frequency region in the Exposed group, we compared the average distance between each site and its nearest neighbor from full field maps. We observed no significant differences in nearest-neighbor distance between Naïve and Exposed animals in any frequency bin for any field, confirming that differences in frequency representation were not due to differences in sampling (mixed effects two-way ANOVAs with Group and Bin as factors. A1: mean distance Naïve = 323.72 ± 6.82 μm, Exposed = 326.21 ± 7.14μm, Interaction $F(4,579) = 1.00$, $p = 4060$. Main effect of Group $F(1,17.26) = 0.06$, $p = .8038$, n = 605 sites within 19 rats. AAF: mean distance Naïve = 336.20 ± 11.56μm, Exposed = 329.73 ± 10.77μm, Interaction $F(4,246.8) = 1.04$, $p = .3863$. Main effect of Group $F(1,13.31) = 0.17$, $p = .6887$, n = 260 sites within 15 rats. VAF: mean distance Naïve = 355.58 ± 11.85μm, Exposed = 338.55 ± 10.09μm, Interaction $F(4,269.5) = 1.73$, $p = 0.1445$. Main effect of Group $F(1,14.9) = 1.20$, $p = .2911$, n = 287 sites within 16 rats). The above results document for the first time that noise-induced CP plasticity extends to A1 and VAF, but not AAF.

It is possible that not every sound-exposed rat will exhibit CP-like plasticity. However, if 7kHz map expansion is a reliable indicator, it could be used to distinguish rats that show phenotypic CP plasticity from those that do not. We explored this possibility using a linear discriminant analysis to test the hypothesis that exposed and naive rats could be distinguished based on a linear combination of the 7kHz percent map area in more than one auditory field (**Fig. 4.1C**). Only animals with full maps in both A1 and VAF were included (7 Naïve and 8 Exposed).

The 7kHz percent map areas in A1 and VAF were positively correlated, $r = 0.53$, $p = .0408$, $n = 15$ rats (**Fig. 4.1C top**). This is in contrast to A1 and AAF, $r = 0.09$, $p = .8068$, $n = 10$ rats, and VAF and AAF, $r = 0.22$, $p = .5964$, $n = 8$ rats, which were not significantly correlated. The canonical function resulting from the linear discriminant analysis was statistically significant (canonical correlation = 0.79, Wilks' Lambda = 0.38, $F(2,12) = 9.68$, $p = .0031$, $n = 15$ rats, **Fig. 4.1C bottom**). Reclassification of the rats based on the new canonical variable using leave-one-out cross-validation was successful: 88.10 ± 1.2% of the rats were correctly classified into their exposure condition. The canonical function was positively correlated with both 7kHz percent map area in A1, $r = 0.99$, $p < .0001$, $n = 15$ rats, and VAF, $r = 0.66$, $p = .0069$, $n = 15$ rats. This result was approximately equivalent to performing a linear discriminant analysis using the 7kHz percent map area in A1 alone and better than using VAF alone. When including only A1, the canonical function was significant (canonical correlation = 0.77, Wilks' Lambda = 0.41, $F(1,17) = 24.15$, $p = .0001$, $n = 19$ rats), cross-validated reclassification led to 89.47 ± 0.41% correct classification. When including only VAF, the canonical function was also significant but less successful (canonical correlation = 0.53, Wilks' Lambda = 0.72, $F(1,14) = 5.38$, $p = .0360$, $n = 16$ rats). Cross-validated reclassification led to 71.25 ± 1.4% correct classification. These results show that the degree of map expansion is relatively consistent within each animal; rats with high map expansion in A1 are likely to have high map expansion in VAF. This characteristic also allows rats that have undergone sound exposure to be classified with high accuracy, suggesting that degree of map expansion is a reliable indicator of CP plasticity whether taking into account only A1 or A1 and VAF together.

To establish the electrophysiological correlates of 7kHz map expansion, we continued to compare neural response properties in five CF bins using data from both full and partial maps.

We predicted that the 7kHz-tuned neurons of exposed animals would show additional evidence of plasticity. We compared the receptive field bandwidth 20dB above threshold (BW20), a measure of response specificity, in each auditory field (**Fig. 4.1E**). In A1, we observed no significant change in BW20 following exposure for any CF bin (mixed effects two-way ANOVA with Group and Bin as factors. Interaction $F(4,687.7) = 1.10$, $p = .3545$. Main effect of Group $F(1,70.18) = 0.26$, $p = .6093$, n = 701 sites within 52 positions and 23 rats). In AAF we found significantly narrower BW20s for the 7kHz and 38kHz bins (mixed effects two-way ANOVA with Group and Bin as factors. Interaction $F(4,278.1) = 3.32$, $p = .0113$ followed by simple main effects for 7kHz $F(1,194.5) = 6.25$, $p = .0132$ and 38kHz $F(1,127.9) = 11.94$, $p = .0007$. No other CF bin was significant $F(1,160.3\text{-}234.5) \leq 1.42$, all $p \geq .2360$, n = 292 sites within 47 positions and 22 rats). In VAF, on the other hand, we observed broader BW20s for the 7kHz bin (mixed effects two-way ANOVA with Group and Bin as factors. Interaction $F(4,327.1) = 2.74$, $p = .0288$, followed by simple main effects for 7kHz $F(1,242.8) = 5.42$, $p = .0207$. No other CF bin was significant $F(1,121.5\text{-}278.7) \leq 2.78$, all $p \geq .0966$, n = 342 sites within 45 positions and 23 rats). These differences demonstrate a reduction in tuning specificity for VAF neurons tuned to 7kHz following sound exposure and an increase in specificity for AAF neurons tuned to 7kHz and 38kHz.

Cortical thresholds measure a neuron's sensitivity to low intensity sounds and can provide an approximate estimate of hearing thresholds. We compared the average cortical thresholds of neurons in each CF bin between experimental groups (**Fig. 4.1F**). We observed no group differences in A1 or VAF for any CF bin (mixed effects two-way ANOVAs with Group and Bin as factors A1: Interaction $F(4,657) = 1.28$, $p = .2761$. Main effect of Group $F(1,49.79) = 0.01$, $p = .9190$, n = 701 sites within 52 positions and 23 rats. VAF: Interaction $F(4,310.9) =$

0.71, p = .5867. Main effect of Group F(1,43.54) = 0.02, p = .8924, n = 342 sites within 45 positions and 23 rats). In AAF, however, we found that average thresholds were significantly lower for the 7kHz bin (mixed effects two-way ANOVA with Group and Bin as factors. Interaction F(4,255.6) = 2.41, p = .0494 followed by simple main effects for 7kHz (F(1,124.8) = 4.70, p = .0320. No other CF bin was significant F(1,67.93-156.3) ≤ 2.28, p ≥ .1334, n = 292 sites within 47 positions and 22 rats). These results show that after sound exposure, AAF became more sensitive to the 7kHz frequency. Importantly, the cortical thresholds of the Exposed group were either the same or lower than Naïve for all fields, demonstrating that the exposure intensities were non-traumatic and did not cause any apparent hearing loss. Taken together, the changes in BW20 and cortical thresholds observed in sound-exposed animals may highlight differences in the receptive field properties of AAF and VAF. VAF neurons tend to have narrow tuning curves with low thresholds while AAF neurons tend to have broad tuning curves with relatively high thresholds. Plasticity following sound exposure appears to have reduced these field-specific qualities for 7kHz-tuned neurons.

Sound-evoked firing rates are elevated in hyperacusis (Sun et al. 2012; Aazh et al. 2014; Hickox and Liberman 2014). We compared the tone-evoked firing rate between exposed and naive animals (**Fig. 4.1G**). The average firing rate in response to the full range of tonal stimuli (66 frequencies presented at 8 intensities) was considered. The rate was computed from the number of spikes counted between 8-58ms after tone presentation minus the number of spikes counted in the 50ms preceding tone presentation. As could be expected, firing rate was positively correlated with sound intensity, r = 0.11, p < .0001, n = 7093 observations. We also found that onset latency was negatively correlated with firing rate, r = -0.20, p <.0001, n = 7093 observations, possibly because a fixed epoch window resulted in less spikes being counted for

sites with later latencies. We did not observe a significant difference in onset latency between naïve and exposed animals for any field (mixed effects two-way ANOVAs with Group and Bin as factors. A1: Interaction $F(4,678.3) = 1.74$, $p = .1403$. Main effect of Group $F(1,64.63) = 0.39$, $p = .5325$, n = 701 sites within 52 positions and 23 rats. AAF: Interaction $F(4,253.2) = 1.74$, $p = .1414$. Main effect of Group $F(1,34.73) = 3.00$, $p = .0920$, n = 292 sites within 47 positions and 22 rats. VAF: Interaction $F(4,317.9) = 0.13$, $p = .9698$. Main effect of Group $F(1,37.34) = 0.09$, $p = .7683$, n = 342 units within 45 positions and 23 rats). As a result, we performed two-way ANCOVAs with intensity and latency as covariates to determine whether the tone-evoked firing rate differed between experimental groups controlling for these two variables. We did not find a significant difference between groups for any CF bin in A1 or VAF (mixed effects two-way ANCOVAs with Group and Bin as factors. A1: Interaction $F(4,3662) = 0.35$, $p = .8456$. Main effect of Group $F(1,50.68) = 0.01$, $p = .9073$, n = 3697 observations within 52 positions and 23 rats. VAF: Interaction $F(4,1937) = 1.38$, $p = .2393$. Main effect of Group $F(1,40.82) = 0.10$, $p = .7516$, n = 1965 observations within 45 positions and 23 rats). In AAF, however, we observed a significantly higher tone-evoked firing rate for the 3.5kHz, 7kHz, and 38kHz bins (mixed effects two-way ANCOVAs with Group and Bin as factors. Interaction $F(4,1398) = 9.02$, $p < .0001$ followed by simple main effects for 3.5kHz $F(1, 60.06) = 4.39$, p .0403, 7kHz $F(1, 67.83) = 4.99$, $p = .0288$, and 38kHz $F(1,47.66) = 8.03$, $p = .0067$. No other CF bins were significant $F(1,51.18\text{-}60.75) \leq 0.23$, both $p \geq .63$, n = 1431 observations within 47 positions and 22 rats) For all of the ANCOVAs above, intensity ($F(1,1383\text{-}3646) \geq 35.56$, all $p \leq .0001$) and latency ($F(1,1406\text{-}3666) \geq 78.36$, all $p \leq .0001$) remained significant factors. These results show that after sound exposure, tone-evoked firing rate was greater within AAF for neurons tuned to a broad range of frequencies.

	Group	Field	Rats	Positions	Sites/Units per CF bin					
					1.4	3.5	7	14	38	Total
All data	Naive	A1	10	22	55	29	21	52	155	312
		AAF	11	22	31	16	19	35	50	151
		VAF	10	18	15	15	17	48	52	147
		PAF	10	17	-	-	-	-	-	69
		All	***11***	***24***						***679***
	Exposed	A1	13	30	47	29	67	59	187	389
		AAF	11	23	23	17	27	31	43	141
		VAF	13	27	19	21	46	62	47	195
		PAF	11	21	-	-	-	-	-	105
		All	***13***	***30***						***830***
Full fields only	Naïve	A1	10	21	53	28	21	52	136	290
		AAF	7	16	22	15	18	32	44	131
		VAF	7	12	11	13	12	43	42	121
		PAF	5	10	-	-	-	-	-	49
		All	***11***	***24***						***591***
	Exposed	A1	9	19	47	21	59	47	141	315
		AAF	8	19	23	17	25	27	37	129
		VAF	9	19	17	20	41	49	39	166
		PAF	8	18	-	-	-	-	-	99
		All	***13***	***30***						***709***
Sorted units	Naïve	A1	10	19	77	32	34	77	197	417
		AAF	11	19	33	23	18	29	65	168
		VAF	9	15	17	17	17	62	76	189
		PAF	10	17	-	-	-	-	-	110
		All	***11***	***20***						***884***
	Exposed	A1	13	25	76	36	90	80	263	545
		AAF	11	18	28	17	28	36	45	154
		VAF	13	22	25	33	62	91	59	270
		PAF	10	18	-	-	-	-	-	164
		All	***13***	***25***						***1133***

Table 4.1: *Summary of data*. Number of rats and recording positions from which data were obtained for each auditory field and experimental group. Number of cortical sites (or units, for sorted data) per CF bin in each field. PAF units were not assigned CFs.

Tinnitus and hyperacusis are associated with higher spontaneous firing rates (Wang et al. 2011; Kaltenbach 2011), and tinnitus in particular is associated with more burst firing in the auditory pathway including the auditory thalamus (Kalappa et al. 2014) and auditory cortex (Syka and Rybalko 2000; Noreña and Eggermont 2003). From five-minute-long recordings of spontaneous activity during silence, we computed the spontaneous firing rate and inter-spike intervals (ISIs) of single-unit activity (**Fig. 4.2**). Each sorted unit was assigned an auditory field

and CF based on sound-evoked responses in the same recording position resulting in a total of 1,743 units from A1, AAF, and VAF combined. The number of units included in each auditory field and group is listed in **Table 4.1**. Because the distribution of firing rates was positively skewed (**Fig. 4.2A**), we applied a natural logarithmic transform before statistical analyses. Back-transformed means are plotted in **Fig. 4.2B**. The average spontaneous firing rates of our naïve animals were as follows: A1 = 5.33 ± 0.33, AAF = 4.77 ± 0.58, VAF = 5.67 ± 0.57 spikes/second. After sound exposure, we did not observe any difference in spontaneous firing rates in A1 or VAF regardless of CF bin (mixed effects two-way ANOVAs with Group and Bin as factors. A1: Interaction $F(4,932.2) = 0.85$, $p = .4951$. Main effect of Group $F(1,40.85) = 1.04$, $p = .3134$, n = 962 units within 44 positions and 23 rats. VAF: Interaction $F(4,442.9) = 1.65$, $p = .1596$. Main effect of Group $F(1,37.86) = 0.34$, $p = .5626$, n = 459 units within 37 positions and 22 rats). In AAF, on the other hand, sound exposure led to a significant and uniform increase in firing rate for all CF bins (mixed effects two-way ANOVA with Group and Bin as factors. Interaction $F(4,304.7) = 1.06$, $p = .3759$. Main effect of Group $F(1,39.57) = 14.67$, $p = .0004$, n = 322 units within 37 positions and 22 rats). The increased spontaneous firing rate in AAF indicates strong, tuning-independent hyperactivity resulting from sound exposure.

The ISI coefficient of variation (CV) was used to estimate the bursting activity of auditory neurons. This measure was obtained by dividing the standard deviation of each unit's ISI distribution by its mean (Longenecker and Galazyuk 2016). A high CV indicated more irregular spiking intervals, suggestive of bursting. Again, the distribution of CVs was positively skewed (**Fig. 4.2C**) so a natural logarithmic transform was applied before statistical analyses and back-transformed means are plotted in **Fig. 4.2D**. We did not observe any difference in the average CV of any field after sound exposure (mixed effects two-way ANOVAs with Group and

Bin as factors. A1: Interaction F(4, 932.3) = .07, p = .9908. Main effect of Group F(1,39.63) = 0.01, p = .9126, n = 962 units within 44 positions and 23 rats. AAF: Interaction F(4,306.8) = 1.40, p = .2325. Main effect of Group F(1,36.99) = 0.78, p = .3842, n = 322 units within 37 positions and 22 rats. VAF: Interaction F(4,433.8) = 0.32, p = .8627. Main effect of Group F(1,33) = 0.30, p = .5886, n = 459 units within 37 positions and 22 rats). From this, we concluded that burst firing was unchanged in the auditory cortex following sound exposure.

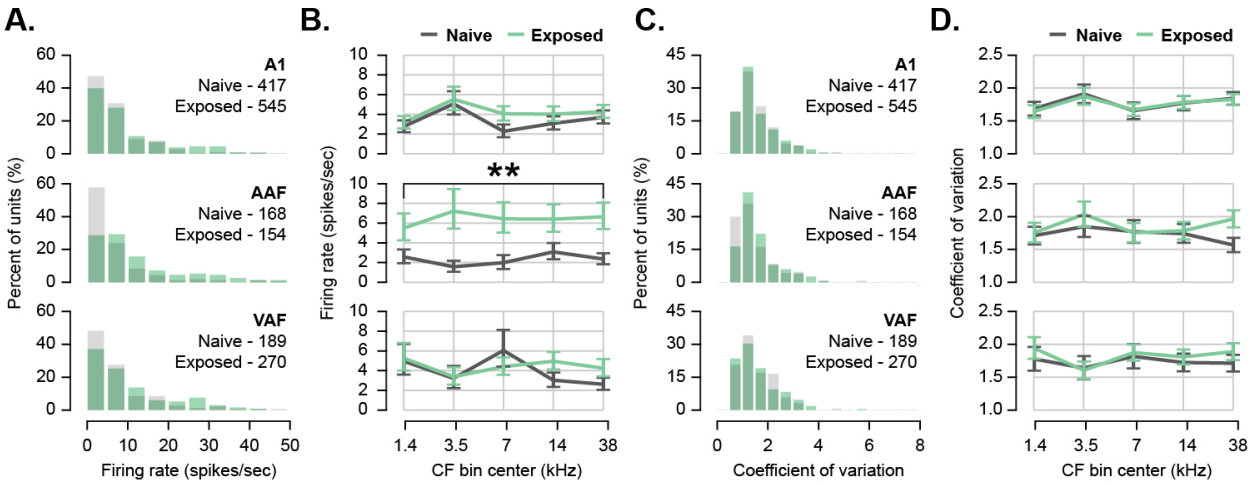


Figure 4.2: *Effect of sound exposure on spontaneous firing rate and burst firing*. **A.** Histogram of firing rates for each auditory field. N units per field and group in inset. **B.** Back-transformed mean firing rate with characteristic frequency (CF) in five frequency bins for each auditory field. **C.** Histogram of coefficient of variation for each auditory field. N units per field and group in inset. **D.** Back-transformed mean coefficient of variation in five frequency bins for each auditory field. ** p < .01. Error bars represent SEM. A1 – primary auditory field, AAF – anterior auditory field, VAF – ventral auditory field. See Table 1 for number of rats, recording positions, and units per auditory field and group.

Tinnitus has also been associated with hypersynchronization in animal models. Hypersynchronization typically appears immediately after noise trauma in a frequency-specific manner (Eggermont and Roberts 2004) and is evidence of increased connectivity, either thalamocortical or corticocortical, between neurons. To assess whether the Exposed group

displayed hypersynchronization, we calculated normalized cross-correlograms between single-unit pairs recorded in silence (**Fig. 4.3**). From the 1,743 units detected above, we identified 16,441 unit pairs in separate channels. We limited our analysis to pairs with a peak between -150 and 150ms, falling within approximately ±2.3 standard deviations of the mean peak, resulting in a total of 14,008 unit pairs for all fields. **Figure 4.3A** shows histograms of the cross-correlogram peak lag times demonstrating that peaks tend to fall near 0ms and **Figure 4.3B** shows the average cross-correlogram for all pairs in each field. The peak value of the cross-correlogram tended to decrease with greater inter-unit distance, $r = -0.24$, $p < .0001$, $n = 14{,}008$ pairs, as well as greater ΔCF, $r = -0.24$, $p < .0001$, $n = 14{,}008$ pairs. Distance and ΔCF were positively related, $r = 0.42$, $p < .0001$, $n = 14{,}008$ pairs. As a result, we performed mixed-effects two-way ANCOVAs with distance as a covariate to determine whether the peak correlation coefficient differed between exposure groups while controlling for differences in inter-unit distance (**Fig. 4.3C**). As the distribution of peaks was positively skewed, we multiplied the data, originally on a 0-1 scale, by 100 and applied a natural logarithmic transform before statistical analyses. In Fig. 3C, the difference between back-transformed group means for each CF bin combination is depicted by a heatmap. The interaction was significant for A1, AAF, and VAF (mixed effects two-way ANCOVAs with Group and Combined CF Bin as factors and Distance as covariate: A1 $F(24,9632) = 5.35$, $p < .0001$, AAF $F(24,1407) = 2.16$, $p = .0009$, VAF $F(24,2730) = 1.88$, $p = .0060$). The simple main effect of Group was evaluated over each level of Combined CF Bin and the significant comparisons are outlined in bold on the heatmap in Fig. 3C. Distance remained a significant covariate in each ANCOVA (A1 $F(1,9650) = 358.29$, $p < .0001$, AAF $F(1,1406)$, $= 47.43$, $p < .0001$, VAF $F(1,2740) = 47.48$, $p < .0001$). From the heatmaps, we observed few significant differences in synchronization strength. Sound-exposed A1 and VAF tended to have

shorter cross-correlograms for most frequency combinations, with peak values being significantly smaller for low-to-medium frequency combinations only. In AAF, differences with respect to naïve animals were less consistent. Only synchronization between unit pairs where both units had CFs in the 38kHz bin was significantly greater.

The strength of synchronization can also be estimated by the width of the cross-correlogram, with wider functions representing greater synchronization at longer lag times. The width at half-height of each peak was compared between exposure groups as a function of CF bin (**Fig. 4.3D**). Width could not be computed for 38 pairs for which the function did not dip below half-height, resulting in 13,970 analyzed pairs. Width was found to weakly but significantly increase with inter-unit distance, $r = 0.03$, $p = .0009$, $n = 13{,}970$ pairs, and ΔCF, $r = 0.03$, $p = .0013$, $n = 13{,}970$. However, distance did not remain significant when included as a covariate for any field (mixed effects two-way ANCOVAs with Group and Combined CF Bin as factors and Distance as covariate. Effect of Distance: A1 $F(1,9651) = 0.22$, $p = .6381$, AAF $F(1,1403) = 0.003$, $p = .9545$, VAF $F(1,2739) = 0.41$, $p = .5239$). As a result, we removed the covariate and performed mixed-effects two-way ANOVAs. The interaction was significant for A1, AAF, and VAF (mixed effects two-way ANOVA with Group and Combined CF Bin as factors: A1 $F(24,9499) = 4.12$, $p < .0001$, AAF $F(24,1422) = 2.60$, $p < .0001$, VAF $F(24,2741) = 1.67$, $p = .0214$). The simple main effect of Group was evaluated over each level of Combined CF Bin and the significant comparisons are outlined in bold on the heatmap in Fig. 3D. In the heatmaps we observed clear wider cross-correlograms in the sound-exposed A1, AAF, and VAF. In A1, this trend showed units in low-to-mid frequency bins having wider cross-correlograms with units in the highest frequency bins. In AAF, almost every frequency bin combination tended to have wider cross-correlograms, with significant differences in the mid-to-high frequency

combinations, and notably with the 7kHz bin showing the greatest increase in width. Interestingly, VAF showed an opposite trend, where only the lowest frequency bins had significantly wider cross-correlograms when paired with the highest frequency bins. The mid-range bins, including 7kHz, showed either no change in width or a slight decrease in width for VAF.

A greater average cross-correlogram width could result from either more pairs with broad cross-correlograms or more pairs with off-centered peaks. To investigate the contribution of pairs with off-centered peaks to the wider cross-correlograms we observed in each field, we conducted mixed-effects two-way ANCOVAs with distance as a covariate on the absolute lag of the peak of the cross-correlogram (**Fig. 4.3E**). Distance was positively correlated with absolute lag, $r = 0.23$, $p < .0001$ $n = 14{,}008$ pairs. The interaction between exposure group and CF bin was significant for A1, AAF, and VAF (mixed effects two-way ANCOVA with Group and Combined CF Bin as factors and Distance as covariate: A1 $F(24,9499) = 4.12$, $p < .0001$, AAF $F(24,1422) = 2.60$, $p < .0001$, VAF $F(24,2741) = 1.67$, $p = .0214$). The simple main effect of Group was evaluated over each level of Combined CF Bin and the significant comparisons are outlined in bold on the heatmap in Fig. 3E. Distance remained a significant covariate for all three fields (A1 $F(1,9679) = 305.61$, $p < .0001$, AAF $F(1,1427) = 7.50$, $p = .0062$, VAF $F(1,2748) = 45.29$, $p < .0001$). The heatmaps revealed mostly increases in absolute lag for the Exposed group, suggestive of a greater number of off-centered peaks. However, in A1 and AAF, the CF bins with greater absolute lag did not correspond with those that showed the broadest widths in Fig. 3D. This suggests that a greater number of broadly synchronized unit pairs contributes to the wider cross-correlograms in these fields. In VAF, some CF bins with wider cross-correlograms

corresponded with bins that also had greater absolute lag, indicating a mixed contribution between broader synchronization and off-centered peaks.

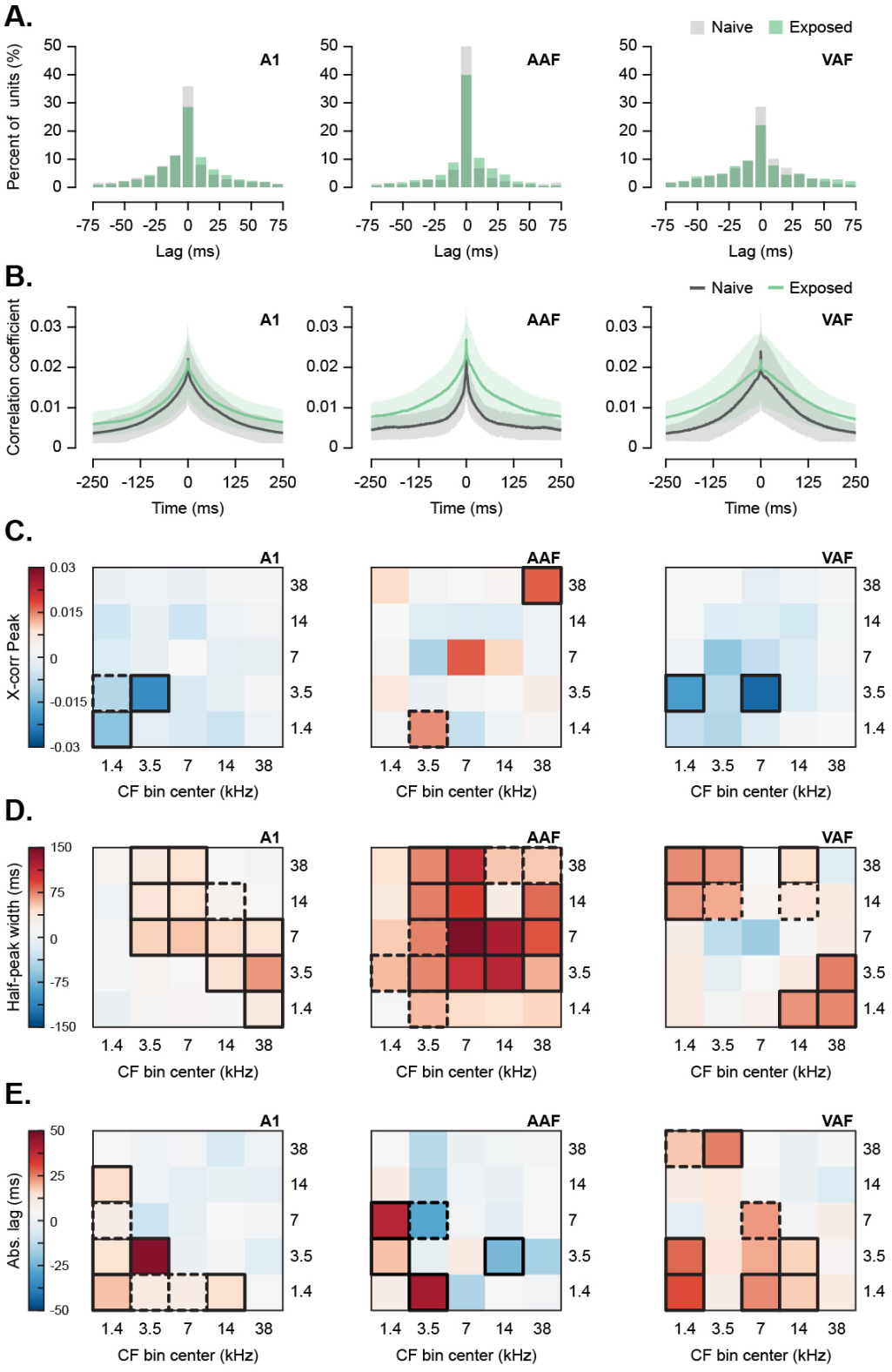


Figure 4.3: *Effect of sound exposure on spontaneous synchronization*. **A.** Histogram of lag times of the peak of the cross-correlogram for all recorded unit pairs in A1, AAF, and VAF. Data for lag times outside -75 and 75 are not shown. **B.** Average cross-correlogram for all unit pairs detected in separate channels in A1, AAF, and VAF. Shaded region represents SEM. **C.** Subtracted (Exposed – Naïve) difference between average peak correlation coefficient for unit pairs with CF in five frequency bins. **D.** Subtracted (Exposed – Naïve) difference between average half-peak width for unit pairs with CF in five frequency bins. **E.** Subtracted (Exposed – Naïve) difference between average time lag in absolute values of the peak of the cross-correlogram. Bolded boxes are significant with $p < .05$. Dashed boxes represent p values < .10. A1 – primary auditory field, AAF – anterior auditory field, VAF – ventral auditory field, PAF – posterior auditory field, CF – characteristic frequency. N unit pairs per auditory field and group: Naïve A1 3614; AAF 746; VAF 1092. Exposed A1 6116; AAF 733; VAF 1707. See Table 1 for number of rats, recording positions, and units per auditory field and group.

4.4.2 Behavioral correlates of sound exposure

A common behavioral measure for detecting tinnitus in rodents is gap-prepulse inhibition of the acoustic startle response (GPIAS), in which a short silent gap within a background sound carrier reduces the magnitude of a rodent's involuntary startle to a subsequent loud noise burst (Brozoski and Bauer 2016). Impaired GPIAS is considered evidence of tinnitus in rodents, meaning that the gap is less effective at reducing the startle response, possibly because the presence of tinnitus interferes with the ability to hear silence. This test is usually accompanied by a similar measure called prepulse inhibition (PPI) of the acoustic startle response. PPI has been proven useful in characterizing hyperacusis and hypoacusis, since a short tonal stimulus will either enhance or dampen inhibition of the startle response in rodents with hyper- or hypoacusis, respectively (Carlson and Willott 1996; Turner and Parrish 2008; Turner and Larsen 2016; Pienkowski 2018). To investigate whether sound exposure could have altered these behavioral measures, we performed GPIAS and PPI testing on two additional groups of naïve (Naïve-BEH, N = 12) and exposed (Exposed-BEH, N = 12) rats.

We found that the Exposed-BEH group did not differ from Naïve-BEH in GPIAS (**Fig. 4.4**). A schematic of the behavioral protocol for GPIAS is presented in **Fig. 4.4A**. We

hypothesized that a deficit in inhibition of the acoustic startle response would be specific to the 7kHz exposure frequency. To test this, we performed testing in the presence of either a 7kHz pure tone background or a 3.5kHz pure tone background with the order of testing counterbalanced between pairs. First, we confirmed that the magnitude of the response to the startle pulse alone was not significantly different between groups for either pure tone condition (7kHz: two-tailed paired t-test t(11) = -.81, p = .4372, Cohen's dav -0.32; 3.5kHz: two-tailed paired t-test t(11) = -.08, p = .9371, Cohen's dav -0.03, n = 12 pairs, **Fig. 4.4B,C bottom left**). Next, we computed the percent reduction in the startle response when the startle pulse was preceded by a silent gap. We found that the average reduction in startle did not differ between groups for either the 7kHz (one-tailed paired t-test t(11) = -0.45 p = .3303, Cohen's dav -0.22, n = 12 pairs, **Fig. 4.4B**) or the 3.5kHz (one-tailed paired t-test t(11) = 0.44 p = .6656, Cohen's dav 0.20, n = 12 pairs, **Fig. 4.4C**) condition. From these results, we concluded that sound exposure did not lead to behavioral evidence of tinnitus in any frequency tested.

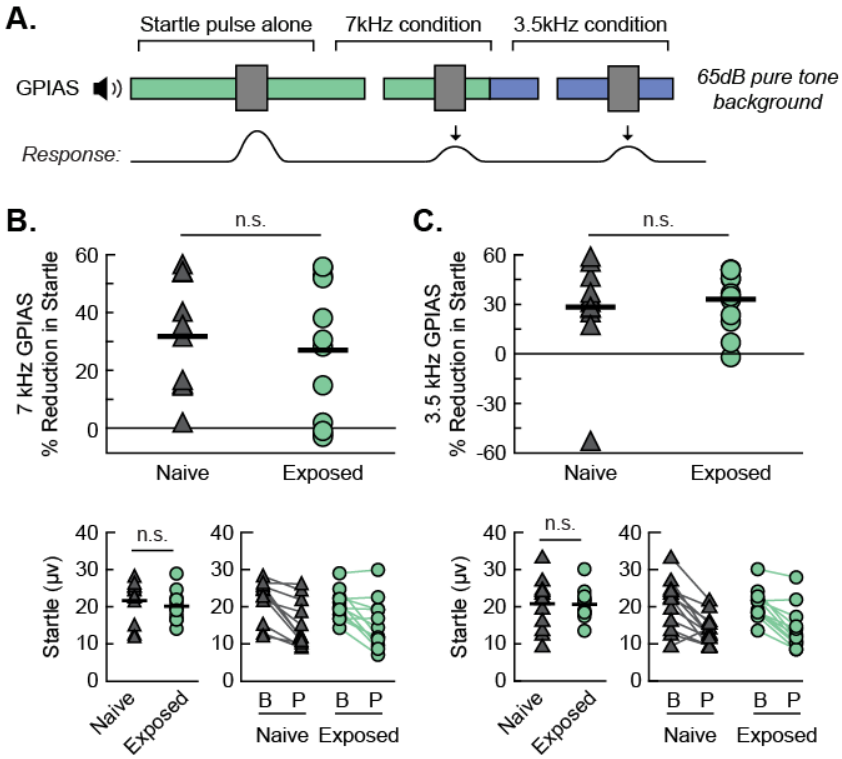


Figure 4.4: *Sound exposed rats demonstrate no change in GPIAS*. **A.** A schematic drawing of the behavioral protocol. Testing takes place in the presence of a 65dB SPL continuous pure tone (3.5 or 7kHz). A 30ms silent gap prepulse preceding the startle sound (40ms white noise burst, 120dB SPL) reduces the magnitude of the acoustic startle response. **B.** Percent GPIAS (Top), baseline startle response (Bottom Left), and comparison of startle response between Baseline (B) and Prepulse (P) trials (Bottom Right) in the presence of a 7kHz pure tone background. Lines connect responses from the same animal. **C.** Percent GPIAS (Top), baseline startle response (Bottom Left), and comparison of startle response between Baseline (B) and Prepulse (P) trials (Bottom Right) in the presence of a 3.5kHz pure tone background. Lines connect responses from the same animal. N.s. = not significant. N rats per group: 12 Naive, 12 Exposed. GPIAS = Gap-Prepulse Inhibition of the Acoustic Startle reflex.

We observed an enhancement in PPI for the Exposed-BEH group when the prepulse was a 7kHz tone (**Fig. 4.5**). A schematic of the behavioral protocol for PPI is presented in **Fig. 4.5A**. Of note, a magnified response to the startle pulse alone is also sometimes taken as evidence of hyperacusis (Chen et al. 2013), but we hypothesized that an improvement in inhibition of the acoustic startle response would be specific to the 7kHz exposure frequency. As a result, we performed PPI testing using either a 7kHz or 3.5kHz pure tone prepulse with the order of 7kHz prepulse, 3.5kHz prepulse, and no prepulse trials randomized within a single testing session. Testing took place in the presence of a 65dB white noise background. We observed that the magnitude of the response to the startle pulse alone was not significantly different between groups (paired t-test $t(11) = -.21$, $p = .8343$, Cohen's d_{av} -0.09, n = 12 pairs, **Fig. 4.5B bottom left**). Next, we compared the average percent reduction in the startle response when the startle pulse was preceded by a prepulse tone. We found that the average reduction in startle was significantly greater for the Exposed-BEH group when the prepulse was a 7kHz tone (one-tailed paired t-test $t(11) = 2.69$ $p = .0105$, Cohen's d_{av} 0.63, n = 12 pairs, **Fig. 4.5B**) but not when the prepulse was a 3.5kHz tone (one-tailed paired t-test $t(11) = 0.66$ $p = .2621$, Cohen's d_{av} 0.29, n = 12 pairs, **Fig. 4.5C**) condition. Our positive findings remained significant when adjusting the alpha value to account for three comparisons using either the Bonferroni or Holms-Bonferroni

correction (both $\alpha = 0.0167$). From these results, we concluded that Exposed-BEH exhibited behavioral evidence of hyperacusis for the 7kHz frequency.

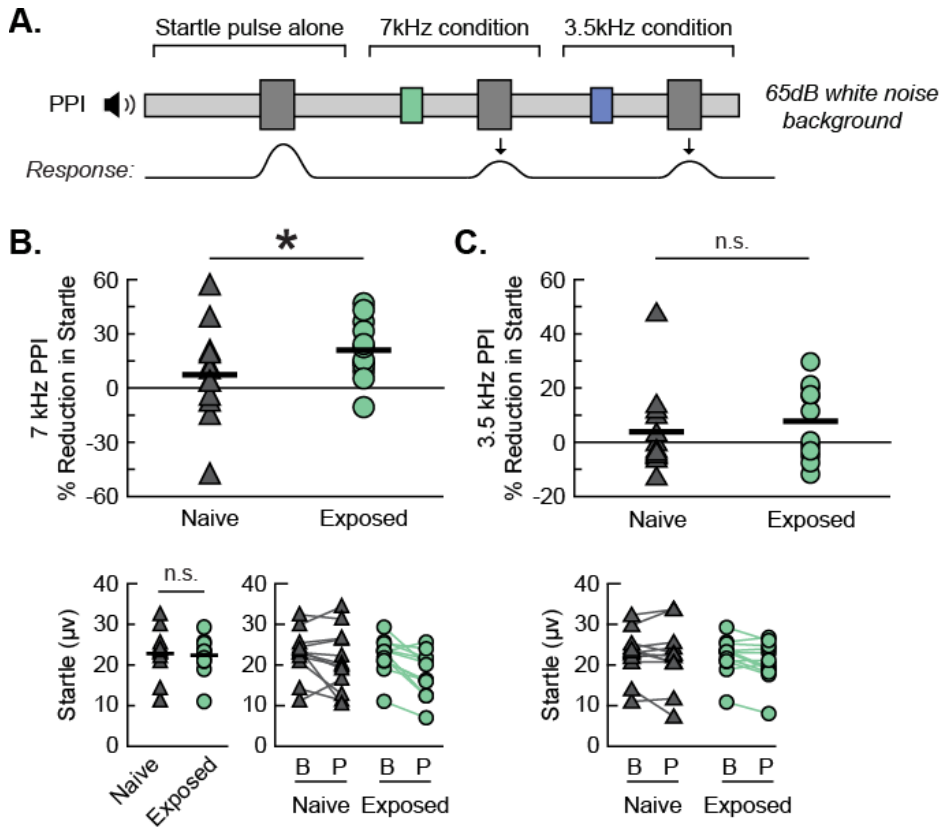


Figure 4.5: *Sound exposed rats demonstrate a frequency-specific enhancement in PPI*. **A.** A schematic drawing of the behavioral protocol. Testing takes place in the presence of a continuous 65dB SPL background noise. A 20ms tone pip (3.5 or 7kHz, 75dB SPL) preceding the startle sound (40ms white noise burst, 120dB SPL) reduces the magnitude of the acoustic startle response. **B.** Percent PPI (Top), baseline startle response (Bottom Left), and comparison of startle response between Baseline (B) and Prepulse (P) trials (Bottom Right) when the prepulse is a 7kHz tone. Lines connect responses from the same animal. **C.** Percent PPI (Top) and comparison of startle response between Baseline (B) and Prepulse (P) trials (Bottom) when the prepulse is a 3.5kHz tone. Lines connect responses from the same animal. Note that the same baseline startle values were used for computing PPI in B and C. * = $p < .05$, n.s. = not significant. N rats per group: 12 Naive, 12 Exposed. PPI = Prepulse Inhibition of the acoustic startle reflex.

4.5 Discussion

Passive exposure to moderate-intensity broadband white noise can be used to open a CP window for frequency tuning in the adult rat auditory cortex, allowing for subsequent frequency-specific reorganization of the tonotopic map. This phenomenon could have profound implications for plasticity-based neurotherapeutics that aim to improve learning and memory or treat disorders of plasticity through non-invasive means. However, frequency-specific tonotopic map expansions and regional changes in excitability have also been described as symptoms of tinnitus and hyperacusis in animal models, leading us to wonder whether noise exposure could increase the risk of developing one or both of these disorders. In the present investigation, we extended previous studies by examining the effects of noise and tone pip exposure on secondary auditory fields and carried out novel experiments to determine whether sound-exposed animals display evidence of tinnitus or hyperacusis.

As in previous studies (Zhou et al. 2011, Thomas et al. 2019), we observed map expansion in the A1 of adult rats passively exposed to moderate-intensity broadband white noise followed by tone pips with no elevation in cortical thresholds. We also showed for the first time that a CP-like window is also opened in VAF as demonstrated by map expansion in this field accompanied by broader receptive field bandwidths for 7kHz-tuned neurons. Apart from map expansion, however, we observed few changes in spontaneous activity or auditory processing in the A1 and VAF of exposed animals. In contrast, we observed strong evidence of hyperactivity in AAF, where there was no map expansion. This included an overall increased spontaneous firing rate, stronger tone-evoked firing rates and narrower receptive field bandwidths for a range of frequencies, and a lower cortical threshold for 7kHz-tuned neurons. Despite changes in AAF affecting multiple iso-frequency bands, the band corresponding to 7kHz showed changes

consistent with heightened sensitivity in all of our measures. Our behavioral results also pointed to enhanced sensorimotor gating for the 7kHz frequency, since exposed rats had improved PPI when the prepulse was a 7kHz pure tone. Taken together, our findings point to a potential hyperacusis for the 7kHz frequency in sound-exposed animals.

We expected hypersynchronization to accompany map expansion given the close link between receptive field overlap and neural synchronization (Noreña and Eggermont 2006; Eggermont 2007; Kilgard et al. 2007). However, we did not observe clear hypersynchronization in any field. The absence of this relationship could be due to the unique manner in which noise induces plasticity. Noise exposure on its own produces lasting desynchronization with shorter cross-correlogram peaks in A1 (Zhou et al. 2011; Kamal et al. 2013; Thomas et al., 2019). The prevalence of lower peaks and broader cross-correlogram widths observed in A1 and VAF could be consistent with these earlier findings, assuming partial recovery of desynchronization potentially hastened by tone pip exposure. The broader widths that we observed in AAF centered on 7kHz are likely the combined result of greater disinhibition and increased firing, since secondary effects independent of connectivity can also affect the width of the cross-correlogram. These include firing patterns intrinsic to each neuron, such as burst firing, and global oscillations (Eggermont and Smith 1996; Nowak and Bullier 2005). Although cross-correlograms were normalized with respect to firing rate and significant differences in burst firing measured by CV were not observed, a higher firing rate could increase the impact of secondary effects on cross-correlogram width. Clear evidence of disinhibition or changes in firing were not observed in A1 or VAF in the present study, therefore assumptions about the origin of broader cross-correlogram widths beyond residual effects of noise exposure remain speculative.

The role of the auditory cortex in generating tinnitus and hyperacusis has not been fully established. Although changes in neural activity related to hearing loss begin in the auditory nerve and cochlear nucleus, individuals with clinically normal audiograms can also report these percepts, and electrophysiological signatures of each condition have been reported in cortex in animal models. In addition, studies have primarily identified A1 and the auditory thalamus (medial geniculate body, MGB) as sites of experience-dependent plasticity following non-traumatic passive sound exposures (Pienkowski and Eggermont 2011; Lau et al. 2015; Pienkowski 2018), revealing a possible mechanism by which passive experience could lead to changes in auditory processing in the absence of hearing loss. Here, we observed a significant difference between the electrophysiological response properties of A1 and AAF following noise exposure that may suggest a causal role for AAF in the generation of hyperacusis. Whereas A1 has been studied extensively in the context of passive sound exposure, much less is known about how AAF adapts to such experiences. Sparse findings demonstrate asymmetric plasticity in each field despite both receiving direct inputs from the ventral MGB and displaying similar tone-evoked response properties (Polley et al. 2007). Takahashi and colleagues (2006) found that A1 responses of juvenile mice were more potentiated than those of AAF following exposure to an amplitude-modulated tone for 4-5 weeks but did not observe an over-representation of the exposure frequency in either field. A recent study documented differences in parvalbumin positive (PV+) interneuron and peri-neuronal net (PNN) densities in A1 and AAF following 70dB SPL broadband noise exposure in mice during the first month of life (Reinhard et al 2019). Noise exposure decreased the density of PNNs in A1 but not AAF, showing that inhibitory elements can be differently regulated across these two fields. Given the preliminary nature of our

study, further studies should be undertaken to understand the potential mechanisms by which AAF could contribute to hyperacusis.

The sound exposure paradigm used to induce 7kHz map expansion consisted of two distinct components: white noise exposure and 7kHz tone pip exposure. On its own, chronic exposure to moderate-intensity white noise has been shown to lead to tonotopic disorganization, broadened receptive field bandwidths, decreased neural synchronization, and disrupted temporal processing. These plastic changes develop whether the noise is present for 2 weeks (Thomas et al. 2019), 6-8 weeks (Zhou et al. 2011; Kamal et al. 2013), or on a 10-hour-per-day schedule (Zhou and Merzenich 2012). As long as the noise is broadband, its effects are non-frequency-specific as illustrated by comparison with band-limited noise exposure (de Villers-Sidani et al. 2008). In the present study, the most prominent electrophysiological measure that was affected in a non-frequency-specific manner was the increased spontaneous firing rate in AAF, and it is possible that this change was driven primarily by white noise exposure. Exposure to non-traumatic white noise has been scarcely studied in the context of PPI or GPIAS, especially in contrast to traumatic noise exposures. One exception is a recent study that found that band-limited noise exposure did not produce either hyper- or hypoacusis in mice exposed for three months (Pienkowski 2018).

Taken alone, exposure to pure tones has not been shown to induce strong cortical plasticity leading to map expansion or altered discrimination abilities for the exposure frequency in adult rodents (Zhou et al. 2011; Blundon et al. 2017). This is consistent with the view that the mature cortex is largely resistant to change based on passively experienced stimuli (Keuroghlian and Knudsen 2007). However, extensive research performed in cat auditory cortex has shown convincingly that band-limited tone pip ensembles can lead to frequency-specific changes in

auditory responsiveness after chronic exposure (Pienkowski and Eggermont 2009; Pienkowski and Eggermont 2010; Pienkowski et al. 2011). Specifically, cortical regions tuned to the exposure frequency range show reduced responsiveness while cortical regions outside the exposure frequency range show increased responsiveness. This suggests that there could be detectable differences in the electrophysiological properties of the auditory cortex after tone pip exposure that could also alter behavioral responses to PPI or GPIAS. However, extrapolating from these results one would expect animals exposed to 7kHz to display hypoacusis for this frequency. Consequently, we do not believe that tone pip exposure on its own would cause increased cortical sensitivity to 7kHz.

A limitation of the present study is that in the interest of reducing the number of animals used, the electrophysiological data for the Exposed group came from combining two groups of sound-exposed animals that underwent slightly different 7kHz exposures (i.e. 7kHz tone pips vs. 7-8.3kHz tone pip clouds. See Methods). It is likely that these exposures would produce different electrophysiological signatures. For example, we would expect tone pip clouds to lead to map expansion for a broader frequency range. To account for this, we used relatively coarse (≥ 1 octave) CF bins in our analysis so that the 7kHz bin spanned 5-10kHz and presumably encompassed all neurons that would have shifted their CFs to the exposure frequencies. However, the combined group contained a greater number of rats exposed to tone pip clouds (n = 9) than tone pips (n = 4), so it is possible that the average data is a better representation of the tone pip cloud exposure. The Exposed-BEH group, on the other hand, was not heterogeneous; every rat was exposed to the tone pip stimulus. As a result, the Exposed group used for electrophysiology is not a perfect analogue for the Exposed-BEH group. Furthermore, because the animals used for behavioral testing in our study were not the same animals that were used for

electrophysiological recording, we were unable to correlate auditory response properties with PPI or GPIAS. This additionally prevents us from making any direct conclusions about cortical properties, such as degree of map expansion, that may have influenced inhibition of the acoustic startle response.

A second limitation is that classifying auditory fields based purely on functional characteristics will always result in an imperfect classification. It is possible that some cortical sites, especially those on borders with CF gradient reversals (such as A1 and AAF), were misclassified as being in neighboring fields. Of note, we were unable to distinguish VAF and SRAF based purely on functional properties and therefore pooled the data from these fields. These challenges are not unique to our study, and there is precedence for pooling VAF and SRAF with sparse datasets (Takahashi et al. 2011). Without accompanying anatomical tracer data or similar, conclusions about the response properties of any auditory field should only be drawn from multiple independent replications. Importantly, an experimenter blind to the identity of the experimental groups performed field classification for the present study. The average response properties reported for each field in Supplementary Fig. 1 are in strong agreement with the published literature on the adult rat auditory cortex (Polley et al. 2007; Profant et al. 2013).

Finally, stress is another factor that could have played a role in our results, as it is known to affect PPI (Guercio et al. 2014). Importantly, chronic noise exposure, even at moderate intensities, is a known stressor for humans and animals and has a complex interplay with tinnitus, mostly exacerbating its symptoms (Eggermont 2017a). The chronic sound exposures used in our study could have caused stress that could affect the acoustic startle response or PPI. The main argument against this, however, is that we did not observe differences in baseline

startle response between exposed and unexposed animals. Furthermore, any stress-induced differences in PPI or GPIAS would likely not have been specific to the 7kHz frequency.

In summary, our study examines the phenomenon of noise-induced map expansion and demonstrates that prolonged exposure to moderate-intensity noise could be considered a risk factor for hyperacusis in adulthood. Our results could have implications for noise levels presently deemed 'safe' in occupational, private, and public settings (Pienkowski and Eggermont 2012; Gourévitch et al. 2014; Eggermont 2017b). Rather than suggesting that noise exposure should not be used for neurotherapeutic purposes, however, we would urge continued investigation into this subject. For one, sensorimotor gating measured by PPI is impaired in some neuropsychiatric disorders, most notably schizophrenia (Swerdlow et al. 2000; Swerdlow and Light 2018). Noise-induced map expansion could thus be a way to target and reverse this specific preattentional deficit (Braff and Light 2004). Additional candidate strategies to drive plasticity in a sensory-specific manner are vagus nerve stimulation paired with the presentation of pure tones (Engineer et al. 2011) and cognitive training programs designed to improve basic sensory processing (Cramer et al. 2011; Merzenich et al. 2014). Exciting or inhibiting specific brain areas through sensory experience is a more targeted and non-invasive means of driving plasticity than purely pharmaceutical strategies such as those presently used in the treatment of schizophrenia (Guercio et al. 2019) and dementia (Farlow and Cummings 2007, Massoud and Léger 2011). By focusing on 'retuning' cortical maps, sensory deprivation or stimulation paradigms in other systems could potentially be developed to treat or reverse symptoms of sensory disorders such as phantom sensations or chronic pain (Flor et al. 2001, Tabot et al. 2015). Through both electrophysiological and behavioral measures, the results of our study suggest that map expansion induced by passive sound exposure opens windows of plasticity that can also be

understood as windows of vulnerability. However, as our understanding of the rules that regulate plasticity and the opening and closure of critical periods progresses, our hope is that we will one day be able to harness them to treat a variety of brain disorders.

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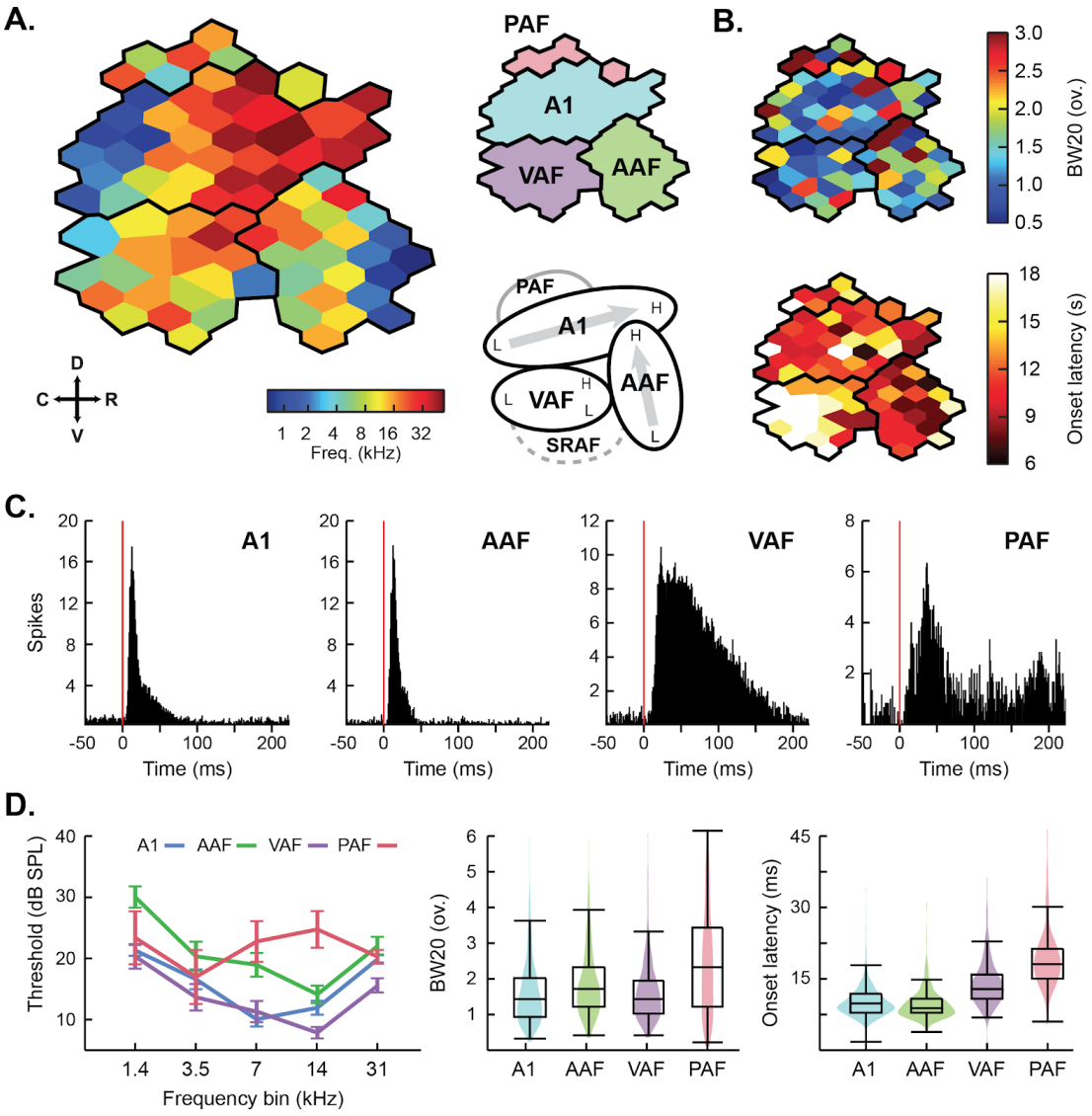
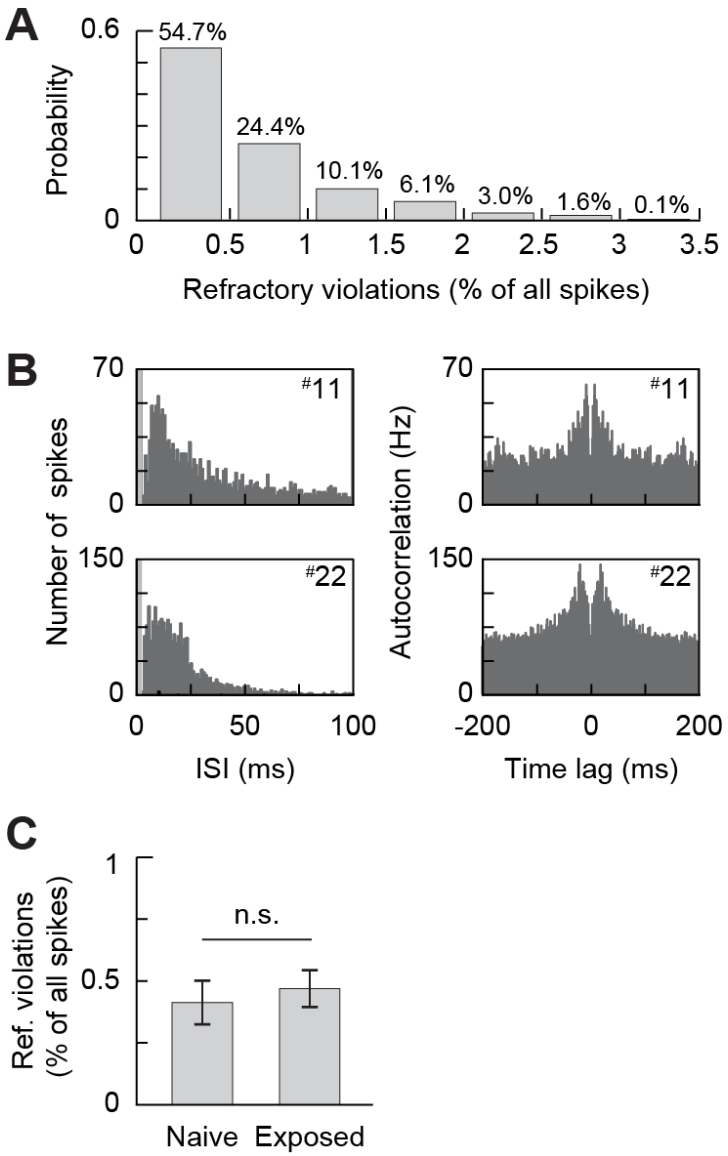
4.7 Supplementary figures



Supplementary Figure 4.1: *Classification of multiple fields within the rat auditory cortex*. **A.** Example of auditory field classification for a tonotopic map from a representative rat with separate fields outlined in black (L = Tuning to low sounds, H = Tuning to high sounds). SRAF was not distinguishable from VAF in our data, so the presumed location of SRAF is represented by a dotted line. **B.** Receptive field bandwidths 20dB SPL above threshold (top) and onset latencies (bottom) for the same rat as in A. **C.** Average tone-evoked peri-stimulus time histograms (PSTH) for each field from a single rat. N sites per field: 26 A1, 7 AAF, 11 VAF, 2 PAF. **D.** Average receptive field threshold (left), distribution of receptive field bandwidth 20dB SPL above threshold (middle), and distribution of onset latency (right) for 24 rats. N sites per field: 701 A1, 292 AAF, 342 VAF, 174 PAF. A1 = primary auditory cortex, AAF = anterior auditory field, VAF = ventral auditory field, PAF = posterior auditory field, SRAF = suprarhinal auditory field.



Supplementary Figure 4.2: *Quality assessments for spike sorting*. A. Histogram of the fraction of refractory period violations out of all spikes for 2017 identified clusters in A1, AAF, VAF, and PAF. B. Representative histograms for two isolated units (#11 and #22) of inter-spike interval (ISI) times (left) and autocorrelation functions (right). Bin widths are 1ms and gray shaded line represents the 2ms refractory period bin. C. Mean fraction of refractory period violations out of all spikes for experimental groups. No difference was found between groups using a mixed-effects test $F(1,41.13) = 0.63$, $p = .4297$, $n = 2017$ units within 45 positions and 24 rats. Error bars represent S.E.M. Number of animals, recording positions, and units per group: Naïve 11,20,884, Exposed 13,25,1133.

Chapter 5: General Discussion

The studies presented in the preceding chapters investigate the ever-present interaction between the brain and its sensory environment. In each chapter, the random acoustic signal white noise was used to probe the impact of chronic periods of uninformative and disruptive sensory stimulation on mature cortical function. The results of this thesis refine current theories about how such experiences could result in robust plasticity in the adult brain and raise questions about the perceptual and functional significance of this capacity for the organism. As the results of each chapter are discussed extensively in their respective Discussion sections, the following section will review the major conclusions of this body of work, consider additional limitations, and discuss the implications of its findings for human health and brain function.

5.1 Summary of the findings

Prior to the undertaking of this thesis, only a sparse number of studies had examined the plasticity-inducing role of prolonged exposures to moderate-intensity white noise. Drawing parallels between noise exposure and earlier studies of sensory deprivation or deafferentation, Zhou and colleagues (2011) suggested that "…the absence of patterned activity, rather than a decrease of activity per se, may be the key for reinstating plasticity in the adult cortex," but this idea remained to be directly tested. The primary contribution of Study 1 was thus to establish whether noise exposure drives plasticity by reducing the amount of patterned inputs reaching the brain. Through contrasting exposures to unmodulated and modulated noise, it was demonstrated that only exposure to unmodulated noise is capable of inducing CP-like plasticity despite the homogeneous masking of spectrotemporal inputs by both stimuli. This finding implies that

noise-induced plasticity is not a unique phenomenon, but rather a consequence of reducing correlated activity in the cortex that could be elicited by 'noise' in other sensory modalities as well. These results also point to the existence of a mechanism that continuously monitors the signal-to-noise ratio in the brain, triggering CP-like plasticity after chronic periods of uncorrelated activity. Potential candidates for this mechanism are the distinct classes of inhibitory interneurons, which are sound responsive and each uniquely poised to react to and control levels of excitation in the cortex (Moore and Wehr 2013; Li et al. 2014; Takesian et al. 2018). Study 1 further extended studies of noise exposure by assessing PV+ and SST+ cellular activity after different durations of noise exposure. These experiments corroborated previous findings that implicate reduced PV+ cell activity in experience-dependent plasticity following both unmodulated (Kamal et al. 2013) and pulsed noise exposure (Zhou and Merzenich 2012) and contributed new evidence that modulated inputs may limit the extent of this plasticity by recruiting SST+ neurons.

A strong motivation for studying noise-induced plasticity is the ability to recover youth-like plasticity in the adult auditory cortex, which has clear implications for learning and memory. In Study 2, the hypothesis that perceptual learning for a specific frequency could be improved by modifying the adult rat tonotopic map with sound exposure was tested, marking the first attempt to understand the perceptual consequences of map expansion following noise-induced plasticity. Instead of improving learning, however, it was observed that the early induction of map expansion led to impaired frequency discrimination. In conjunction with other studies, this finding lends credence to the view that the mode of induction (e.g. sound-driven vs. neuromodulatory-driven) of map expansion plays a key role in determining the perceptual outcome of this plasticity (Pienkowski and Eggermont 2011). Despite not confirming the initial

hypothesis, Study 2 provided encouraging evidence that training reversed electrophysiological measures of reduced discriminability. This supports a number of studies that show that cognitive training strategies may be effective in the treatment of auditory processing disorders (Merzenich et al. 1996; Zhou and Merzenich 2007; Merzenich et al. 2014; Mishra et al. 2014).

Another possible consequence of prolonged sound exposure is the potential for maladaptive plastic changes. Study 3 aimed to further uncover the perceptual consequences of noise-induced map expansion by assessing symptoms of tinnitus and hyperacusis following noise and tone pip exposure. This study established that sound-exposed rats exhibited electrophysiological and behavioral evidence of hyperacusis, but not tinnitus. The main impact of these findings was to link noise-induced plasticity and hyperacusis for the first time and to implicate the anterior auditory field in the generation of this disorder. In conjunction with the findings of Studies 1 and 2, these results illustrate the potential negative consequences of reopening CP windows in adulthood. Altogether, the work presented in this thesis underlines the important and continuous role of high-fidelity sensory experience for mature cortical function.

5.2 Limitations

While the individual limitations of each study were discussed in their respective chapters, the following section considers additional limitations that are relevant to all three studies.

5.2.1 Role of anesthesia

The electrophysiological recordings obtained for this thesis were carried out under isoflurane anesthesia. Isoflurane is widely used in neuroscience research because it gives researchers the ability to quickly and easily control the depth of anesthesia. However, a concern

with any anesthetic agent is that findings obtained in anesthetized animals won't generalize to awake animals. Like other anesthetics, isoflurane is known to reduce excitation and enhance inhibition (Alkire et al. 2008). In the auditory cortex, this has the effect of prolonging the onset latency and increasing minimum response thresholds to sound in both rat and cat (Cheung et al. 2001; Ruebhausen et al. 2012; Noda and Takahashi 2015). Furthermore, isoflurane has been documented to broaden receptive field bandwidths and increase neural synchrony in rat A1 (Noda and Takahashi 2015). For this reason, care was taken to maintain a similar depth of anesthesia for all groups of animals in all experiments, although group differences in this respect could not be examined. Importantly, the results of Study 1 replicated the findings of Zhou and colleagues (2011), which were performed under pentobarbital anesthesia, lending credibility to the findings of both studies. Furthermore, the CF of individual neurons and coarse tonotopic mapping have been repeatedly shown to not be affected by various anesthetized or awake states (Cheung et al. 2001; Schumacher et al. 2011; Noda and Takahashi 2015), allowing for strong confidence in the findings related to tonotopic organization, which represent a majority of the results.

5.2.2 Role of stress

Stress is known to modulate cortical plasticity through multiple neurotransmitter and endocrine systems, having widespread effects on learning and memory (McEwen 2000; Radley and Morrison 2005). In the auditory system, stress has been shown to impair acoustic learning (Dagnino-Subiabre et al. 2005) and in Study 3 the special role of stress in the generation and modulation of tinnitus was discussed. Since environmental noise is known to cause stress in both animals and humans (Geber et al. 1966; Staples 1996), a primary question pertaining to the

results of this study would be whether the effects of noise-induced plasticity could be accounted for by stress alone. A previous study of 82dB continuous white noise exposure maintained for 40 days in adult rats found that the effects of noise on long-term potentiation and long-term depression were the opposite of those observed in established animal models of stress, and concluded that stress did not have a major impact on thalamocortical plasticity in their animals (Speechley et al. 2007). As an assay for stress, Zheng (2012) analyzed animals' behavioral patterns during operant training after four weeks in a 65dB sound environment and additionally did not find differences between exposed and naïve rats. These findings would suggest that the substantially shorter two-week duration of noise exposure at 70dB used here would not elicit stressful conditions strong enough to confound electrophysiological or behavioral results. Furthermore, in Study 3 there was no effect of sound exposure on the baseline acoustic startle response, a measure easily affected by stress. Apart from this, however, stress was not directly measured in the studies presented here. A full understanding of noise-induced plasticity would certainly be improved by more thoroughly investigating its link with stress.

5.2.3 Role of sex

Exclusively female rats were used for the experiments contained within this thesis. Since sex differences in basic auditory processing, higher cognitive processing, and the modulation of plasticity regulators are known to exist (McFadden 1998; P. Voss et al. 2017), a point of concern might be that the findings presented within this thesis do not generalize to both sexes. During traumatic sound exposure, female rodents have been shown to be significantly protected from the effects of noise-induced hearing loss when compared to males (Willott and Bross 2004; Milon et al. 2018), possibly due to the neuroprotective effects of estrogen (Wise 2002). At present, sex

differences in experience-dependent plasticity following non-traumatic sound exposure have not been explicitly investigated. Importantly, noise-induced plasticity has been documented in both male (Zheng 2012) and female (Zhou et al. 2011; Kamal et al. 2013) rats with largely concordant findings. Overall, the role of sex differences in many aspects of neuroscience research has been underexplored (Shansky and Woolley 2016) and future studies of noise-induced plasticity would benefit from including both male and female animals and reporting any differences that emerge.

The sex of the experimenter can also have an effect on behavioral measures when conducting studies with rodents (Sorge et al. 2014). In Study 2, behavioral training took place on average over a period of 12 weeks with more than 60 one-hour training sessions per rat. Both male and female experimenters transferred rats from their home cages to the behavioral training boxes over the course of this period, however this information was not recorded. Over such long durations of training, it is not expected that the sex of the experimenters would have influenced overall performance on the adaptive tone discrimination task, because the rats would have ample time to become accustomed to the demands of the experiment, in contrast to a task in which measures are collected during a single session. In Study 3, behavioral testing of the acoustic startle response did take place during single testing sessions. One female and one male experimenter performed all behavioral testing together and rats were acclimatized in a room where both experimenters were present before testing.

5.3 Future directions

The studies presented in this thesis demonstrate the broad range of questions that can be asked using the relatively simple technique of white noise exposure, and should encourage the

continued investigation of this phenomenon. In this section, a selection of pertinent questions that remain about noise-induced plasticity are discussed.

5.3.1 What are the mechanisms of tonotopic map expansion following noise exposure?

CP plasticity induced by noise is quantitatively and qualitatively different from typical adult experience-dependent plasticity. This is evident in the ability for an uninformative and repetitive stimulus (e.g. 7kHz pure tone) to become over-represented in the tonotopic map. A still unanswered question is how a frequency can become over-represented following this type of experience. I would like to propose that the answer lies in altered stimulus-specific adaptation during heightened states of plasticity. Stimulus-specific adaptation (SSA) is the reduction of neural responses to a common, repeated stimulus but not to other, rare stimuli (Nelken 2014). Our group has observed that impaired SSA and the capacity for CP-like plasticity are present in both noise-exposed adult rats and aged rats (Kamal et al. 2013; Cisneros-Franco et al. 2018) suggesting that reduced SSA might be a common mechanism for uninformative stimuli to have strong effects on tonotopic plasticity later in life. This inability to adapt to uninformative stimuli is likely regulated by inhibitory cortical circuits, and further unpublished work by our lab has demonstrated that reducing PV+ cell activity for one week using chemogenetics is sufficient to impair SSA and induce CP-like plasticity in the adult rat A1 (Cisneros-Franco and de Villers-Sidani, In preparation). Future experiments should establish whether a similar mechanism is present in noise-induced plasticity.

5.3.2 Does noise-induced plasticity exist in other sensory systems?

Despite the use of acoustic noise in the present experiments, noise can be understood as a random signal and is therefore present in other sensory modalities as well. It would be interesting

to know whether CP-like plasticity could be reinstated in other sensory cortices using equivalent stimuli, and if so, whether the mechanism is the same or different. In 2001, Chichilnisky introduced a white noise stimulus for the visual domain, which could in theory be used to test the effects of noise exposure on the visual cortex. The stimulus looks like a "flickering coloured checkerboard pattern with no spatial, temporal, or chromatic structure" (Chichilnisky 2001 page 201). Each square of the checkerboard changes in contrast and color in a random and independent sequence, thus mimicking the key features of white noise in the spatial, chromatic, and temporal domains. It would be relatively trivial to present such a stimulus during short-term experiments, in which an animal is fixed. However, assessing its impact over prolonged durations requires more creative methods of stimulus presentation. A number of experiments have accomplished long-term exposure to altered visual stimuli, most notably in the study of visual cortex orientation maps, using opaque striped goggles (Stryker et al. 1978), striped cylindrical housing (Sengpiel et al. 1999), or cylindrical lenses (Tanaka et al. 2006; O'Hashi et al. 2007; Tanaka et al. 2009; Yoshida et al. 2012) in cats, mice, and rats. With technology available today, I propose that electronic goggles or cylindrical environments displaying a continuously changing image, such as the stimulus introduced by Chichilnisky, could be used to study the effects of long-term exposure to visual noise in adult rodents or cats. These efforts could determine whether noise-induced plasticity is indeed present across sensory domains.

5.3.3 Could sensory 'noise' contribute to dysregulated plasticity in aging?

Commonalities between noise exposure and aging have led our group to propose that an increase in sensory noise may contribute to dysregulated plasticity in the aged brain (Kamal et al. 2013; Patrice Voss et al. 2017; Cisneros-Franco et al. 2018). Age-related deficits in auditory

processing such as trouble understanding speech in noisy environments are thought to originate from age- or trauma-related deterioration of the auditory periphery (Vasama and Mäkelä 1995; Caspary et al. 2005; Caspary et al. 2008), although deficits may also be present even when hearing thresholds are normal (Mendelson and Ricketts 2001; Tremblay et al. 2003; Harris and Dubno 2017). Studies of induced hearing loss in adult animals have revealed that peripheral damage leads to reduced inhibition in the central auditory pathway and increased cortical plasticity (Robertson and Irvine 1989; Schwaber et al. 1993; Irvine et al. 2000; Syka 2002; Eggermont 2017). We have additionally shown that inhibitory elements including GABAergic interneuron populations, myelin-associated proteins, and peri-neuronal nets are downregulated with normal aging, corresponding to an increase in functional plasticity and unstable frequency representations in A1 (Kamal et al. 2013; Ouellet and de Villers-Sidani 2014; Cisneros-Franco et al. 2018). Increasing inhibition with diazepam, a GABA-A receptor agonist, is sufficient to restore frequency representation stability in aged rats (Cisneros-Franco et al. 2018). Our earlier results (Kamal et al. 2013) combined with the studies described in this thesis show that the structural and functional changes observed in aging are mirrored in the noise-exposed A1, yet with an absence of age-related peripheral damage. Together, these findings might suggest that degraded or 'noisy' auditory inputs regardless of origin could contribute to reduced inhibition and dysregulated plasticity in the mature brain, potentially hastening age-related changes in central auditory processing. In support of this, a small number of studies have documented impairments in perceptual discrimination, concentration, memory, and attention in humans exposed to either short or chronic durations of low-frequency noise (Gomes et al. 1999; Pawlaczyk-Łuszyńska et al. 2005). Encouragingly however, our work also suggests that since central auditory changes resulting from noisy inputs are plastic in nature, they may be inherently

reversible (Study 2). Increasing inhibition in the aged brain or during periods of sensory deprivation, hearing loss, over-stimulation, or noise exposure may be a viable strategy for preventing maladaptive plastic changes due to altered sensory experience.

5.4 Implications

The findings of the present thesis highlight the profound impact of altered sensory experience on adult brain function with implications at the individual, environmental, and societal levels. For individuals, this work provides evidence that high-fidelity sensory inputs are supportive of perceptual and cognitive function. As a result, care should be taken to prevent and correct sensory deficits as much as possible. This is especially relevant with respect to noise-induced and age-related hearing loss, which together represent a public health problem that is estimated to affect 20% of adults aged 19 to 79 in Canada (2012 and 2013 Canadian Health Measures Survey). Furthermore, hearing loss has been identified as a major risk factor for dementia (Peters et al. 1988; Livingston et al. 2017; Thomson et al. 2017). Improving auditory inputs through the use of hearing aids may slow the rate of cognitive decline and improve quality of life in seniors (Amieva et al. 2015; Maharani et al. 2018) as does improving visual perception through increasing room brightness and contrast for patients with dementia (Shikder et al. 2012). The present research may be helpful in understanding losses of sensory function as factors that increase neural noise, providing altered sensory information to downstream brain areas that may impact cognitive functioning (Voss et al. 2018).

At the environmental level, the evidence presented in this work is unambiguous: prolonged exposure to noise has potentially harmful effects on adult cortical plasticity even at levels typically deemed safe for hearing. The U.S. National Institute for Occupational Safety and

Health recommends that occupational noise exposure not exceed an 8-hour time-weighted average of 85 dB per day (NIOSH, 1998), yet a significant body of animal studies have shown negative effects on cortical representations, gap detection, pitch discrimination, and temporal processing at sound levels much lower than that (Zheng 2012; Zhou and Merzenich 2012; Kamal et al. 2013; Jiang et al. 2015). It is clear that the full extent of the impact of low-grade noise exposure on perceptual and cognitive functioning remains to be determined. By leveraging these insights, the present thesis should inform a better understanding of our modern lives, from designing our architectural and city spaces to be more conscious of the negative effects of noise exposure, to informing policy that recognizes the importance of understanding of noise pollution as a matter of public health (Gourévitch et al. 2014; Attarha et al. 2018).

Finally, it is also useful to understand the work presented in this thesis at the sociological level. Individuals who live or work in noisy environments have an increased risk of experiencing the negative health outcomes of such deleterious exposure. Both day and nighttime noise pollution are more prominent in neighborhoods that are near highways, airports, railroads, or industrial zones (Goines and Hagler 2007; Zuo et al. 2014), and occupational noise reaching hazardous levels is most likely to be encountered in the industries of construction, mining, agriculture, manufacturing, utilities, transportation, and in the military (May 2000). Such areas and occupations are more common among socioeconomically disadvantaged and otherwise vulnerable groups (Blishen 1967; Casey et al. 2017), including on the island of Montreal (Dale et al. 2015; Carrier et al. 2016). Furthermore, such groups have been shown to have greater incidences of noise-induced hearing loss (Dobie 2007), workplace accidents (Picard et al. 2008), and poorer cardiovascular health (Belojevic and Evans 2012; Gan et al. 2012) as a result. Vulnerable groups may also be understood as extending to the elderly, the chronically ill, the

hearing-impaired, shift-workers, pregnant women, and individuals who may otherwise be more sensitive to noise than the general population (van Kamp and Davies 2013; Auger et al. 2018). In this context, the goal of improving public health outcomes for at-risk populations can be informed by the broadened understanding of the effects of environmental noise exposure provided here.

5.5 Conclusion

In its simplest form, noise is a random signal, yet as a concept, noise transcends various frameworks of meaning. It is used both literally and colloquially in our day-to-day lives to describe distortions produced by unwanted or unexplainable phenomena, and is an integral part of fields from electronics to statistics to computer science. It is clear that noise is everywhere. The present thesis embraced noise as a tool with which to study experience-dependent plasticity in the adult auditory cortex. Its findings describe the profound impact of noise on brain structure and function, advance our present understanding of plasticity in mature sensory cortices, and demonstrate how sensory environments may powerfully influence the brain throughout life.

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Appendix 1: Permissions

Permissions are listed in order of appearance in the thesis text.

Chapter 1: General Introduction

Sections 1.1 and 1.2.1, Figure 1.1

- Voss P, Thomas M, Cisneros-Franco JM, de Villers-Sidani E. Dynamic Brains and the Changing Rules of Neuroplasticity: Implications for Learning and Recovery. *Frontiers in Psychology*, 2017. 8:1657.
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Figure 1.3

- Figure 2 in Eggermont JJ, Roberts LE. The neuroscience of tinnitus. *Trends in Neurosciences*, 2004 27(11), 676-682.
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Chapter 2: Study 1

Full text and figures

- Thomas ME, Friedman NHM, Cisneros-Franco JM, Ouellet L, de Villers-Sidani E. The Prolonged Masking of Temporal Acoustic Inputs with Noise Drives Plasticity in the Adult Rat Auditory Cortex. *Cerebral Cortex,* 2018, 29(3):1032-1046.
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Chapter 4: Study 3

Full text and figures

- Thomas ME, Guercio GG, Drudik KM, de Villers-Sidani E. Evidence of hyperacusis in adult rats following non-traumatic sound exposure. *Frontiers in Systems Neuroscience*, 2019, 13:1-17.
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