Mechanisms of Movement-Related Changes in Auditory Detection

by

Janani Sundararajan

Department of Neurobiology
Duke University

Date:_____

Approved:

_____
Richard Mooney, Supervisor

_____
Marc Sommer

_____
Lindsey Glickfeld

_____
Scott Soderling

Dissertation submitted in partial fulfillment of
the requirements for the degree of Doctor
of Philosophy in the Department of
Neurobiology in the Graduate School
of Duke University

2019

ABSTRACT

Mechanisms of Movement-Related Changes in Auditory Detection

by

Janani Sundararajan

Department of Neurobiology
Duke University

Date:_____
Approved:

_____
Richard Mooney, Supervisor

_____
Marc Sommer

_____
Lindsey Glickfeld

_____
Scott Soderling

An abstract of a dissertation submitted in partial
fulfillment of the requirements for the degree
of Doctor of Philosophy in the Department of
Neurobiology in the Graduate School of
Duke University

2019

Abstract

To successfully navigate the world, our sensory systems must process stimuli accurately during both rest and movement. Indeed, movements have been shown to modulate sensory systems at different levels. In audition, studies in humans and other animals have shown that movements strongly suppress auditory cortical responses to acoustic stimuli relative to rest. A largely untested idea is that this cortical suppression works to suppress responses to predictable acoustic consequences of movements, while enhancing sensitivity to novel stimuli. How this cortical suppression influences auditory perception and whether this suppression functions predictively as widely theorized remain unknown. Here, I trained head-fixed mice to lick in response to tones of different intensities during rest or running on a quiet treadmill. I observed that auditory detection was impaired during running compared to rest. Inactivating the auditory cortex impaired detection, and optogenetically activating secondary motor cortical axons in the auditory cortex during rest degraded detection similar to movement. Finally, movement-related impairment of auditory detection was specific to expected sounds following predictable sensorimotor experience. Overall, these findings support the idea that movement-related modulation of auditory cortical activity is behaviorally adaptive, selectively suppressing predictable movement-related sounds while enhancing sensitivity to novel stimuli.

Contents

List of Figures

Acknowledgements

I would like to first thank my advisor Rich Mooney for the guidance, motivation and encouragement he has provided over the past six years. His mentorship has played a crucial role in shaping my scientific thought process and building my confidence as a scientist. I will continue to remember the many valuable things I have learnt from him throughout my career. Thanks to David Schneider for being the best mentor, colleague and collaborator. I am grateful to him for training me to do everything from surgeries to Matlab coding and for patiently answering my never-ending stream of questions. Thanks to all the members of the Mooney lab for their support during my time in the lab and for sincerely protecting me during every bird attack. Special thanks to Matt, Erin and Mor for making me feel at home in Durham. I am grateful to Dale Purves for inducting me into neuroscience and for always encouraging me to think outside the box, and my committee for providing me with valuable feedback regarding my project, that has helped shape this project. A big thanks to my husband Sudarsun, for being part of this journey and persevering with me through the many highs and lows. Finally, I couldn't have done this without the unconditional support of my friends and family both in the US and in India, especially my mother Vijayalakshmi, who was always there for me, even if it meant taking 30 hour long flight journeys or staying up through the night to listen to me rant from half way across the world.

1. Introduction

1.1 Overview

Sensory stimuli can arise from our own movements as well as from the environment. For example, when walking along a leaf-covered path, our brain must distinguish the sounds generated by our footsteps from those of a stalking predator. Corollary discharge signals between the motor and auditory systems are theorized to play a role in distinguishing between stimuli produced by our own movements (reafference) and stimuli arising from external sources (exafference) (Sperry, 1950; Crapse and Sommer, 2008; Schneider and Mooney, 2018). Recent work in the mouse has described a corollary discharge circuit that suppresses auditory cortical neurons during movements: A subset of motor cortical neurons sends axons to the auditory cortex, where they engage feedforward inhibition that suppresses the activity of excitatory cells. Moreover, this circuit appears to be both necessary and sufficient to drive movement-related suppression of auditory cortical activity (Nelson et al., 2013; Schneider et al. 2014). However, the behavioral significance of this movement-related modulation of auditory cortical activity remains unknown.

Using a combination of behavioral, optogenetic and viral strategies, I showed that auditory detection thresholds in mice are increased during running compared to rest. Motor cortical projections to the auditory cortex can account for this increase

providing a circuit basis for this movement-related change in auditory perception. Furthermore, using a custom designed acoustic virtual reality setup I showed that mice were differently sensitive to auditory stimuli that were predictably coupled with their movements compared to stimuli that were not. These experiments illustrate the behavioral significance of corollary discharge signals in the mouse auditory cortex and shed light on how these signals can facilitate normal hearing during movements.

1.2 Corollary discharge signals

An organism that moves must be able to distinguish between the sensory consequences of its own movements and sensory stimuli arising from changes in the external environment to successfully navigate the world (Crapse and Sommer, 2008). Early understandings of how the nervous system accomplishes this come from the work of von Holst and Mittelstaedt who suggested that the brain engages an 'efference copy' signal, i.e. a copy of the motor signal is relayed to sensory regions, allowing sensory systems predict and prepare for upcoming reafference (Von holst and Mittelstaedt, 1950). While the term efference copy refers to a copy of the final motor signal sent by motor neurons to muscles, signals conveying movement-related information to sensory systems have been observed at multiple levels of the neuraxis. 'Corollary discharge' is a

general term that refers to movement-related signals relayed to sensory systems occurring at any level of the neuraxis (Figure 1; Sperry, 1950).

Examples of corollary discharge signals have been observed in several species ranging from invertebrates with relatively simple nervous systems to higher order species engaging in complex behaviors such as sensorimotor learning (Poulet and Hedwig, 2002; Bell, 1981; Sommer and Wurtz, 2002; Ford and Mathalon, 2004; Schneider et al., 2014). Corollary discharge signals have also been observed at several levels of the nervous system. At lower levels, these signals are transmitted through direct copies of the actual motor commands routed to the sensorimotor periphery and can serve functions such as sensory filtration. At higher levels, these signals can arise from regions involved in motor planning and patterning, and communicate with higher order sensory regions controlling perception. Higher order corollary discharge signals have been implicated in many functions including sensory filtration, maintaining sensorimotor stability and shaping sensorimotor behaviors (Crapse and Sommer, 2008).

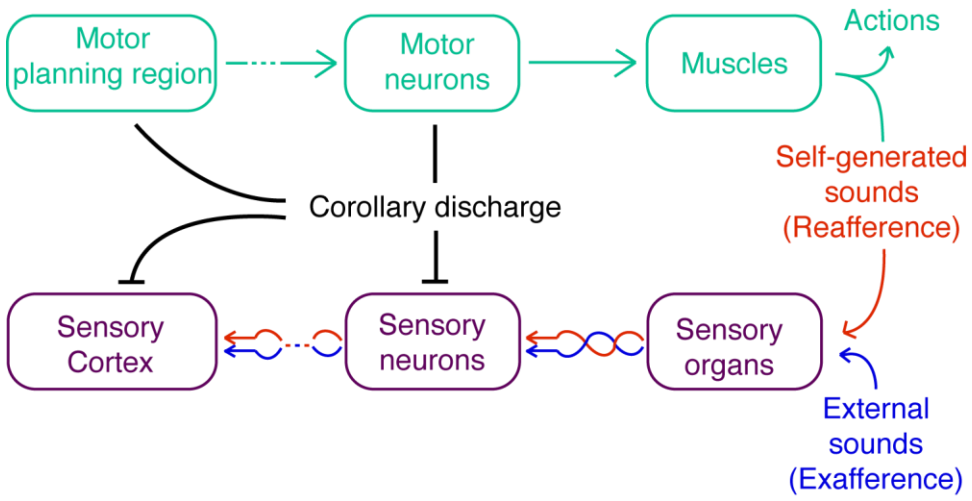


Figure 1: Corollary discharge signals in the nervous system. Corollary discharge signals can occur at different levels of the neuraxis in sensorimotor circuits. (adapted from Crapse and Sommer, 2008)

1.3 Movements and auditory reafference

The problem of distinguishing between reafferent and exafferent stimuli is one that is faced by all sensory systems. Specifically in the auditory system, this problem arises because many movements themselves produce sounds (e.g. during vocalizations, locomotion, etc.). Therefore, the signal arriving at the auditory periphery is a mixture of reafferent movement-related sounds and exafferent sounds generated by external sources in the environment. In the absence of movement-related information, the auditory system would weigh both these categories of sounds equally and therefore be unable to differentiate between them. In the next two sections, I will elaborate on why this could be problematic and explain how corollary discharge signals conveying movement-related information to the auditory system help maintain auditory sensitivity and also potentially play bigger roles in shaping sensorimotor behaviors by facilitating feedback monitoring and error detection.

1.3.1 Sensory filtration in the auditory system

During movements, it is important that the auditory system not be overwhelmed by the magnitude of self-generated sounds, potentially resulting in self-induced desensitization to externally generated sounds. Evidence for how the auditory system deals with this has emerged from several species and several stages of auditory

processing (Carmel and Starr, 1964; Suga and Shimozawa, 1974; Poulet and Hedwig, 2002; Houde et al., 2002; Eliades and Wang, 2003; Zhou et al., 2014; Williamson et al., 2015; Singla et al., 2017). In invertebrates, one example where the neural mechanisms that deal with this issue have been well characterized is during chirping in male crickets. Male crickets engage in a process known as stridulation, where they rub their forewings to produce a chirping noise used to attract mates or engage in rivalry. Their peripheral auditory machinery is however located in close proximity on their forelegs and is subject to potentially damaging levels (>100dB) of sounds during chirping bouts that can last for several hours. The cricket therefore risks temporary desensitization of its auditory system and the possibility of losing relevant information from the environment between chirp bouts that could potentially be critical for survival e.g. sounds that indicate an approaching predator. Using intracellular recordings, Poulet and Hedwig demonstrated that the cricket brain gets around this issue by engaging a corollary discharge mechanism that is time-locked with chirping bouts resulting in the inhibition of auditory responses to self-generated sounds, thereby allowing the cricket to maintain sensitivity to external sounds during chirp intervals (Poulet and Hedwig, 2002).

Studies in mammals have also found evidence of sensory filtration occurring in higher order structures such as the auditory cortex (Eliades and Wang, 2003; Zhou et al., 2014; Schneider et al., 2014; Rummell et al., 2016). One behavior where the issue of

sensory filtration is particularly relevant is during vocal communication. In humans and other animals, vocalizations serve a fundamental role in social communication and recognition. However, the sounds produced during vocalizations can quickly overwhelm the auditory system and overshadow other auditory stimuli, presenting the auditory system with a similar problem as in the cricket. While sensory filtration during vocalizations has been observed at many levels of the auditory system, the auditory cortex is of particular interest here given its major role in processing speech and music in humans (Hickok & Poeppel 2007, Peretz et al. 1994, Zatorre et al. 2002). Indeed, studies using electroencephalography (EEG) or magnetoencephalography (MEG) recordings in vocalizing humans indicate that auditory cortical responses are strongly suppressed during vocalizations compared to passively listening to the same sounds (Curio et al., 2000; Ford et al., 2001; Houde et al., 2002). To dissect how responses vary at the single neuron level, Eliades and Wang recorded the action potential activity of individual auditory cortical neurons in vocalizing marmoset monkeys. They observed that a majority of auditory cortical neurons were suppressed during vocalization. Furthermore, this suppression preceded the onset of vocalizations by hundreds of milliseconds suggesting premotor origins (Eliades and Wang, 2003). A similar modulation of auditory cortical activity has also been observed using intracellular sharp electrode recordings during vocalization and other movements in mice suggesting that

these mechanisms are likely to be conserved across organisms and allow the auditory system to maintain sensitivity during movements (Schneider et al. 2014).

1.3.2 Predictive monitoring of auditory feedback

Corollary discharge signals conveying information about the expected sensory consequences of motor signals can be compared with incoming sensory reafference to generate error signals that can guide sensorimotor learning. One behavior where such feedback monitoring mechanisms have been implicated in the auditory system is during vocal communication. Eliades and Wang recorded from auditory cortical neurons in vocalizing marmosets while decoupling the vocal motor signal from the resulting auditory reafference by using specialized headphones to pitch-shift auditory feedback. By doing this, they observed that auditory cortical neurons that were previously suppressed during normal or amplified auditory feedback were excited in response to pitch-shifted auditory feedback (Eliades and Wang, 2008). Similar observations have also been made in separate experiments in human subjects presented with altered auditory feedback during speech (Houde et al., 2002). These results suggest that error signals are generated in the auditory system to detect deviations from expected sensory feedback during motor behaviors.

In a future set of experiments in marmosets, Eliades and Tsunada noted that error signals were most pronounced in auditory cortical neurons that also exhibited movement-related suppression during normal vocalizations, suggesting a mechanistic role for movement-related modulation in feedback monitoring (Eliades and Tsunada, 2018). A further question is how do these error signals shape sensorimotor behaviors? Human subjects and marmoset monkeys presented with distorted auditory feedback during vocalizations compensate for differences between expected sensory feedback and actual feedback by rapidly adjusting their vocalizations, signifying a behavioral correlate for these error signals (Burnett et al., 1998; Houde and Jordan, 1998; Bauer et al., 2006; Eliades and Tsunada, 2018). Eliades and Tsunada further observed that vocal compensation in marmoset monkeys during altered auditory feedback was proportional to the magnitude of the overall error signal in auditory cortical neurons suggesting a functional role of these signals in shaping sensorimotor behaviors (Eliades and Tsunada, 2018). Overall, these experiments provide evidence for how movement-related modulation in the auditory cortex can play a role in feedback monitoring and guide sensorimotor behaviors, though the exact circuit mechanisms that constitute the integration of predictive movement-related information with afferent sensory input remain to be delineated.

1.4 Movement-related modulation at subcortical stages of auditory processing

Movements have been shown to modulate the auditory system at multiple nodes, beginning at the auditory periphery (Figure 2; Schneider and Mooney, 2018). At the periphery, studies in both humans and other animals have shown that vocalizations and other body movements are accompanied by increased contraction of the middle ear muscles. This contraction precedes movement-onset by several hundred milliseconds and persists throughout the period of movement, diminishing the sound intensity propagating to the inner ear. Though the time period of this attenuation is consistent with a premotor mechanism, the exact circuit mechanisms that contribute to this predictive movement-related suppression are yet to be parsed out (Carmel & Starr 1964, Borg and Zakrisson, 1975; Mukerji et al. 2010).

In the central nervous system, attenuation of auditory responses to self-generated sounds has been observed at several stages of auditory processing. Using electrophysiological recordings of single neurons in the auditory brainstem, Singla et al demonstrated that simple acoustic consequences of rhythmic movements such as licking are suppressed in the Dorsal Cochlear Nucleus (DCN). This suppression was contingent upon non-auditory inputs from the spinal trigeminal nucleus that potentially convey lick-related somatosensory information to the DCN, presenting a circuit mechanism by which the predictable acoustic consequences of stereotyped movements are suppressed

within the brainstem (Singla et al., 2017). Neural responses in the auditory thalamus are also suppressed during movements compared to rest, though this suppression is noted to be less robust compared to suppression in the primary auditory cortex. Further work is required to delineate the circuit mechanisms of movement-related modulation in the auditory thalamus and determine the extent to which thalamic suppression during movements is mediated by corticothalamic projections versus other subcortical circuit mechanisms (Williamson et al., 2015; Guo et al., 2017; Williamson and Polley, 2019).

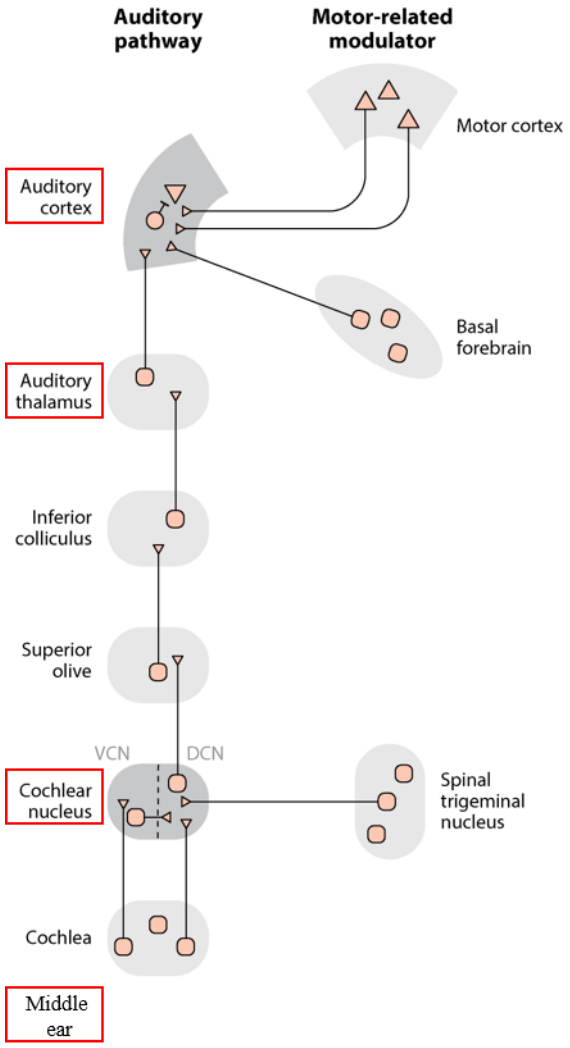


Figure 2: Movement-related modulation at different levels of the auditory system. Schematic showing the ascending auditory pathway and motor-related inputs at different levels. Nodes that are known to be modulated by movement are indicated with red boxes (adapted from Schneider and Mooney, 2018).

1.5 Movement-related modulation in the auditory cortex and the underlying circuit mechanisms

Although subcortical mechanisms acting at various levels of the auditory system have been shown to suppress auditory responses during movements, movement-related suppression also arises independently in the auditory cortex. In humans, functional imaging studies show that the primary auditory cortex is suppressed during vocalizations compared to passive listening (Curio et al., 2000; Ford et al., 2001; Houde et al., 2002). Movement-related modulation of auditory cortical activity is not limited to vocalizations alone but also accompanies a variety of other movements such as manual musical gestures and other body movements (Martikainen at al., 2005; Zatorre et al., 2007; Weiss et al., 2011; Reznik et al., 2014). Furthermore, studies in non-human primates and mice characterizing single neuron as well as population level responses of primary auditory cortical neurons during behaviors such as vocalization or locomotion have shown that auditory cortical responses are suppressed during movements compared to rest (Muller-Preuss and Ploog, 1981; Eliades and Wang, 2003; Schneider et al., 2014; Zhou et al., 2014; Rummell et al., 2016).

Movement-related suppression in the auditory cortex has been shown to initiate in a time window that precedes movement-onset, suggesting the role of corollary discharge signals with premotor origins (Eliades and Wang, 2002; Schneider et al. 2014). Such signals could potentially originate from many motor-related centers in the brain

that send direct projections to the auditory cortex, including the basal forebrain and prefrontal cortical areas associated with motor planning (Nelson et al., 2013; Schneider et al. 2014; Nelson and Mooney, 2016).

In humans, evidence of corollary discharge signals in the auditory cortex comes from measurements of gamma band coherence of event related potentials (ERPs) between the frontal (motor) cortex and the auditory cortex, revealing a significantly greater value during speech compared to passive listening. This coherence was decreased in schizophrenic patients with auditory hallucinations suggesting a role for this pathway in normal auditory processing during movements (Ford et al., 2001; Ford and Mathalon, 2004). Furthermore, a host of other behavioral, electrophysiological and anatomical tracing studies in both primates and rodents have suggested functional links between the motor and auditory cortex suggesting a possible circuit mechanism for the movement-related modulation in the auditory cortex (Alexander et al., 1976; Leichnetz and Astruc, 1975; Mottonen and Watkins, 2009; Budinger et al., 2006).

The synaptic and cellular bases of corollary discharge signals in the auditory cortex have been only recently studied in the mouse brain. Using a host of viral tracing techniques, Nelson et al. showed that the mouse auditory cortex receives direct projections from several cortical and subcortical movement-related regions. Of these regions, the secondary motor cortex (M2) was identified to be of particular interest,

given the implications of M2 in premotor activity and sensorimotor planning, and the high density of projections from deep layer M2 neurons to the auditory cortex that also project to downstream motor regions implicated in movement-execution such as the striatum, periaqueductal gray, mesencephalic reticular nucleus, etc. Using a combination of electrophysiological and intersectional tracing techniques, Nelson et al demonstrated that M2 neurons send axons to both excitatory and inhibitory classes of auditory cortical neurons and engage a circuit mechanism that suppresses auditory cortical activity through feedforward inhibition during movements (Figure 3; Nelson et al., 2013).

In a following study, Schneider et al. determined that more than half of the auditory cortical suppression during movements could be accounted for by mechanisms that were local to the cortex. Optogenetically activating M2 axons in the primary auditory cortex of resting mice recapitulated movement-like suppression of auditory cortical neurons, and unilaterally silencing M2 in a moving mouse switched auditory cortical responses to a rest-like state indicating that M2 projections to the auditory cortex are sufficient for modulating auditory cortical activity during movements. In a complementary set of experiments, they further showed that M2 driven suppression of auditory cortical activity is likely mediated by local inhibitory neurons within the auditory cortex (Schneider et al., 2014). These experiments provide the cellular and circuit bases for the movement-related suppression of auditory cortical neurons.

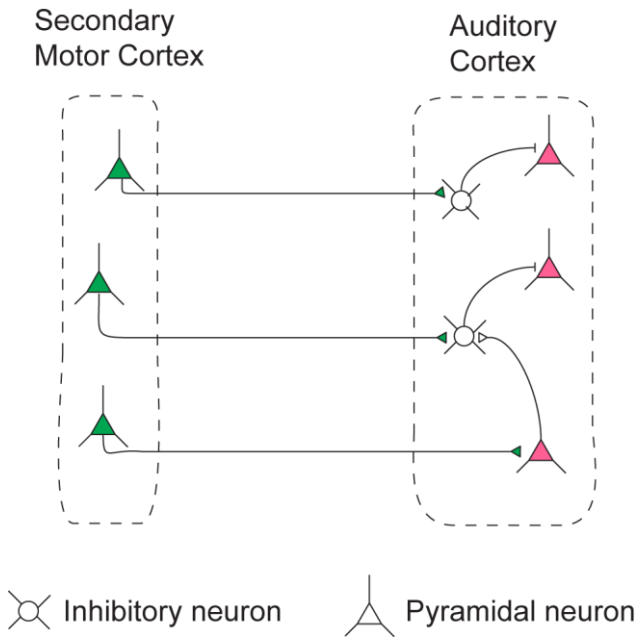


Figure 3: Circuit mechanism for the movement-related modulation of auditory cortical activity. The secondary motor cortex sends direct projections to excitatory and inhibitory neurons within the primary auditory cortex and suppresses auditory cortical activity during movements through feedforward inhibition.

While M2 to auditory cortical projections can account for most of the movement-related changes in auditory cortical activity, the auditory cortex also receives modulatory cholinergic inputs from the basal forebrain that are active during movements. During movements, these inputs have a more modest effect on acute auditory cortical activity compared to M2 neurons, and subtly normalize the receptive fields of auditory cortical neurons (Nelson and Mooney, 2016). Cholinergic inputs from the basal forebrain to the auditory cortex have been extensively studied in the context of modulating auditory cortical activity during learning and arousal states (Froemke et al., 2007; Kuchibhotla et al., 2016; McGinley et al., 2015). These inputs might therefore serve the broader role of driving auditory cortical plasticity during movements by modulating movement-related signals from the motor cortex to form new sensorimotor associations. Furthermore, basal forebrain neurons that project to the auditory cortex receive inputs from brainstem regions implicated in movements and arousal suggesting that these projections could relay information regarding internal state and movements during sensory processing. This is distinct from the information conveyed by M2 neurons projecting to the auditory cortex that receive inputs from cortical regions involved in decision-making, motor planning, etc., and are well-poised to provide top-down information during sensory processing (Nelson and Mooney, 2016).

1.6 Context-dependent gain modulation in the auditory cortex

Whereas movements are one source of auditory cortical modulation, auditory cortical responses to sounds are also influenced by other factors such as arousal states, task engagement and habituation (McGinley et al., 2015; Kuchibhotla et al., 2017; Fritz et al., 2003; Kato et al., 2015; Otazu et al., 2009; King et al., 2018; David, 2018). In a recent study, by using pupillometry to assay changes in the arousal states of mice, McGinley et al demonstrated that membrane potentials of auditory cortical neurons vary as a function of arousal levels, exhibiting a U-shaped relationship with pupil diameter. Auditory cortical responses to sounds also showed a strong dependence on arousal levels, displaying strongest and most reliable responses during intermediate levels of arousal, which in turn corresponded with 'optimal' behavioral performance compared to lower or hyper-aroused states. Interestingly, walking and running are correlated with hyper-aroused states compared to resting. Therefore, one possibility is that while motor-modulation of auditory cortical activity has directed roles in sensory filtration and guiding sensorimotor behaviors, global changes in internal state may also contribute to changes in auditory processing during movements (McGinley et al., 2015).

Auditory cortical responses to sounds are also modulated by behavioral engagement. Experiments in mice and rats have demonstrated that active task engagement suppresses auditory cortical responses to sounds compared to passive

listening (Otazu et al., 2009; Kuchibhotla et al., 2016). Using two-photon imaging of different sub-populations of neurons in the auditory cortex, Kuchibhotla et al demonstrated that switching from passive listening to active behavioral engagement is correlated with increased recruitment of local inhibitory neuronal populations within the auditory cortex, which in turn is mediated by increased cholinergic drive from nucleus basalis. While majority of excitatory neurons are suppressed during behavioral engagement, a smaller proportion of neurons also increase their responses during task performance, presumably to maintain sensitivity to auditory stimuli (Kuchibhotla et al., 2016; Hattori et al., 2017).

The auditory cortex can also modulate responses to individual stimuli in a context-specific and experience-dependent manner. Auditory cortical receptive fields have been shown to dynamically reorganize to enhance responses to specific target sounds during active task engagement compared to passive listening (Fritz et al., 2003). Furthermore, the auditory cortex also shows short-term stimulus specific adaptation as well as long-term habituation changes that accompany repeated experience with the same sound stimulus (Ulanovsky et al., 2003; Kato et al., 2015). Kato et al demonstrated that passive experience with the same pure tone stimulus across several days decreased the overall excitation and increased inhibition of auditory cortical neurons in response to that particular tone frequency. This was also accompanied by an increase in the response

of somatostatin positive interneurons to the habituated tone frequency. Furthermore, these effects were rapidly reversed when switching from passive listening to active task engagement, indicating that auditory cortical responses to stimuli are dynamically modulated in a context-specific manner by both passive auditory experience and task engagement (Kato et al., 2015).

1.7 Role of the auditory cortex in auditory perception

The auditory cortex is well poised to influence behavior and perception given its role in integrating sensory input with a wide variety of signals conveying both top-down information about upcoming body movements and behaviors, and modulatory input providing information about the internal state of the animal and behavioral relevance of sensory stimuli (Nelson and Mooney, 2016; Schneider et al., 2014; David, 2018; King et al., 2018). Therefore, auditory cortical activity not only encodes the physical characteristics of sensory stimuli, but also encodes information about movements, attention, arousal states, past experience, behavioral relevance, etc. that actively influence behavior. Indeed, studies have demonstrated that auditory cortical activity correlates with final behavioral and perceptual choices in addition to relaying information about stimulus features (Bizley et al., 2013; Niwa et al., 2012; Francis et al., 2018; King et al., 2018).

In humans, the auditory cortex is an important center for auditory processing, playing a critical role in normal hearing and the perception of speech and music (Hickok & Poeppel 2007, Peretz et al. 1994, Zatorre et al. 2002; Schreiner and Malone, 2015). Patients with unilateral and bilateral auditory cortical lesions show effects that range from complete deafness to partial hearing loss including deficiencies in comprehending speech and discriminating pitch (Peretz et al., 1994; Tramo et al., 2002; Zatorre, 1988; Zatorre, 2007; Johnsrude et al., 2000; Dykstra et al., 2012). Studies of auditory cortical lesions in non-human primates also report defects in auditory perception, including elevated detection thresholds and decreased frequency discrimination and sound localization abilities (Heffner and Heffner, 1986, 1990a, b; Thompson and Cortez, 1983; Strominger et al., 1980).

In rodents, reports of perceptual effects following auditory cortical inactivation have been mixed. Some studies report little or no changes in perceptual abilities following auditory cortical inactivation and lesions (Kelly and Glazier, 1978; Hunter and Willot, 1993; Gimenez et al., 2015). In other works, acute inactivation of the auditory cortex by the application of muscimol ($GABA_A$ agonist) has been shown to significantly and reversibly affect the ability of rodents to detect and discriminate between tone stimuli (Talwar et al., 2001; Kuchibhotla et al., 2016). Optogenetic suppression of

auditory cortical activity has also been shown to impair the ability of mice to detect sound offsets (Kato et al., 2015).

In recent years, studies in rodents and other species have demonstrated correlations between auditory cortical activity and behavioral measurements during a variety of auditory tasks. The auditory cortex shows pronounced capacity for experience-dependent plasticity that is associated with perceptual correlates even in the absence of subcortical changes. Repeatedly pairing tone stimuli with electrical stimulation of nucleus basalis in the rat brain results in enhanced auditory cortical representation and responses to paired sounds while also increasing detection levels of paired sounds (Kilgard and Merzenich, 1998; Froemke et al., 2013). Additionally, the auditory cortex also encodes information about behavioral relevance and undergoes changes to selectively enhance the processing of behaviorally relevant stimulus features. Studies both in rodents and primates have shown that auditory cortical representation and response magnitudes are preferentially increased for behaviorally relevant sounds following training in a wide variety of auditory tasks, and behavioral learning and perceptual abilities in auditory tasks are correlated with the magnitude of changes in auditory cortical representation following training (Recanzone et al., 1993; Rutkowski and Weinberger, 2005; Polley et al., 2006). Similar changes have also been noted at other nodes of the auditory neuraxis such as the inferior colliculus and are possibly induced

by engaging corticofugal pathways (Gao and Suga, 2000; Suga, 2008; Edeline and Weinberger, 1991; Williamson and Polley, 2019).

1.8 The mouse as a model system for studying movement-related changes in auditory perception

While studies from humans and non-human primates have shed light on the basic neural correlates of movement-related changes in auditory cortical processing, recent studies in the mouse brain using a wide variety of techniques to measure and selectively manipulate neural activity have been instrumental in parsing out the circuit mechanisms underlying the motor modulation of auditory cortical activity (Nelson et al., 2013; Schneider et al., 2014). However, the behavioral significance of this movement-related suppression in the auditory cortex remains unexplored.

Movement-related suppression of auditory cortical activity in the mouse brain occurs on a time scale similar to human and non-human primates, and potentially engages similar circuit mechanisms, suggesting a conservation in the neural mechanisms underlying movement-related modulation in the auditory cortex (Eliades and Wang, 2003; Schneider et al., 2014). Several studies have demonstrated that both head-fixed and freely behaving mice are capable of learning and performing complex auditory tasks including tone detection and frequency discrimination (Kato et al., 2015; Kuchibhotla et al., 2016; Rummell et al., 2016; McGinley et al., 2015). Additionally, head-fixed mice

positioned atop a treadmill transition naturally and spontaneously between periods of rest and running, allowing the ability to combine precise behavioral measurements with manipulations of neural activity (Schneider et al., 2014; Zhou et al., 2014). Finally, mice are useful experimental organisms, given the availability of a vast variety of genetic tools optimized for the mouse brain and techniques that allow the monitoring and manipulation of neural activity in different brain regions of interest. Given these reasons, I will use the mouse as my model system to study the effects of movement on auditory perception in my dissertation work.

In summary, corollary discharge signals in the auditory cortex are thought to facilitate sensory filtration, distinguish between self-generated and external sounds, and guide learning during sensorimotor behaviors (Crapse and Sommer, 2008; Schneider and Mooney, 2018). However, how these signals affect auditory perception and whether they help the auditory system maintain sensitivity to external sounds during movements, is not understood. Here, using the mouse as my model system, I studied how movements affect auditory perception, the circuit mechanisms underlying movement-related changes in auditory perception and whether movement-related changes to auditory perception are behaviorally adaptive. Specifically, I hypothesized that movements degrade auditory detection and secondary motor to auditory cortical projections can account at least in part for this degradation. I further tested the idea that the movement-related modulation

of auditory cortical activity following temporally coupled sensorimotor experience is behaviorally adaptive and facilitates the distinction between predictable acoustic consequences of movements and unexpected stimuli.

2. Movement-related changes in auditory detection

2.1 Introduction

Auditory responses to sounds are suppressed during movements compared to rest (Schneider et al., 2014; Zhou et al., 2014; Williamson et al., 2015; Singla et al., 2017). However, the behavioral significance of this suppression remains unknown. The first goal of my thesis work was to determine how movements affect auditory detection. Given the various experimental advantages of using a mouse model and the recent characterization of the synaptic and circuit mechanisms underlying movement-related modulation in the auditory cortex of the mouse brain, I used the mouse to address this. I designed an auditory task in which mice were trained to detect tones while either resting or running on a quiet treadmill. I compared psychophysical performance during rest and running to establish how running affects loudness perception, testing the hypothesis that auditory detection is degraded during running compared to rest. The findings of these experiments have been previously published (Schneider, Sundararajan & Mooney, 2018).

2.2 An auditory task to determine detection thresholds during rest and running.

To study the effect of movement on auditory detection, the task design required that the same test conditions be presented during both rest and movement. To achieve

this, I trained head-fixed mice to do an auditory detection task while they were either resting or running on a quiet treadmill. Mice were first implanted with a metal headpost and acclimated to a treadmill setup (Figure 4A) for 2-3 days. Following a period of 24 hours of water restriction, mice were trained using operant methods to lick in response to tones of a fixed frequency (8kHz) and duration (25ms), but varying sound intensities while they were either at rest or running on the treadmill.

The training protocol consisted of presentation of only the highest intensity tones (70 and 80 dB) at variable inter-tone intervals (8 - 15s). These intensities were chosen for training, as they are well above detection thresholds for mice (Ison et al., 2007). During training, tone presentations were paired with the delivery of a water reward (4-8uL) through a reward port after a delay of 1s (Figure 4B). The reward port was integrated with a custom-designed infrared lick-detector placed in front of the mouse's face. To differentiate licks that were in response to tone presentations from non-specific licking, as well as to deter mice from continuously licking in a non-specific fashion, a variable 'no-lick-period' (3 - 8s) was imposed before the presentation of each tone. Licking within the 'no lick period' resulted in the reset of the inter tone interval acting as a 'time out' delaying the presentation of tones and consequently the delivery of rewards. As with classical conditioning paradigms, mice initially tended to lick following reward delivery (mean lick latency from tone onset: 1.24 +/- 0.06s). With several days (3.8 +/- 1.4 days) of

training with paired presentations of tones and rewards, their licks tended to follow tone presentation, but preceded reward delivery (mean lick latency from tone onset: 0.86 +/- 0.07s), demonstrating a learned association between the tone and reward.

Following training, mice were transitioned to the testing protocol, where they were required to lick within a 1 s window following tone presentation in order to receive a reward (Figure 4C). During this transition, tones of lower intensities (10 to 60 dB, in steps of 10 dB) were gradually introduced over a period of 5 days. During testing, tones were presented using a block design where each block consisted of tone intensities ranging from 10 to 80dB in steps of 10dB, presented in random order at variable inter-tone-intervals (8 – 15 s). To measure the differences in spontaneous lick rates and false alarm rates between rest and running states, the task also included 'catch' trials (0 dB trials) where no stimulus was presented. A variable 'no-lick-period' (3 – 8 s) similar to the training paradigm was also incorporated to differentiate non-specific licks from licks made in response to tone presentation.

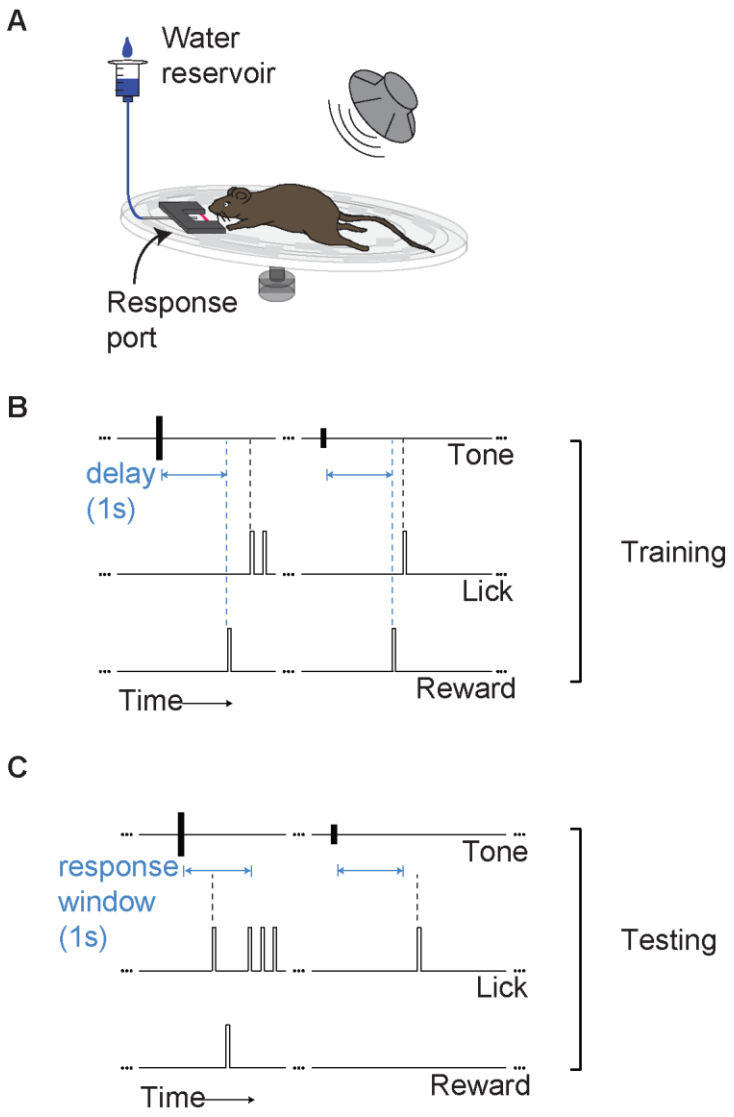


Figure 4: Experimental design for testing auditory detection in mice during rest and running. A) Mice were trained to lick a response port upon hearing a tone while resting or running on a treadmill. B) Training phase: 70dB and 80dB tones were presented at random inter-tone intervals following a period without spontaneous licking. A reward was delivered 1s following every tone presentation. C) Tones from 0 – 80dB were presented at random inter-tone intervals following a period without spontaneous licking. Mice were rewarded for licking within 1s following tone presentation.

Most mice spontaneously transitioned between periods of running and rest, allowing us to measure psychometric functions for each mouse under both conditions on each day (Figure 5). A subset of mice had a tendency to either rest or run continuously throughout an entire daily session. For mice that always rested, we temporarily enforced that tones were only presented during periods of locomotion, such that mice could not receive a reward unless they ran (and vice versa for mice that always ran). This transient adjustment of the task criteria was sufficient to alter a mouse's behavior such that they began to spontaneously transition between periods of running and resting. Following training/testing each day, each mouse received a supplement of 1.5 ml of water. Each mouse was weighed daily to ensure that its weight did not fall below 80% of its pre-water-restriction weight.

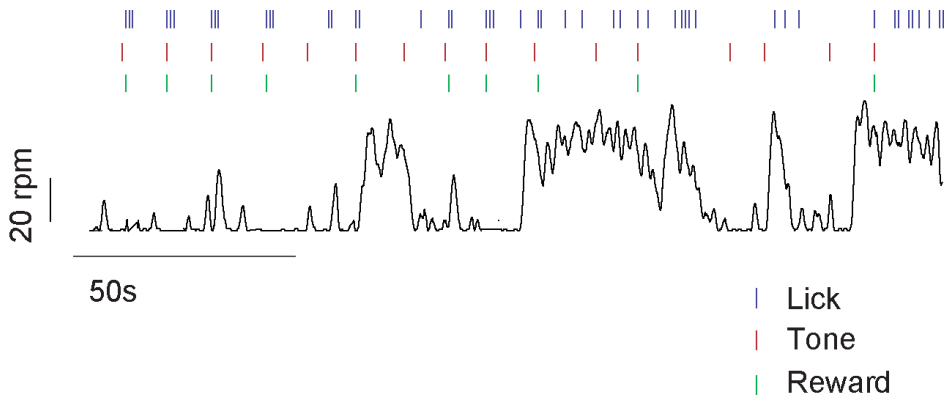


Figure 5: Example running trace from a single mouse during tone detection. Example trace of locomotion (black line), timing of individual licks (blue ticks), tone presentations (red ticks) and reward delivery (green ticks).

2.3 Movement degrades auditory detection

2.3.1 Psychometric curves during rest and running

To create psychometric functions for each mouse, trials were separated based on whether mice were running or resting at the time of tone presentation and trials were pooled across all testing sessions (12.4 ± 5.4 sessions). To ensure that the mice were engaged in the task, blocks during which the mouse did not lick were removed from analysis. Mice performed an average of 215 trials per session, consisting of, on average, 170 trials during rest and 45 trials during running. For each tone intensity, hit rate was calculated as the number of correct detections divided by the total number of tone presentations at that intensity during rest or running. Mice were significantly worse at detecting tones at intermediate intensities (10–40 dB) while running than at rest, and displayed similar levels of motivation in these two states (Figure 6; RM 2-way ANOVA at non-zero intensities, p (intensity x behavioral state) = 0.0003, F (7, 126) = 4.22, followed by post hoc Tukey test at individual intensities) This suggests that, similar to the suppression of neural responses to auditory stimuli during running compared to rest, perceptual responses to auditory stimuli are also degraded during running compared to rest.

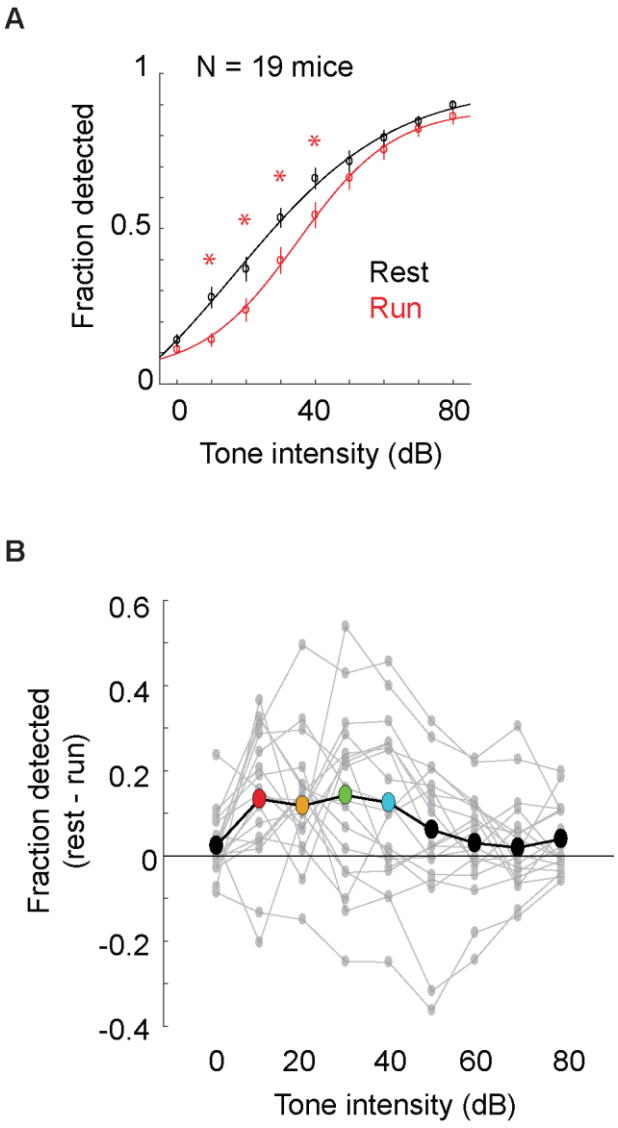


Figure 6: Psychometric curves during rest and running. A) Average psychometric functions (N = 19 mice) showing detection rates as a function of tone intensity while mice were resting (black) and running (red). Red asterisk: $p < 0.005$ (RM 2-way ANOVA followed by post hoc Tukey test). B) Difference in performance as a function of intensity for each mouse (gray dots). Large connected dots show mean difference in performance (N = 19 mice) and colored dots indicate intensities where performance was significantly different ($p<0.005$, RM 2-way ANOVA followed by post hoc Tukey test) across conditions.

2.3.2 Auditory detection thresholds during rest and running

In order to understand how running affects auditory detection thresholds, I calculated behavioral thresholds for each mouse during rest and running by estimating the intensity at which performance reached 50% in each condition. This value was determined by linear interpolation of surrounding intensity values. To account for differences in thresholds due to difference in false alarm rates between conditions, the fraction of correct trials that could be accounted for by false alarms for each condition was removed. Not applying this correction and estimating thresholds on the raw data did not change the findings (data not shown). Behavioral thresholds were significantly increased during running (mean threshold: 42.07 +/- 2.73 dB) compared to rest (mean threshold: 32.57 +/- 2.73 dB), indicating that mice were worse at detecting tones during running compared to rest (Figure 7; comparisons with two-sided paired t-test. P = 0.009).

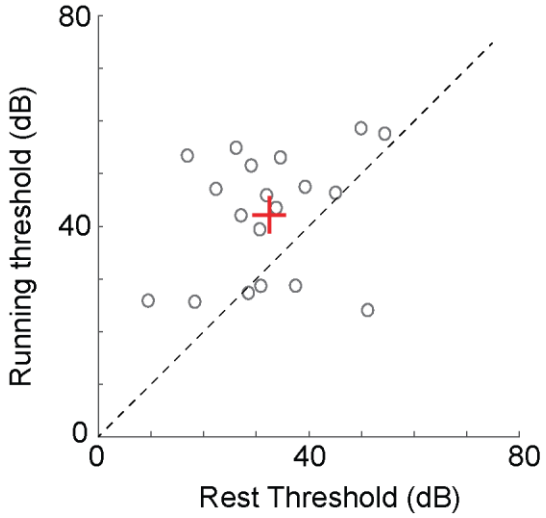


Figure 7: Auditory detection thresholds during rest and running. Behavioral threshold (intensity at 50% performance) during resting and running for each mouse denoted by black circles; red + denotes mean thresholds during rest and running. Dashed line shows unity. Auditory detection thresholds are higher during running compared to rest (N = 19 mice, P=0.009, paired t-test).

2.3.3 Lick latencies during rest and running

I calculated lick latencies from tone onset as a function of tone intensity during rest and running to test whether lick latencies were increased during running compared to rest. Lick latencies were calculated as the time elapsed from the presentation of a tone to the first lick in response to the tone on correct trials during rest and running. Lick latencies were not significantly different between rest and running conditions (Figure 8; RM 2-way ANOVA at non-zero intensities). There was however a significant decrease in latencies with an increase in tone intensity, indicating that mice were quicker to respond to louder tones compared to softer tones (RM 2-way ANOVA at non-zero intensities, p (intensity) < 0.0001, $F(7, 126) = 5.751$).

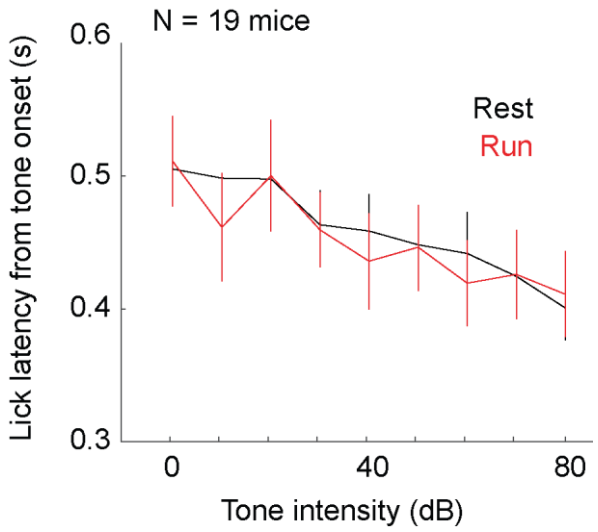


Figure 8: Lick latencies during rest and running. Lick latencies from time of tone presentation (N = 19 mice) as a function of tone intensity during resting and running. Lick latencies were not significantly different between rest and running conditions during auditory detection. (RM 2-way ANOVA)

2.4 Chapter 2 conclusions

In summary, I have designed and implemented an auditory detection task to test how auditory detection thresholds in mice change during rest and running. Mice were significantly worse at tone detection during running compared to rest. This behavioral result is consistent with the observation that action potential responses in the auditory cortex are suppressed in response to sounds during movements compared to rest (Schneider et al., 2014; Zhou et al. 2014; Rummell et al., 2016). Overall, these results also agree with findings from other studies that support the idea that sensory processing and perception are heavily influenced by the behavioral state of animals (McGinley et al., 2015; Kuchibhotla et al., 2017).

3. The role of the mouse auditory cortex in auditory detection

3.1 Introduction

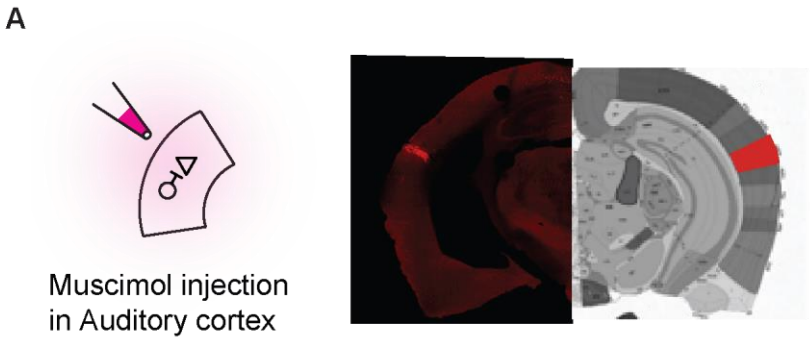
The auditory cortex is of special interest here, given its role in the perception of speech, music, and other acoustic qualities in humans (Hickok & Poeppel 2007, Peretz et al. 1994, Zatorre et al. 2002; Schreiner and Malone, 2015; Belin et al., 2000; Bouchard et al., 2013). Though studies in humans and non-human primates have indicated a role for the auditory cortex in detecting and perceiving different qualities of acoustic stimuli (Peretz et al. 1994; Heffner and Heffner, 1986, 1990a,b; Tramo et al., 2002; Dykstra et al., 2012), it is unclear whether the auditory cortex is important for auditory perception in rodents. Whereas some studies report minimal or no perceptual role of the rodent auditory cortex (Kelly and Glazier, 1978; Hunter and Willot, 1993; Gimenez et al., 2015), other studies indicate that the auditory cortex in rodents is important for detection and discrimination of auditory stimuli (Talwar et al., 2001; Kato et al., 2015; Kuchibhotla et al., 2016). One possibility is that ablating the auditory cortex engages compensatory mechanisms involving parallel subcortical auditory pathways that are recruited over time, thereby alleviating some of the initial effects of the ablation (Talwar et al., 2001). It was therefore important to test whether the mouse auditory cortex is involved in this particular auditory detection task using reversible methods to suppress auditory cortical activity.

To determine if the auditory cortex is a component of the tone detection circuit in mice, I combined behavioral methods with pharmacological and optogenetic tools to suppress auditory cortical activity in a temporally precise and reversible manner during auditory detection. These experiments have been included in a previously published manuscript (Schneider, Sundararajan and Mooney, 2018).

3.2 Suppression of auditory cortical activity impairs auditory detection in the mouse.

3.2.1 Pharmacological silencing of auditory cortical activity

To determine whether the mouse auditory cortex is involved in auditory detection, I used pharmacological methods to reversibly silence the auditory cortex of mice during auditory detection. Mice were first trained to detect tones of different intensities using operant conditioning methods described in Chapter 2.2. Following training, the auditory cortex was bilaterally identified using stereotaxic coordinates and by measuring electrophysiological responses to tones. Saline (150nl) or muscimol (2 μg/μl, 150 nl) was pressure injected using a Nanoject system bilaterally into the auditory cortex on alternate days. Injection of muscimol silenced auditory cortical activity abolishing both spontaneous activity and tone-evoked responses (Figure 9).



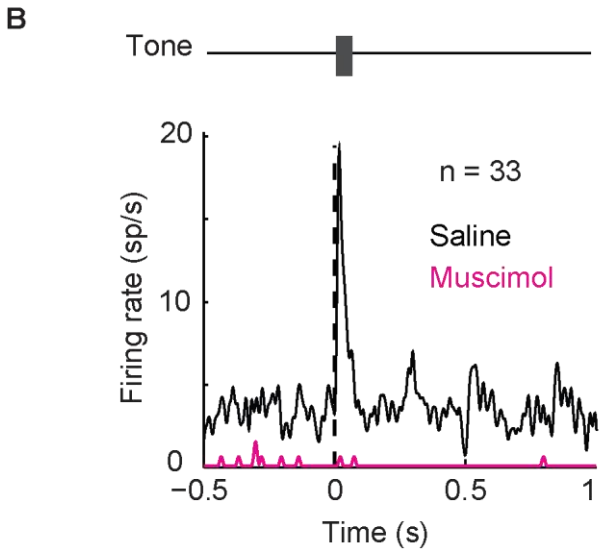


Figure 9: Muscimol injection silences auditory cortical activity. A) *Left:* Muscimol injection in auditory cortex. *Right:* Coronal section of mouse brain showing infusion of muscimol-BODIPY in auditory cortex. B) Tone evoked responses from putative excitatory neurons in auditory cortex during infusion of saline (black) or muscimol (magenta). Infusion of muscimol suppresses spontaneous and tone-evoked responses in auditory cortex.

Mice were allowed to recover in their home cage for 30 minutes after injection of muscimol or saline. At the end of this period, mice were reintroduced to the testing chamber where they were tested on their ability to detect tones of different intensities. Psychometric curves were calculated for saline and muscimol conditions during rest to determine if silencing the auditory cortex impacted auditory detection (Figure 10). Auditory detection was significantly degraded in the muscimol condition compared to the saline condition suggesting that the auditory cortex is involved in auditory detection in the mouse.

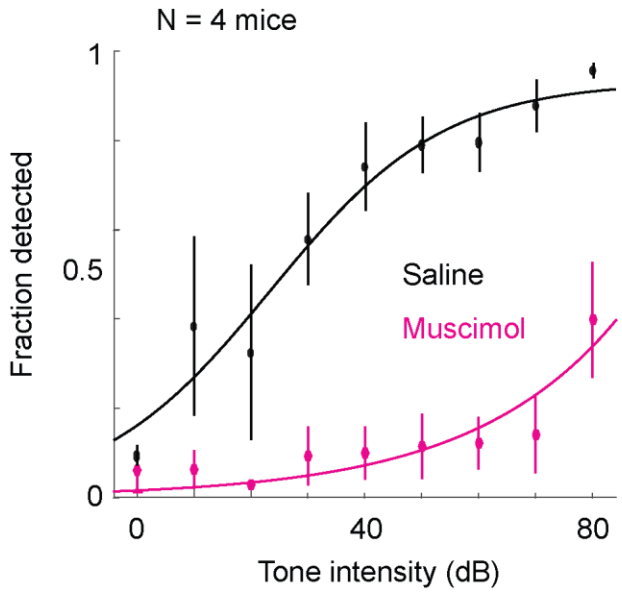


Figure 10: Psychometric curves during pharmacological silencing of auditory cortex. Average psychometric metric functions showing detection rates as a function of tone intensity for trials performed during rest with infusion of either saline (black) or muscimol (magenta) into the auditory cortex. Data points show mean and SE of detection rates. Auditory detection is degraded during muscimol infusion compared to saline.

3.2.2 Optogenetic inhibition of auditory cortical activity

Local inhibitory neurons in the auditory cortex have been shown to dynamically modulate auditory cortical activity during a variety of behaviors, including during movement (Hattori et al., 2017). Does suppressing auditory cortical activity by activating local inhibitory neurons in a reversible and temporally specific manner affect auditory detection? To address this question, I used optogenetic methods to activate inhibitory neuron populations in the auditory cortex of VGAT-ChR2 mice (a mouse line genetically engineered to express channelrhodopsin in GABAergic interneuronal populations in the brain). I first used extracellular electrophysiological recordings to confirm that optogenetic stimulation of inhibitory neurons in the auditory cortex of VGAT-ChR2 mice using blue light suppressed both baseline activity and tone-evoked responses (Figure 11; paired t-test, $p < 0.05$).

For behavioral testing, VGAT-ChR2 mice were first trained on the auditory detection task described in Chapter 2.2. Following training, cranial windows were bilaterally implanted over the auditory cortex using stereotaxic coordinates and by measuring electrophysiological responses to tone presentations. During behavioral testing, a blue (420 nm) laser (Shanghai) was coupled to a pair of optical fibers (Doric optical splitter), which were positioned bilaterally over the auditory cortex cranial windows (Figure 12). Mice were tested on their ability to detect a subset of tone

intensities (0, 30, 40 and 60 dB). Laser pulses (25 Hz, 50% duty cycle, 15–30mW) were presented on 50% of sound trials. Laser stimulation began 200 msec prior to tone presentation and continued for 1.2 sec, which covered the entire response window. This laser stimulation time period was chosen to avoid behavioral effects of rebound neural activity within the reward window that could occur with smaller laser stimulation periods. To accurately estimate the mouse's chance performance on optogenetic trials, a laser-only 'catch' stimulus was added. To deter mice from using light as a cue on optogenetic trials, 50% of stimuli were laser-only trials, and a short air puff (100 ms, Picospritzer II) was directed towards the mouse's face as negative reinforcement on trials where the animal licked in response to laser-only trials.

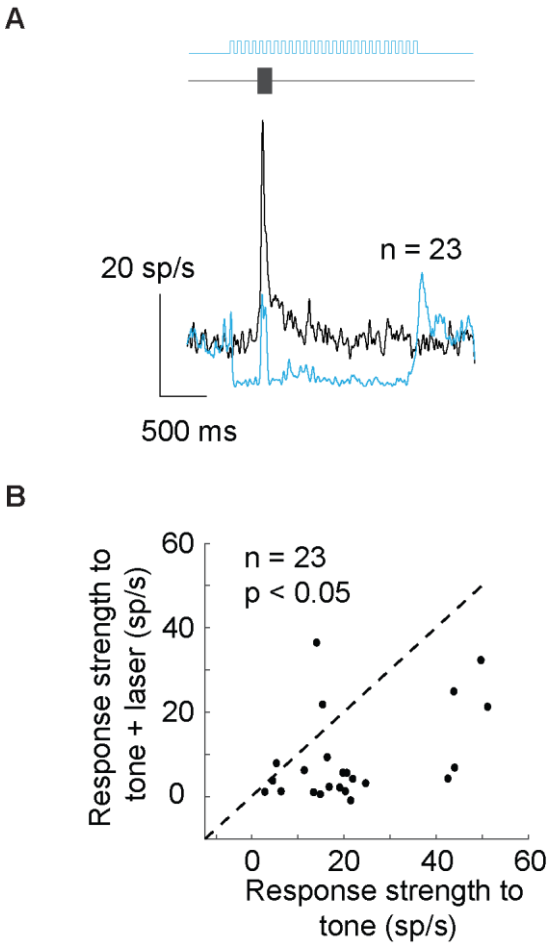


Figure 11: Optogenetic suppression of auditory cortex. A) Tone-evoked responses from putative excitatory neurons recorded from auditory cortical neurons in a VGAT::ChR2 mouse without (black) and with (blue) simultaneous blue laser stimulation. Optogenetic activation of inhibitory neurons decreases the spontaneous and tone-evoked firing rates of excitatory neurons. B) Tone-evoked responses are weaker during optogenetic activation of inhibitory interneurons. Dashed line is unity. ($p<0.05$, paired t-test).

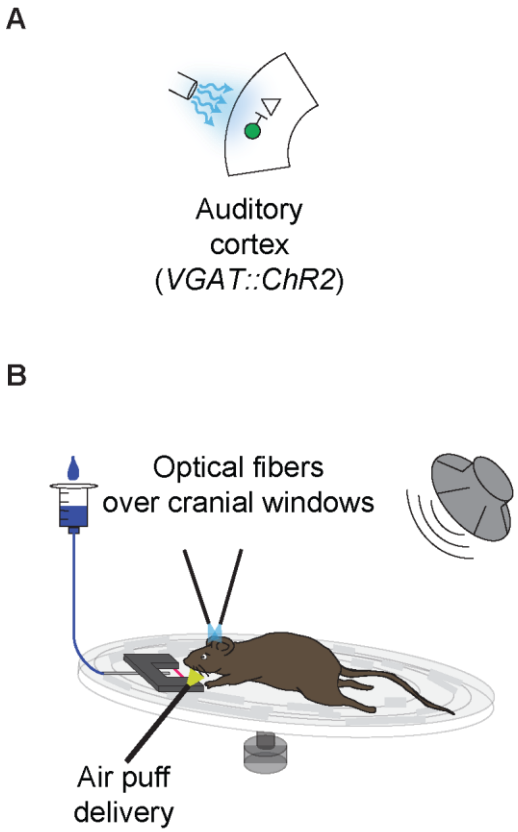


Figure 12: Optogenetic suppression of auditory cortex during auditory detection. A) Optogenetic activation of inhibitory neurons in the auditory cortex of a VGAT::ChR2 mouse B) Optogenetic suppression of auditory cortex during auditory detection.

Psychometric curves were calculated for rest, running and laser trials during rest. Mice were significantly worse at detecting tones during running compared to rest (RM 2-way ANOVA at non-zero intensities, p (intensity x behavioral state) = 0.03, F (2, 6) = 6.60, post hoc Tukey test). Mice were also significantly worse at detecting tones in the laser condition compared to resting performance (Figure 13A; RM 2-way ANOVA at non-zero intensities. p (intensity x laser state) = 0.01, F (2, 6) = 14.27, post hoc Tukey test, ANOVA p values were corrected using the Holm Bonferroni method). In a subset of mice, we could only obtain psychometric curves for rest and laser trials. Overall, mice also showed impaired detection on laser trials compared to non-laser trials at rest (Figure 13B; RM 2-way ANOVA, p (intensity x laser state) =0.0028, F (2, 10) = 11.23, post hoc Tukey test). These results indicate that suppressing auditory cortical activity using local inhibitory neuronal populations in a temporally specific and reversible manner degrades auditory detection in the mouse suggesting that the auditory cortex is a part of the auditory detection circuit in the mouse and that dynamic auditory cortical modulations by engaging local inhibitory neuronal populations within the auditory cortex can influence auditory detection.

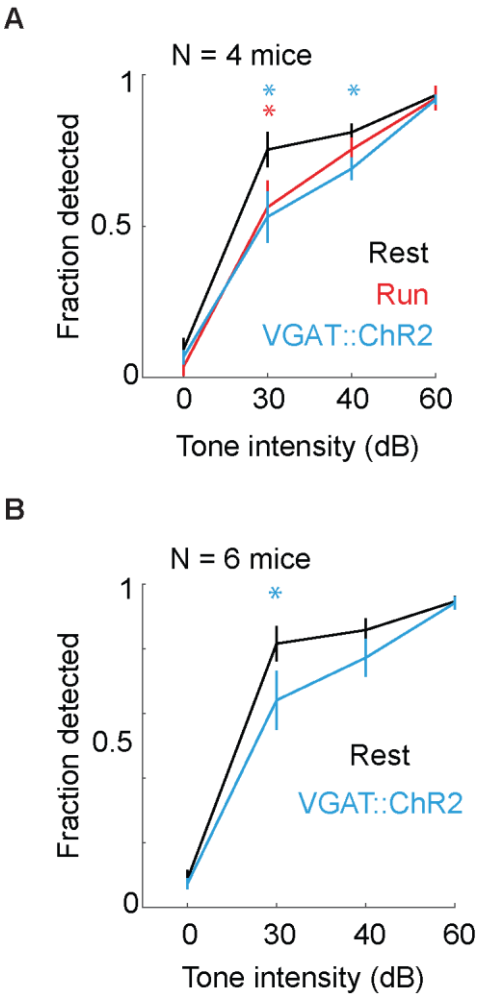


Figure 13: Optogenetic suppression of auditory cortex impairs auditory detection. A) Tone detection performance (N = 4 mice) during rest (black), running (red) and rest with optogenetic activation of auditory cortical inhibitory neurons (blue). Mice were worse at detecting tones during optogenetic trials compared to non-optogenetic trials at rest, (blue asterisk $p<0.05$, RM 2-way ANOVA, post hoc Tukey test) and during running compared to rest. (red asterisk $p<0.05$, RM 2-way ANOVA, post hoc Tukey test). B) Tone detection performance (N = 6 mice) during rest (black) and rest with optogenetic activation of auditory cortical inhibitory neurons (blue). Mice were worse at detecting tones on optogenetic trials compared to rest. (blue asterisk: $p<0.05$ on laser trials. RM 2-way ANOVA, post hoc Tukey test)

3.2.3 Optogenetic suppression of visual cortical activity

One possibility is that optogenetically activating inhibitory neurons in the cortex generates an internal neural distractor signal, impairing tone detection on laser trials compared to non-laser trials. If this is true, suppressing any part of the cortex by activating inhibitory neurons should impair tone detection. To confirm that the behavioral effect observed above is indeed a consequence of auditory cortical modulation, I optogenetically activated inhibitory neurons in the visual cortex of VGAT-ChR2 mice engaged in the tone detection task. Mice were implanted with circular glass coverslips bilaterally over visual cortex (identified using stereotaxic coordinates) at the same time as an annular headpost was attached to the skull. The same experimental protocol described in Chapter 3.2.2 was used to train and test the ability of these mice to detect tones during rest and laser stimulation during rest. In contrast to suppression of auditory cortical activity during tone detection, optogenetic suppression of visual cortical activity did not impair tone detection compared to performance at rest (Figure 14; RM 2-way ANOVA at non-zero intensities). These results suggest that non-specific neural distractor effects cannot account for the diminished tone detection performance during suppression of auditory cortical activity.

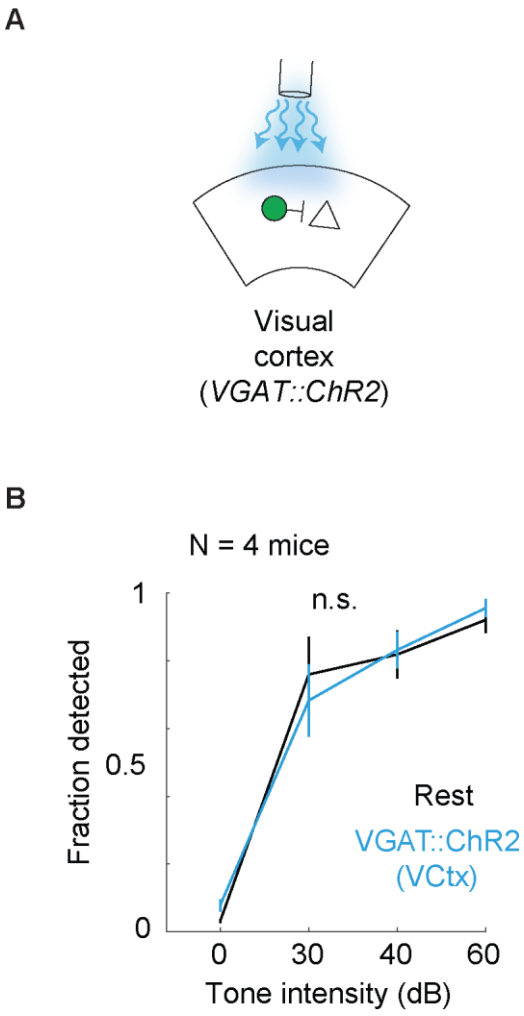


Figure 14: Optogenetic suppression of visual cortex during auditory detection. A) Optogenetic activation of inhibitory neurons in the visual cortex of VGAT::ChR2 mice. B) Tone detection performance (N = 4 mice) during rest (black) and rest with optogenetic activation of visual cortical inhibitory neurons (blue). Auditory detection was not significantly impaired during optogenetic suppression of visual cortex. (RM 2-way ANOVA)

3.2.4 Sham controls

Is the degradation in auditory detection during optogenetic suppression of auditory cortical activity due to visual distractors? Specifically, were mice distracted by the light stimulus on optogenetic trials, leading to lower detection rates on optogenetic trials compared to non-optogenetic trials? To address this, I conducted sham experiments in a subset of VGAT ChR2 mice that were also used for optogenetic suppression of auditory cortical activity during auditory detection. The same experimental protocol used for optogenetic stimulation during auditory detection (described in Chapter 3.2.2) was also adapted for sham stimulation in these mice, and sham experiment days were interleaved with days consisting of actual optogenetic stimulation. On sham days, blue light was flashed over intact skull during tone presentation on 50% of the trials (Figure 15A). Pyschometric curves were calculated for rest and light trials during rest. There was no significant difference in detection rates between rest and laser conditions (Figure 15B; RM 2-way ANOVA at non-zero intensities), indicating that visual distractors cannot account for the impaired auditory detection during optogenetic suppression of auditory cortex.

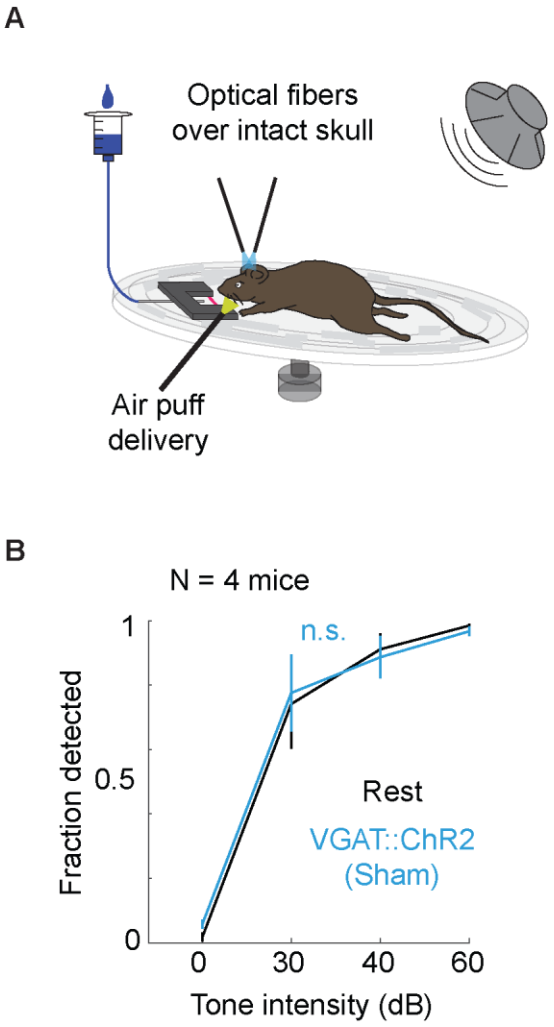


Figure 15: Laser stimulation over intact skull does not impair auditory detection. A) Shining blue laser over intact skull during auditory detection. B) Tone detection performance (N = 4 mice) during rest (black) and during rest with laser activation (blue) when optical fibers were placed over intact skull near, but not directly over auditory cortex. Sham laser stimulation (which is visible to the mouse) does not impair auditory detection. (RM 2-way ANOVA)

3.3 Chapter 3 conclusions

Here I have used pharmacological and optogenetic methods in combination with behavioral testing to demonstrate that the auditory cortex is a component of the auditory detection circuit in the mouse. Auditory detection is degraded during pharmacological or optogenetic suppression of auditory cortex, and this impairment cannot be attributed to non-specific neural or visual distractor effects. Furthermore, these experiments also demonstrate that modulating auditory cortical activity by activating local inhibitory neurons, which are recruited during a variety of behavioral states including movements, can influence auditory perception.

4. The role of secondary motor cortical projections to the auditory cortex in movement-related changes in auditory detection

4.1 Introduction

What are the circuit mechanisms underlying the movement-related degradation of auditory detection? Experiments in Chapter 3 have indicated a role for auditory cortical modulation in changes in auditory detection. Auditory cortical responses to sounds are strongly suppressed during movements, and projections from the secondary motor cortex (M2) to the auditory cortex account for more than half of this suppression (Schneider et al., 2014). To test if this circuitry also plays a role in degrading auditory detection during running compared to rest, I activated M2 terminals in the auditory cortex during tone detection at rest. Specifically, I tested the hypothesis that engaging motor cortical terminals in the auditory cortex during rest, changes tone detection in a manner comparable to movement. The results of these experiments have been previously published (Schneider, Sundararajan and Mooney, 2018).

4.2 Activating secondary motor cortical projections to the auditory cortex recapitulates movement-related changes in auditory detection.

4.2.1 Optogenetic activation of secondary motor cortical terminals in auditory cortex

I used optogenetic methods to activate secondary motor cortical axon terminals in the auditory cortex of mice during auditory detection. I first injected ~300nl of AAV.1.hsyn.ChR2.EYFP.WPRE bilaterally into the secondary motor cortex of C57 mice, at the same time implanting a Y-shaped headpost on the skull. These mice were then trained on the auditory detection task described in Chapter 2.2. 2-4 weeks following viral injection, cranial windows were bilaterally implanted over the auditory cortex. Mice were tested on their ability to detect a subset of tone intensities (0, 30, 40, and 60dB). Optogenetic stimulation of M2 terminals in the auditory cortex during auditory detection was done using the same protocol described in Chapter 3.2.2 (Figure 16).

Psychometric curves were calculated for rest, running and laser trials during rest. Mice were significantly worse at detecting tones on laser trials, compared to non-laser trials at rest (RM 2-way ANOVA at non-zero intensities, p (intensity x laser state) = 0.04, F (2, 6) = 5.84, followed by post hoc Tukey test). Mice were also significantly worse at detecting tones on running trials, compared to rest trials (Figure 17A; RM 2-way ANOVA at non-zero intensities, p (intensity x behavioral state) = 0.04, F (2, 6) = 8.29, followed by post hoc Tukey test). In a subset of mice, we presented tone pips of a

different frequency (4kHz) during rest and laser trials. Mice showed impaired detection on laser trials compared to non-laser trials at rest regardless of tone frequency (Figure 17B; RM 2-way ANOVA, p (intensity x laser state) =0.0028, F (2, 10) = 11.23, post hoc Tukey test).

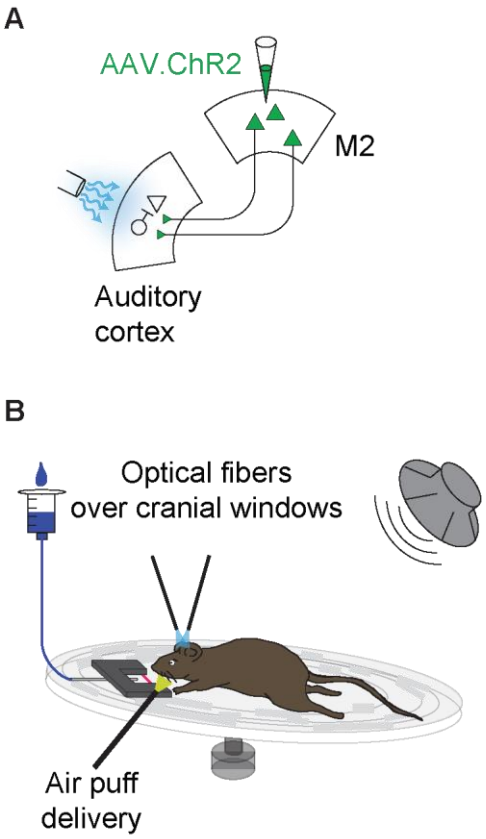


Figure 16: Optogenetic activation of M2 terminals in auditory cortex during auditory detection. A) An AAV encoding Channelrhodopsin was injected into the secondary motor cortex of mice for optogenetic activation of terminals in the auditory cortex B) Optogenetic activation of secondary motor cortical terminals in the auditory cortex during auditory detection.

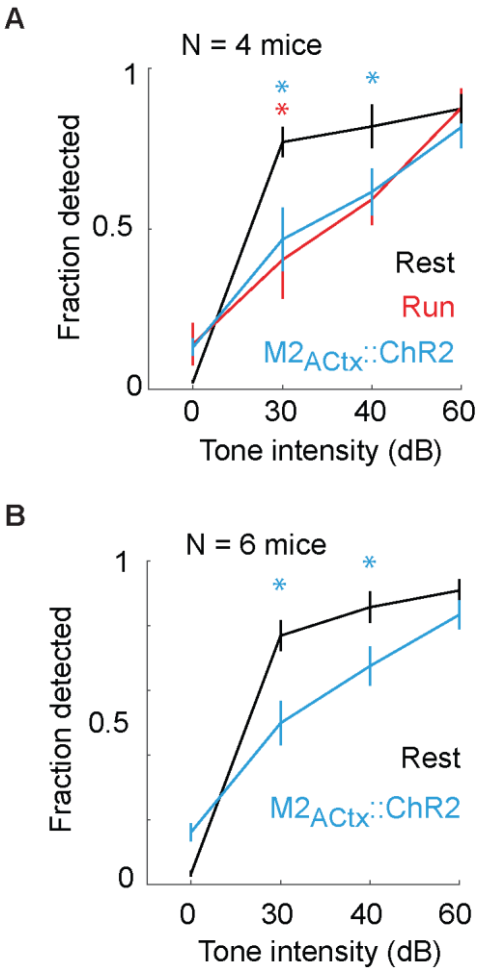


Figure 17: Activating M2 terminals in auditory cortex impairs auditory detection. A) Tone detection performance (N = 4 mice) during rest (black), running (red) and rest with optogenetic activation of M2 terminals in auditory cortex (blue). Mice were worse at detecting tones during optogenetic trials compared to non-optogenetic trials at rest, (blue asterisk, $p<0.05$, RM 2-way ANOVA, post hoc Tukey test) and during running compared to rest (red asterisk, $p<0.05$, RM 2-way ANOVA, post hoc Tukey test). B) Tone detection performance (N = 6 mice) during rest (black) and rest with optogenetic activation of M2 terminals in auditory cortex (blue). Four of these mice were presented with 8kHz tones and the remaining two were presented 4kHz tones. Mice were worse at detecting tones on optogenetic trials regardless of the tone frequency. (blue asterisk $p<0.05$ on laser trials, RM 2-way ANOVA, post hoc Tukey test).

In a subset of mice, I also optogenetically activated M2 terminals in the auditory cortex during running trials. Performance was degraded on both laser and non-laser trials during running compared to baseline performance at rest (Figure 18; RM 2-way ANOVA, p (intensity x condition) = 0.0018, F (6, 24) = 5.03, followed by post hoc Tukey test). Performance on laser trials during running was however not significantly different from performance on non-laser trials during running, indicating that over-engaging M2 terminals during movement does not further impair performance compared to running. Overall, these observations suggest that secondary motor cortical modulation of auditory cortical activity, which has been shown to play a role in the movement-related suppression of auditory cortical activity, also degrades auditory detection in mice.

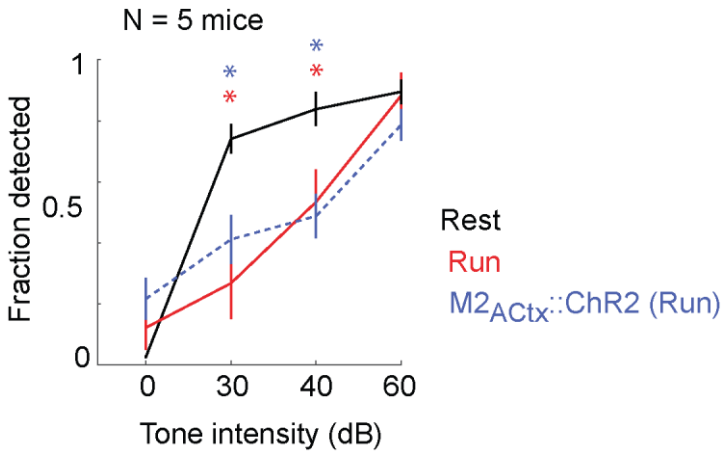


Figure 18: Activating M2 terminals in auditory cortex during running. Tone detection performance (N = 5 mice) during rest (black), running (red) and running with optogenetic activation of M2 terminals in auditory cortex (blue dashed line). Four of these mice were presented with 8kHz tones and one of them was presented with 4kHz tones. Mice were worse at detecting tones during running compared to rest (red asterisk, $p<0.05$, RM 2-way ANOVA, post hoc Tukey test), and on optogenetic trials during running compared to baseline performance at rest, (blue asterisk, $p<0.05$, RM 2-way ANOVA, post hoc Tukey test).

4.2.2 GFP controls

Is the degradation in auditory detection during optogenetic activation of M2 axons in the auditory cortex due to non-specific effects of shining blue light over the auditory cortex? To address this question, I conducted a control experiment where I injected ~300nl of AAV.1.CB7.eGFP.WPRE bilaterally into the secondary motor cortex of C57 mice. These mice were then trained on the auditory detection task using operant conditioning methods described above, and auditory cortical cranial windows were bilaterally implanted 2-4 weeks following viral injection. During testing, blue light was bilaterally flashed over auditory cortical cranial windows, on a subset of trials during rest (refer to Chapter 3.2.2 for details of laser stimulation). Performance on laser trials was not different from baseline performance on non-laser trials at rest (Figure 19), suggesting that non-specific neural effects due to shining blue light cannot account for the degradation in auditory detection induced by optogenetic activation of secondary motor cortical terminals in the auditory cortex.

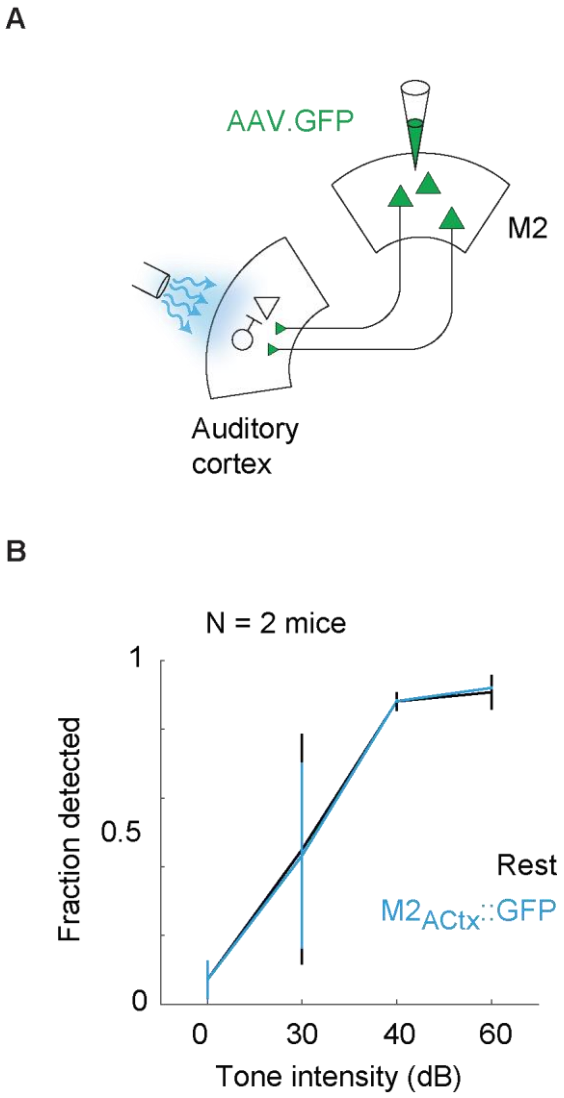


Figure 19: Shining blue light on M2 terminals expressing GFP in auditory cortex does not impair auditory detection. A) Mice were injected with an AAV encoding eGFP in M2. Blue light was shined over auditory cortex during auditory detection. B) Tone detection performance (N = 2 mice) during rest (black) and during rest with laser stimulation over auditory cortex (blue). Laser stimulation of M2-eGFP terminals in auditory cortex does not impair auditory detection.

4.2.3 Sham controls

Similar to the optogenetic experiments described in Chapter 3, it was also important to rule out the role of blue light as a visual distractor on laser trials compared to non-laser trials. I conducted sham experiments on a subset of the mice using the same protocol as detailed in Chapter 3.2.4. On sham days, blue light was flashed over intact skull in conjunction with tone presentation on 50% of the trials. Performance was not degraded on light trials compared to baseline performance on non-laser trials at rest (Figure 20). Interestingly, detection rates were higher on light trials compared to rest (RM 2-way ANOVA at non-zero intensities, p (intensity x laser state) = 0.009, F (2, 8) = 8.86, post hoc Tukey test), indicating that in the absence of neural stimulation, mice used light as a predictive cue on laser trials to boost their performance. This improvement in performance is in the opposite direction compared to the degradation observed during optogenetic activation of M2 terminals in the auditory cortex. This indicates that visual distractor effects cannot account for the degradation in auditory detection observed during the optogenetic activation of motor cortical terminals in the auditory cortex.

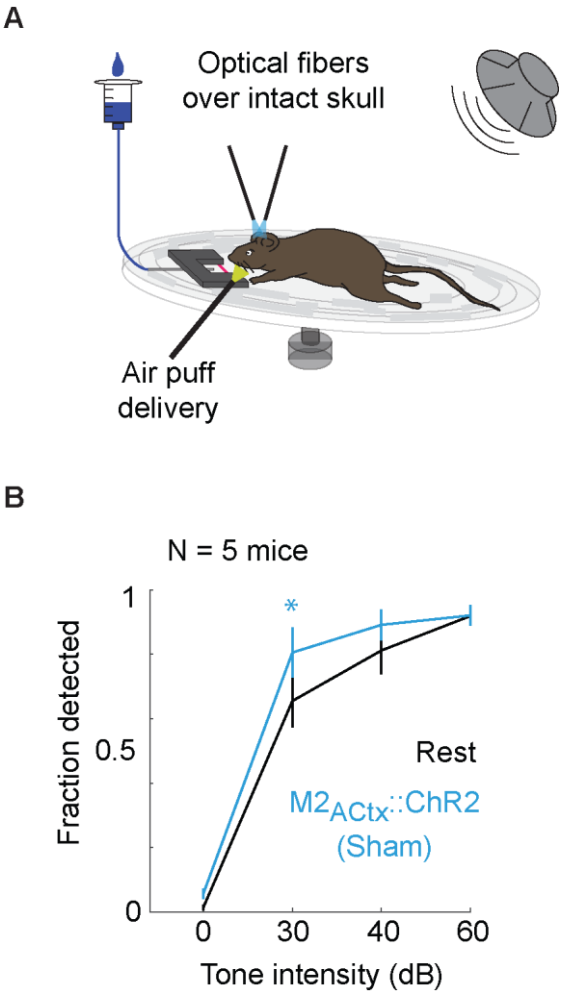


Figure 20: Laser stimulation over intact skull does not impair auditory detection. A) Shining blue laser over intact skull during auditory detection. B) Tone detection performance (N = 5 mice) during rest (black) and during rest with laser activation (blue) when optical fibers were placed over intact skull near, but not directly over auditory cortex. 3 mice were presented with 8kHz tones and 2 with 4kHz tones. Sham laser stimulation (which is visible to the mouse) does not impair auditory detection. Auditory detection is enhanced on laser trials compared to rest. (blue asterisk: $p<0.05$ on laser trials. RM 2-way ANOVA, post hoc Tukey test)

4.3 Chapter 4 conclusions

In these experiments, I have demonstrated that optogenetically activating secondary motor cortical projections within the auditory cortex during rest shifts auditory detection performance in a manner similar to running. Additionally, activating the same projections during running also degrades performance relative to rest, but does not further impair detection compared to baseline performance during running. These results suggest that the same circuit mechanisms that play a role in the movement-related suppression of auditory cortical activity, also account for the movement-related impairment of auditory detection.

5. Movement-related changes in auditory detection are experience-dependent

5.1 Introduction

One idea is that the movement-related suppression of auditory cortical activity functions in a predictive fashion to detect and differentiate self-generated (reafferent) stimuli from environmental (exafferent) stimuli. To explore this idea, I trained mice on a variant of the single frequency auditory detection task. I designed a two-frequency auditory detection task, where one of the frequencies was predictively coupled to the animal's running on the treadmill, by providing real-time, computer-controlled acoustic feedback that was tightly linked to the mouse's running on the treadmill for several days. Following this temporally coupled locomotor-auditory experience, I tested whether perceptual responses to the uncoupled acoustic stimulus were different from responses to the sound predictably coupled with running. These experiments were done in collaboration with Dr. David Schneider and have been previously published (Schneider, Sundararajan and Mooney, 2018). David Schneider performed experiments described in Chapters 5.1.1 – 5.1.3. I performed the remaining experiments.

5.1.1 Movement-related suppression of auditory cortex is selective to expected sound frequencies

To simulate an experimentally adjustable yet predictable form of auditory reafference associated with locomotion, David Schneider developed an acoustic virtual reality (aVR) system where head-fixed mice positioned atop a non-motorized treadmill heard a series of tones (25 ms) of a given frequency (2, 4, 8, 16, 32, or 64 kHz) and intensity, presented at a rate proportional to their running speed (Figure 21). Mice gained aVR experience for ~2 hours per day for ~ 1 week, over which time each mouse cumulatively ran several hundred meters on the treadmill and heard several thousand tones. Following aVR experience, action potential activity of primary auditory cortex neurons was recorded in response to both reafferent (aVR-coupled) and non-reafferent (aVR-uncoupled) tone frequencies during resting and running on the treadmill (Figure 22A). Auditory cortical responses to the reafferent tone frequency were nearly abolished during locomotion while responses to frequencies one or more octaves distant from the reafferent frequency showed a more modest amount of suppression similar to that seen during locomotion in aVR-naïve mice. Notably, this differential suppression only manifested during movement: in resting mice, auditory cortical neurons responded to reafferent and non-reafferent frequencies with equivalent firing rates (Figure 22B). Furthermore, calculating a locomotion-related gain function for each neuron to measure the strength of locomotion-induced suppression at each frequency revealed that,

regardless of a neuron's best frequency, locomotion-related suppression was greater for the reafferent frequency than for tone frequencies one or more octaves higher or lower. Averaging these gain functions across neurons revealed the presence of a notch filter centered at the reafferent frequency indicating that, following aVR experience, locomotion engages a filter that dampens auditory cortical responses to a predictable reafferent sound compared to unexpected sounds (Figure 22C).

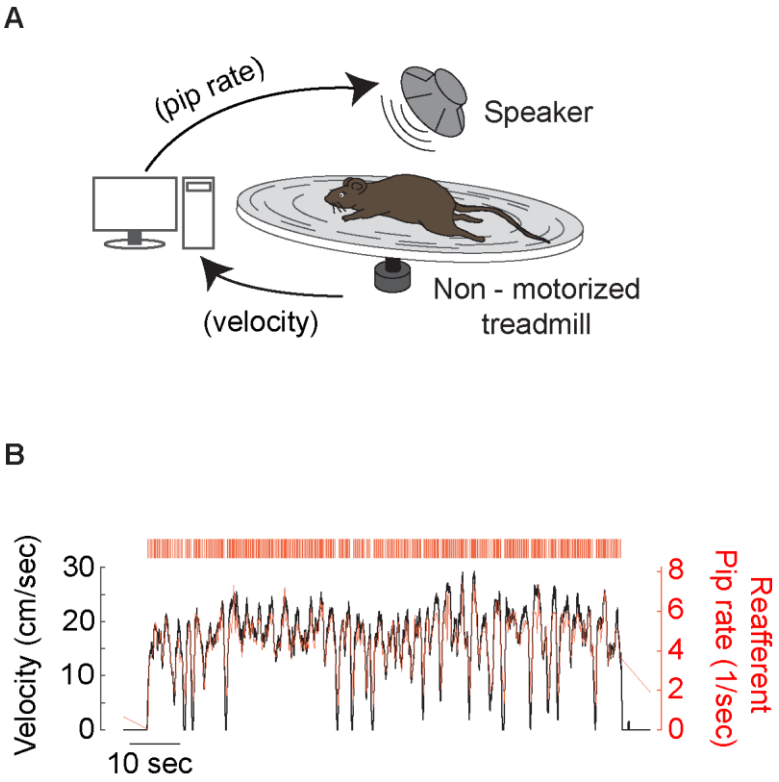


Figure 21: Acoustic virtual reality setup. A) Mice were acclimated to a closed-loop acoustic virtual reality (aVR) system. The rate at which brief (25msec) tones of a fixed pitch were played from a speaker was coupled to the mouse's running velocity. B) Example trace of locomotion (black trace), timing of individual aVR tone pips (red ticks at top), and instantaneous rate of aVR tone pips (red trace).

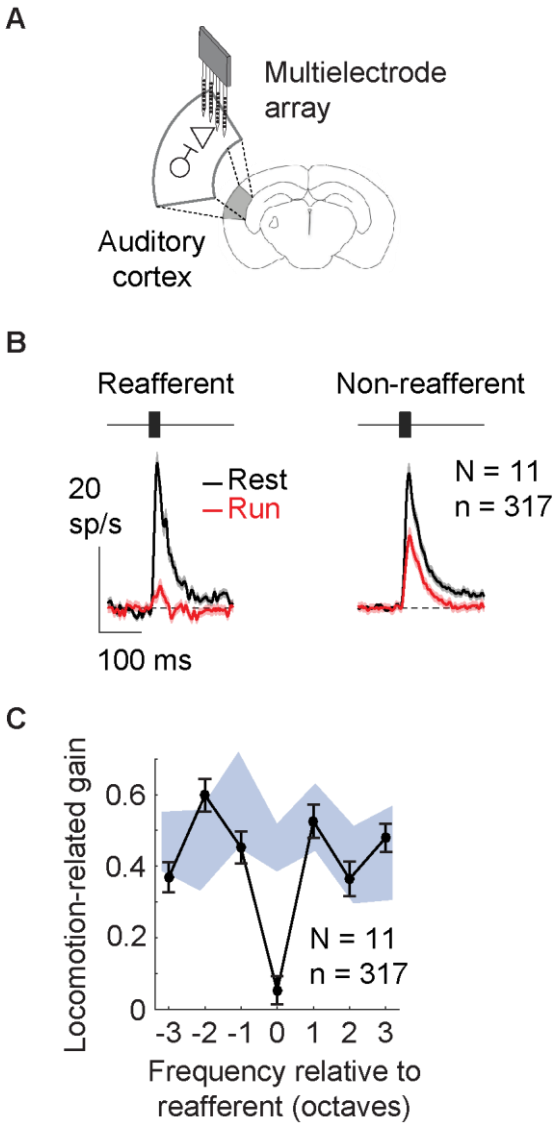


Figure 22: Locomotion-related suppression is specific for the frequency of self-generated sounds. A) Extracellular recordings from auditory cortical neurons after 6-9 days of aVR acclimation. B) Population PSTHs showing neural responses to expected (left) and unexpected (right) frequencies during running and rest. During running, responses to the expected reafferent frequency were suppressed relative to unexpected frequencies ($p<0.0001$). C) Average locomotion-related suppression (black, mean +/- SE) of auditory cortical neurons, centered on the expected reafferent frequency heard by each mouse. Blue area shows 95% confidence bounds from shuffled data with the reafferent frequency of every neuron assigned randomly.

Movement suppresses auditory responses at cortical and subcortical levels of the auditory system in untrained animals. However, unlike the frequency-specific suppression observed in the auditory cortex of aVR experienced mice, movement-related suppression in auditory thalamic neurons remained flat across sound frequencies (Figure 23A), indicating that circuits local to the auditory cortex are likely the source of the reafferent notch filter that arises following aVR experience.

The formation of this auditory cortical filter required a predictable and prolonged association of movement with an ensuing sound. Mice acclimated for ~1 week on a treadmill in which fixed-frequency tones were presented only at rest did not display enhanced cortical suppression at the training frequency during rest or running (Figure 23B). Furthermore, mice acclimated for ~1 week on a treadmill in which tones were presented at a fixed tempo during locomotion regardless of running speed (i.e., "metronome"-experienced mice) showed no enhanced locomotion-related auditory cortical suppression at the training frequency (Figure 23C).

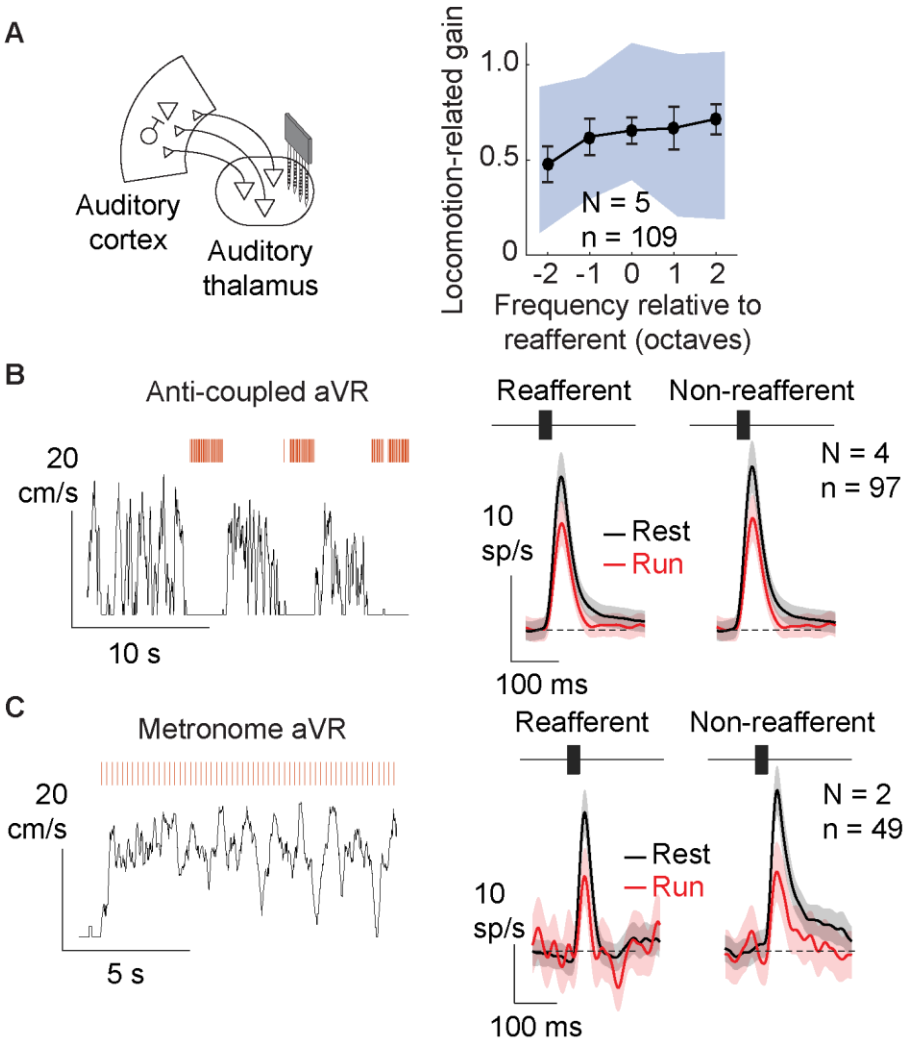


Figure 23: Frequency-specific locomotion-related suppression requires several days of coupled sensory-motor experience. A) *Left:* extracellular recordings from the auditory thalamus following 6-9 days of aVR acclimation. *Right:* Locomotion-related suppression (black, mean +/- SE) of auditory thalamic neurons is not specific to the reafferent frequency B) *Left*: Anti-coupled aVR experience. Tones were played during rest with inter-tone intervals drawn from intervals that mice should have heard while running. *Right:* Population PSTHs showing that anti-coupled aVR experience does not change auditory responsiveness during running or rest. C) *Left:* Metronome aVR experience. Tones were presented during running at a fixed rate (2/sec) during running. *Right:* Population PSTHs showing that metronome aVR experience does not selectively change auditory responsiveness during running or rest.

5.1.2 Auditory cortical responses undergo bidirectional changes to expected and unexpected tone frequencies following aVR experience

To determine more precisely the time course over which the movement-related notch filter arises, David Schneider performed 2-photon calcium imaging experiments to longitudinally image Layer II/III excitatory neurons in the auditory cortex of mice across their first 5 days of aVR experience. CaMKII-Cre mice were first injected with ~300 nl of AAV.1.hSyn.FLEX.GCaMP6f in the left auditory cortex. Three weeks following viral infection a cranial window was implanted over the left auditory cortex, and mice were acclimated to the aVR treadmill setup for 2-photon imaging (Figure 24A). Prior to aVR experience, locomotion-related suppression of aVR-naïve mice at each frequency was independently determined for the neurons in the given field of view. The sound frequency that had the least amount of movement-related suppression was chosen as the reafferent frequency for aVR experience on subsequent days. This approach allowed us to not bias our results toward sound frequencies that were already strongly suppressed in a given field of view.

On each imaging day, mice ran on the aVR treadmill and heard fixed-frequency tones yoked to their running speed for ~2 hours. Following each ~2 hour aVR session, tones of random frequency (2 to 64 kHz) were presented during running (with timing yoked to the mouse's running speed) and rest (with timing chosen from the running inter-tone-intervals) during image acquisition until mice heard at least 50 tones of each

frequency. Calcium traces of all ROIs within an imaging session that were responsive to a particular frequency were averaged together independently for running and rest. With aVR experience, locomotion-related suppression became progressively more specific for the reafferent frequency, indicating a gradual emergence of frequency-specific suppression that parallels the accumulation of sensorimotor experience (Figure 24B). This was accompanied by a bidirectional change, involving both an increase in the suppression of the expected reafferent frequency and a decrease in the suppression of unexpected frequencies during locomotion (Figure 24C).

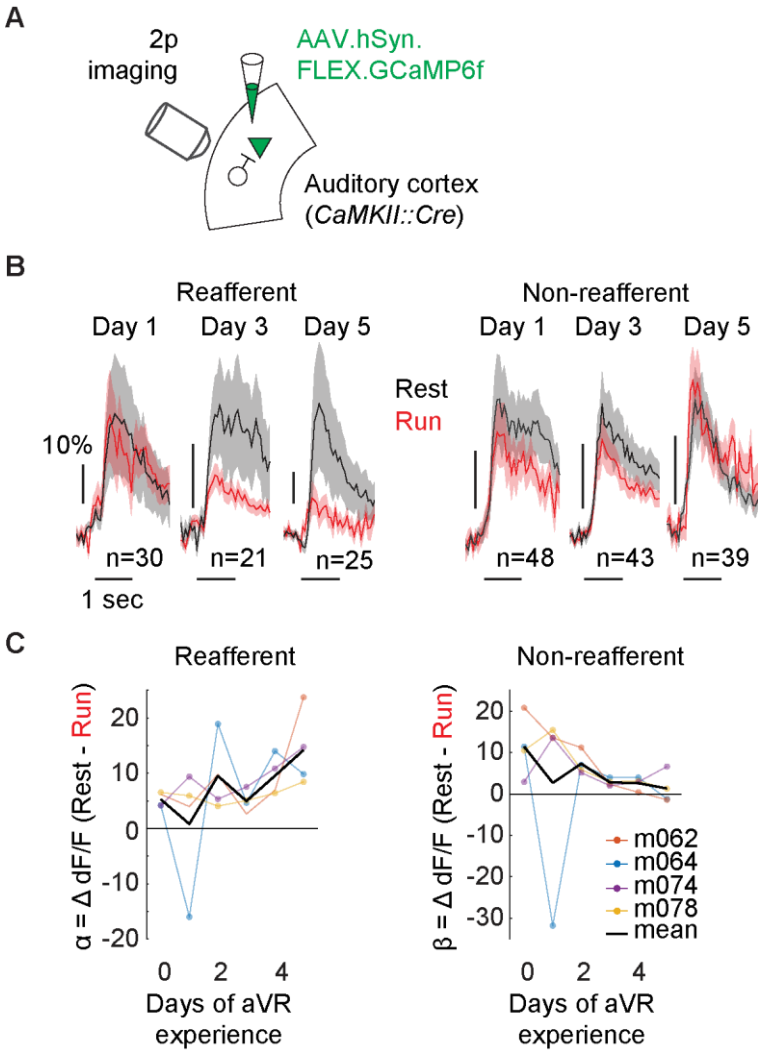


Figure 24: Bidirectional changes in auditory cortical responses to reafferent and non-reafferent frequencies arise in parallel with sensorimotor experience. A) Injection of AAV encoding Cre-dependent GCaMP6f into auditory cortex of CaMK2::Cre mice followed by calcium imaging of L2/3 excitatory neurons during aVR experience. B) Population-averaged calcium transients evoked by the reafferent frequency and a non-reafferent frequency (2 octaves away) during rest and running on days 1, 3 and 5 of aVR experience. Shaded areas show mean +/- SE. C) Magnitude of locomotion-related suppression for reafferent (*left*) and non-reafferent (*right*) frequencies, measured as the difference between population-averaged calcium responses during rest and running. Locomotion-related suppression across days becomes stronger for the reafferent frequency and weaker for non-reafferent frequencies.

5.1.3 Secondary motor cortical projections to the auditory cortex undergo experience-dependent changes in plasticity

Auditory cortical interneurons integrate auditory signals with locomotor-related signals from neurons in the secondary motor cortex (M2), making them attractive candidates where aVR experience could act to drive the formation of the movement-dependent notch filter described above (Nelson et al., 2013; Schneider et al., 2014). One possibility is that following aVR experience, inhibitory interneurons that respond to the reafferent frequency become more strongly recruited during locomotion by M2 inputs to the auditory cortex than do non-responsive interneurons, resulting in the expression of a notch filter on excitatory cells (Figure 25A). To test this idea, we expressed Channelrhodopsin (ChR2) in various types of inhibitory neurons in different mice, subjected them to ~1 week of aVR experience, and then recorded the action potential activity of photo-identified auditory cortical inhibitory cells. Frequency-tuning curves were calculated for photo-identified PV+, SST+, and VGAT+ inhibitory neurons in the auditory cortex. Next, brief current pulses were applied in M2 and resultant action potential activity was measured in these identified interneurons. These measurements revealed that auditory cortical inhibitory neurons that strongly responded to the reafferent frequency were driven more strongly by M2 stimulation than inhibitory neurons that responded only weakly or not at all to the reafferent frequency (Figure 25B). Such functional enhancement was not observed between M2 and auditory cortical

interneurons non-responsive to the reafferent frequency, and there was only a weak relationship between the magnitude of tone-evoked and M2-stimulation-evoked responses in auditory cortical interneurons of aVR-naïve mice. There was also a similar but weaker correlation for auditory cortical excitatory neurons in aVR-experienced mice (Figure 25C). These differential effects of M2 stimulation on auditory cortical excitatory and inhibitory neurons are consistent with the observation that M2 synapses excite both cell types but exert a primarily suppressive effect on auditory cortical activity through feedforward inhibition (Nelson et al., 2013; Schneider et al., 2014; Zhou et al., 2014). These findings advance a model in which aVR experience strengthens motor cortical inputs to reafferent frequency-responsive inhibitory interneurons in the auditory cortex, leading to the locomotor-dependent suppression of auditory cortical responses to predictable self-generated sounds.

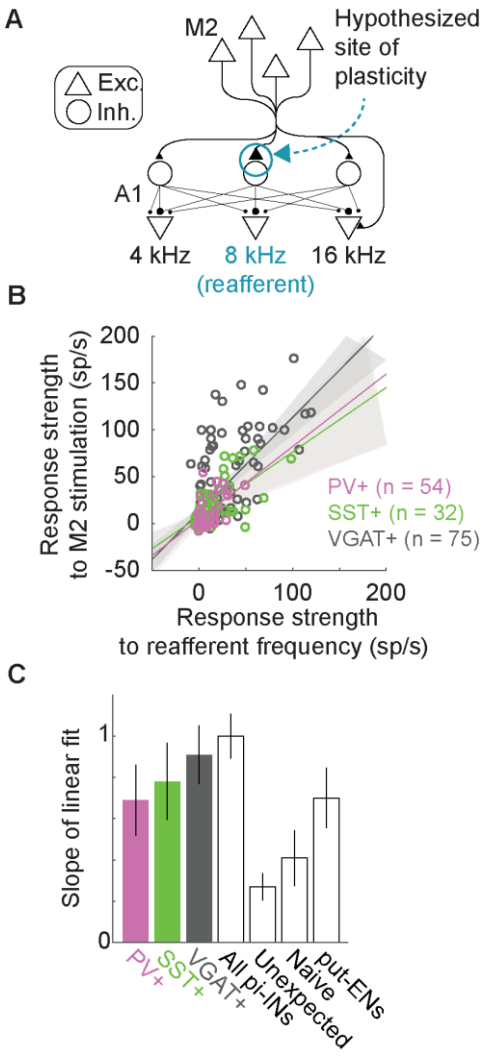


Figure 25: Reafferent-tuned inhibitory neurons receive enhanced M2 input following aVR experience. A) Model of experience-dependent strengthening of M2 inputs onto A1 inhibitory neurons tuned to the reafferent frequency (blue circle). B) PV+, SST+ and VGAT+ pi-INs that were more strongly driven by the reafferent frequency were more strongly recruited by M2 electrical stimulation. Lines and shaded areas show linear regression and 95% confidence bounds from bootstrap analysis. C) Slope of the linear fit for the relationship shown in (B) and four other conditions. All pi-INs: all pi-INs recorded from PV+, SST+ and VGAT+ mice. Unexpected: VGAT+ neurons but for responses to an unexpected tone. Naïve: pi-INs recorded from VGAT+ mice acclimated to a quiet treadmill. put-ENs: putative excitatory neurons in auditory cortex of VGAT::ChR2 mice. Error bars show 95% confidence bounds from bootstrap analysis.

5.2 Movement-related modulation of hearing is experience dependent

5.2.1 Two frequency auditory detection task

To test whether the auditory cortical filter formed by aVR experience differentially impacts the mouse's ability to detect reafferent versus non-reafferent tones during movement, we trained mice on a variant of the single tone frequency auditory detection task explained in Chapter 2. The new task consisted of tones of two frequencies (Tone A and Tone B, separated by 2 octaves), which were randomly interleaved from one trial to the next. Training and testing methods were similar to the single-frequency detection task (described in Chapter 2.2), but using tones of two frequencies (4 and 16 kHz) and a subset of tone intensities (0, 30, 40, 50 and 60 dB). During behavioral testing, trials were presented in a block design in which each block consisted of all the frequency-intensity combinations. Following an initial phase of behavioral testing during rest and running, the lick-detector and reward port were removed from the treadmill and mice received aVR experience with one of the two testing frequencies (Tone A) for ~1 week, 1~2 hours per day. Five mice each received aVR experience with 4 kHz and 16 kHz. After 7 days of aVR experience, behavioral testing was performed again to test the ability of mice to detect both tone frequencies during rest and running. In this phase, at the beginning of each day, mice received ~30 min of experience with aVR, during which time they ran and heard the reafferent

frequency they were acclimated to over the previous 7 days. This brief re-exposure to aVR was followed by a short period of time in their home cage without any aVR or behavioral testing. At the end of this period, we reintroduced them to the behavioral testing chamber where mice were tested on their ability to detect both tone frequencies during rest and running (Figure 26). We tested the hypothesis that, following aVR experience, mice would show different detection rates for the reafferent tone frequency (Tone A) compared to the non-reafferent tone frequency (Tone B) during movement.

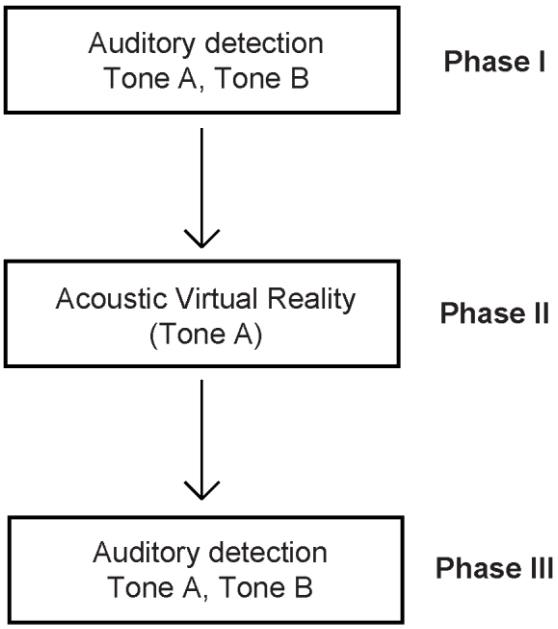


Figure 26: Two frequency auditory detection task. Phase I: Mice were initially tested on their ability to detect two tone frequencies during rest and running. Phase II: Mice received aVR experience with one of the two frequencies from phase I. Phase III: Mice were again tested on their ability to detect both tone frequencies during rest and running.

5.2.2 Baseline psychometric curves

After several days of training, mice were tested on their ability to detect the 2 tone frequencies during both rest and running. Baseline psychometric curves were calculated as a function of intensity for both tone frequencies during rest and running. Similar to the single frequency detection task, mice showed a deficit in detecting both tone frequencies during locomotion compared to rest (Figure 27; RM 2-way ANOVA at non-zero intensities. For Tone A: p (intensity x behavioral state) = 0.0002, F (3, 27) = 11.56. For Tone B: p (intensity x behavioral state) = 0.04, F (3, 27) = 3.85), followed by post hoc Tukey test. ANOVA p values corrected using the Holm Bonferroni method).

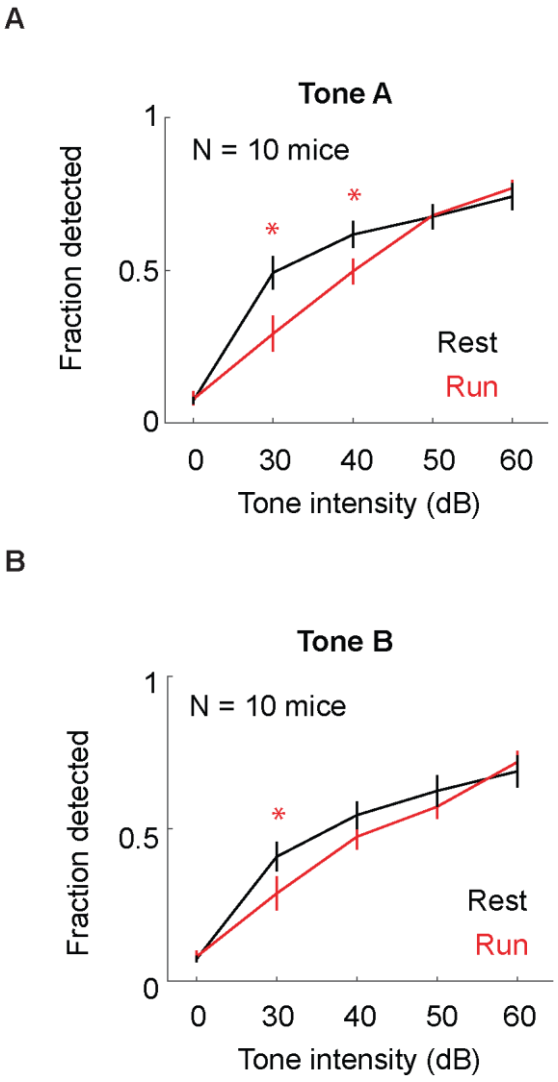


Figure 27: Psychometric curves during rest and running for 2-frequency auditory detection task in naïve mice. Tone detection performance (N = 10 mice) during rest (black) and running (red) for naïve mice detecting Tone A (*top*) or Tone B (*bottom*). Mice were significantly worse at detecting tones during running compared to rest for both tone frequencies (red asterisk $p<0.05$, RM 2-way ANOVA, followed by post hoc Tukey test).

5.2.3 aVR experience

Following the initial behavioral testing phase, in phase II, mice received ~1 week of aVR experience in which only one of the tones (Tone A) was used as a reafferent training stimulus. Over the course of this experience, mice cumulatively heard tens of thousands of tones (average: 90,535 +/- 24,902) that were predictively coupled with their running speed on the treadmill. This sensorimotor experience is similar to the experience received by mice in the electrophysiology and calcium imaging experiments described in Chapters 5.1.1, and 5.1.2, presumably forming a frequency specific notch filter during running compared to rest.

5.2.4 Psychometric curves following aVR experience

In the last phase of this experiment (Phase III), aVR-experienced mice were re-introduced to the behavioral chamber, where they were tested on their ability to detect both the reafferent and non-reafferent tone frequencies during rest and running. In contrast to their performance in phase I in the aVR-naïve state, aVR-experienced mice no longer showed a locomotion-related deficit in detecting the non-reafferent tone frequency (Tone B), even though they continued to show a movement-related deficit in detecting Tone A, the reafferent tone frequency (Figure 28; RM 2-way ANOVA at non-zero intensities. For Tone A: p (intensity x behavioral state) = 0.01, F (3, 27) = 5.62. For

Tone B: p (intensity x behavioral state) = 0.05, F (3, 27) = 2.90), post hoc Tukey test. ANOVA p values corrected using the Holm Bonferroni method). Furthermore, aVR-experienced mice were better at detecting the non-reafferent tone frequency during running compared to the reafferent tone frequency (Figure 29; RM 2-way ANOVA at non-zero intensities. For rest: p (time of testing) = 0.46, F (1, 9) = 0.61. For running: p (time of testing) = 0.04, F (1, 9) = 8.07, ANOVA p values corrected using the Holm Bonferroni method). These results indicate that aVR experience differentially affects the mouse's ability to detect predictable and unpredicted sounds during movement in addition to selectively suppressing auditory cortical responses to predictable reafferent sounds.

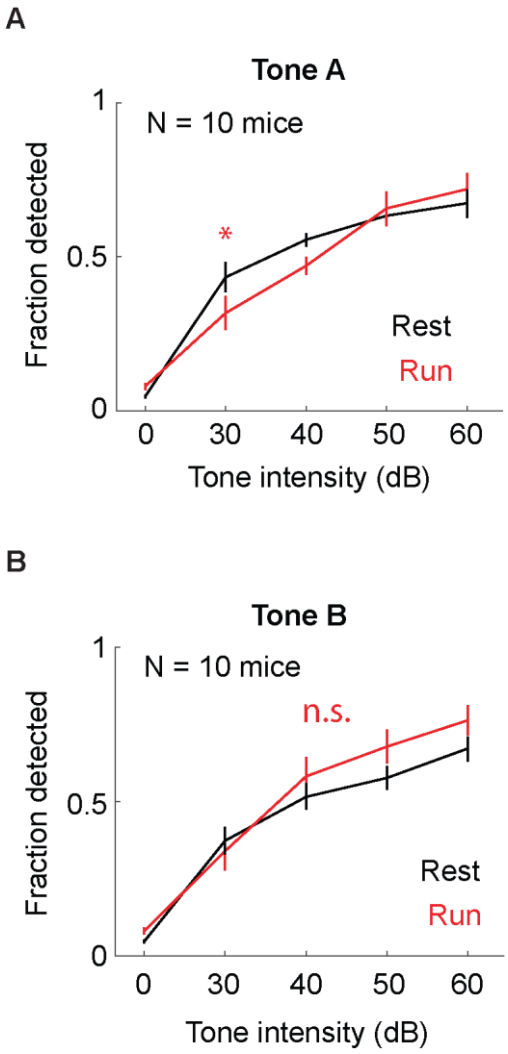


Figure 28: Psychometric curves during rest and running for 2-frequency auditory detection task in aVR-experienced mice. Tone detection performance (N = 10 mice) during rest (black) and running (red) following aVR experience with Tone A. Mice were significantly worse at detecting Tone A during running compared to rest (*top*), but not Tone B (*bottom*). Red asterisk: $p<0.05$ (RM 2-way ANOVA, followed by a post hoc Tukey test).

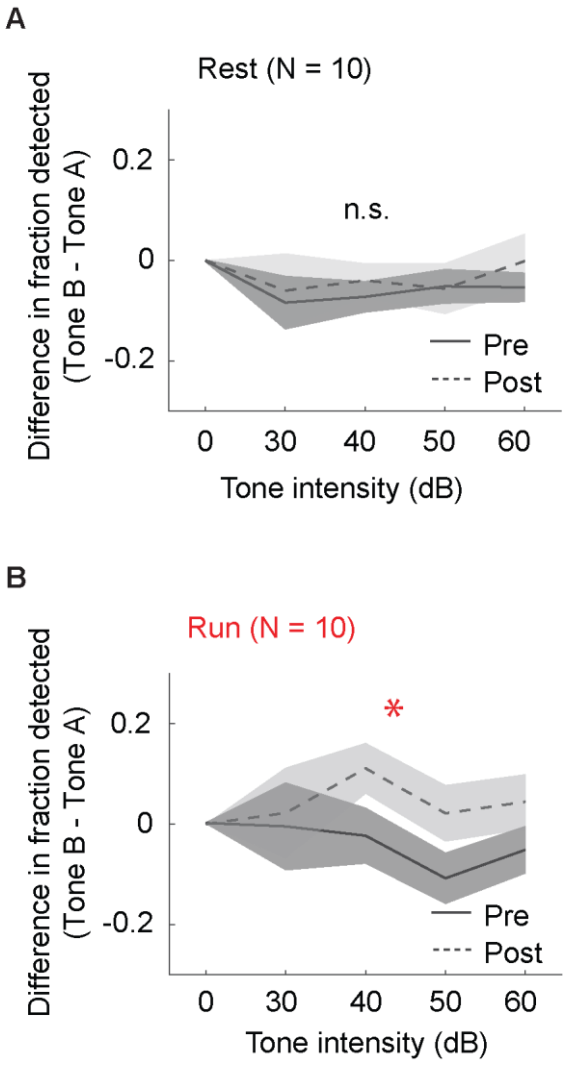


Figure 29: Auditory detection performance during rest and running in naïve and aVR-experienced mice. A) Relative detection of Tone B compared to Tone A during rest before (Pre) and after (Post) aVR experience with Tone A. Lines represent mean difference and shaded regions show SE for N = 10 mice. There is no difference in rest performance before and after aVR experience (RM 2-way ANOVA). B) Similar to (A), but during running. Mice are significantly better at detecting Tone B compared to Tone A after aVR experience (RM 2-way ANOVA).

5.3 Activating secondary motor cortical terminals in auditory cortex predictively shifts behavior

Experiments in chapters 4 and 5.1.3 indicate that the same circuit elements that modulate hearing in a movement-dependent manner, i.e. secondary motor cortical projections to the auditory cortex, are also influenced by aVR experience. A remaining question is whether changes in motor to auditory cortical connectivity are causally related to the behaviorally adaptive changes that accrue with aVR experience. Specifically, we wanted to test if, after aVR experience, optogenetic activation of M2 terminals in the auditory cortex of resting mice more strongly impaired detection of the reafferent tone frequency than of the non-reafferent frequency. To address this question, I combined optogenetic stimulation of M2 terminals in the auditory cortex with the two-frequency auditory detection task. I first bilaterally injected ~300nl of AAV.1.hsyn.ChR2.EYFP.WPRE into the secondary motor cortex of C57 mice. Mice were next trained on the two-frequency auditory detection task using methods described in Chapter 5.2.1. Following training, cranial windows were bilaterally implanted over the auditory cortex, and blue light was shined over the auditory cortex on a subset of trials in a randomly interleaved fashion (see Chapter 3.2.2 for laser stimulation details). Psychometric curves were calculated for rest and laser trials during rest for both tone frequencies before and after aVR experience.

This turned out to be a challenging experiment, presumably because of the many layers of behavioral, viral, and optogenetic manipulations that had to be performed over a long time (weeks to months for each mouse): 7 out of the 8 mice in which we attempted these measurements failed to either learn or perform the task at various stages of the experiment, with the result that we were able to collect data from a single mouse. In this one mouse, optogenetic activation of M2 terminals in the auditory cortex of the resting mouse more strongly impaired detection of the reafferent frequency than of the non-reafferent frequency following aVR experience, consistent with the data collected in the previous psychophysics and electrophysiology experiments (Figure 30).

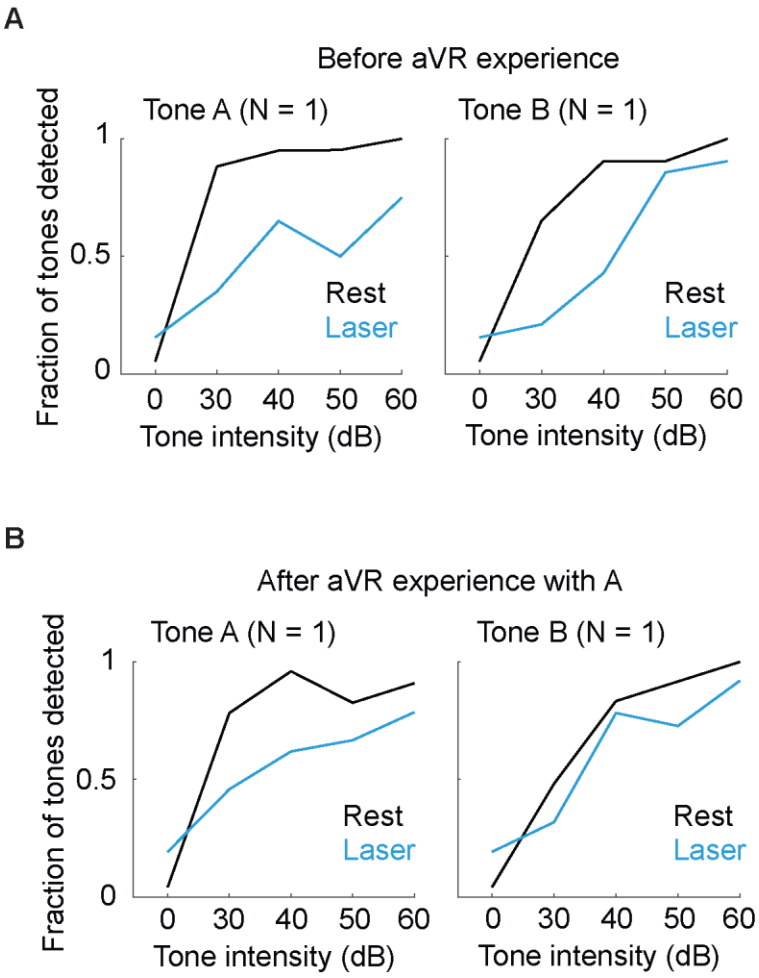


Figure 30: Tone detection behavior during optogenetic activation of M2 terminals before and after aVR experience. A) Tone detection performance during rest (black) and optogenetic stimulation of M2 terminals in auditory cortex (blue) for 1 mouse performing the 2-frequency auditory detection task before aVR experience. Performance on optogenetic trials is worse than resting performance for both Tone A and Tone B. B) As in panel (A), but following aVR experience with Tone A. Optogenetic activation of M2 terminals in the auditory cortex shows a smaller impairment in performance for Tone B.

5.4 Chapter 5 conclusions

Subjecting mice to several days of temporally coupled locomotor – auditory experience results in the formation of a movement-dependent filter that suppresses auditory cortical responses to predictable self-generated sounds. Using behavioral testing methods, I have shown that this sensorimotor experience is behaviorally adaptive, ameliorating the movement-related perceptual deficit for the non-reafferent frequency and resulting in higher detection rates for the non-reafferent frequency compared to the reafferent frequency during running. This was also accompanied by reduced locomotion-dependent suppression at non-reafferent frequencies in layer 2/3 of the auditory cortex, providing an auditory cortical correlate of this adaptive perceptual change. Furthermore, a sparse subpopulation of auditory cortical neurons maintained responsiveness to the reafferent frequency even after aVR experience, which may be sufficient for detecting predictable stimuli.

I have also acquired preliminary evidence suggesting that changes in functional connections between M2 neurons and auditory cortical interneurons responsive to the reafferent frequency following aVR experience could account for the psychometric changes that follow aVR experience. While further work is necessary to pin down the circuit mechanisms underlying these adaptive perceptual changes, these experiments illustrate the behavioral significance of corollary discharge signals in the auditory cortex.

6. Conclusions

The experiments described above are the first to characterize the behavioral significance of corollary discharge signals in the auditory cortex. Using a custom designed auditory task, I showed that auditory detection in mice is impaired during running compared to rest. Performance on this detection task is contingent upon auditory cortical activity. Secondary motor cortical modulations of auditory cortical activity can account for the running-related impairment in detection providing a circuit mechanism for this behavioral change. Finally, movement-related changes in auditory detection are behaviorally adaptive with higher detection rates for unexpected stimuli compared to predictable acoustic consequences of movements. In the next few sections I will go over the conclusions from chapters 2 – 5 and discuss potential implications and future directions.

6.1 Running increases auditory detection thresholds

In the experiments described in chapter 2, I tested the idea that auditory perception changes as a function of movement. I designed an auditory detection task where head-fixed mice were trained using operant methods to detect and report tones of fixed frequency and duration but varying intensities while they were either at rest or running on a quiet treadmill. Comparing their psychophysical performance during rest

and running indicated that running significantly impaired auditory detection by increasing detection thresholds compared to rest.

While experiments in several organisms have shown that a wide variety of movements suppress the auditory system (Eliades and Wang, 2003; Schneider et al., 2014; Singla et al., 2017; Poulet and Hedwig, 2002; Zatorre et al., 2007), the behavioral correlates of this suppression have remained untested. By designing an auditory task that assayed a single perceptual quality (loudness perception) during rest and running I tested three possible outcomes: 1) Auditory detection is impaired during running compared to rest. This is supported by the observation that neural responses in the auditory system are suppressed in response to auditory stimuli during movements compared to rest (Schneider et al., 2014; Singla et al., 2017; Williamson et al., 2015). 2) Auditory detection is improved during running compared to rest. This would be possible if the movement-related suppression in the auditory system is ultimately a mechanism to improve the signal to noise ratio (SNR) of auditory neurons by suppressing spontaneous neural activity to a larger extent compared to auditory-evoked neural activity (Zhou et al., 2014). 3) There is no difference in detection abilities during running compared to rest. One possibility here is that factors such as attention and arousal mask the effects of movement in this task (Kuchibhotla et al., 2016; McGinley et al., 2015). Another possibility is that movement-related modulation of the auditory

system does not impact the perception of simple auditory qualities such as loudness but could have implications for more complex auditory behaviors such as frequency discrimination, auditory categorization, etc., which would warrant future experiments.

Results in chapter 2 indicate that auditory detection is impaired during running compared to rest (Figures 6, 7), implying that the movement-related suppression of neuronal activity in the auditory system is associated with a perceptual cost. While it is counterintuitive why such an overall sensory deficit would be useful to the animal, one possibility is that the brain adopts a strategy whereby it reallocates its resources based on sensory reliability (Sheppard et al., 2013). Changes in head position during locomotion and other kinds of body movements may make dynamic sound localization computations challenging, thereby rendering auditory cues less reliable during movements (Schneider and Mooney, 2018). This idea is further supported by the observation that visual cortical activity is heightened during running compared to rest, directly opposing the auditory system (Niell and Stryker, 2010; Polack et al., 2013).

Auditory processing and perception are also influenced by neuromodulatory inputs that vary as a function of arousal levels during rest and movement. Specifically, locomotion is correlated with periods of heightened arousal and reduced auditory sensitivity (McGinley et al., 2015; Nelson and Mooney, 2016). Therefore, changes in auditory processing during rest and running could simply be an outcome of global

changes in arousal levels. However, movement-related suppression of the auditory system presumably also plays other roles that are important for sensory processing independent of arousal effects. For instance, during movements, it is important that the auditory system not be overwhelmed by self-generated sounds in order to maintain sensitivity to externally generated sounds. Studies in a variety of species have indicated that the brain engages sensory filtration mechanisms that suppress auditory responses during self-initiated movements to achieve this (Poulet and Hedwig, 2002; Eliades and Wang, 2003; Singla et al., 2017; Rummell et al., 2016). Perhaps, the general suppression of the auditory system during movements is a consequence of sensory filtration mechanisms that are engaged during movements. Further questions examining the circuit mechanisms that distinguish between self-generated and external sounds during movements, and their corresponding behavioral correlates are addressed in later sections.

While behavioral assays in head-fixed mice have been commonly used to measure auditory perceptual changes in a variety of contexts (Kato et al., 2015; McGinley et al., 2015; Kuchibhotla et al., 2016), this is the first assay to systematically test how auditory detection varies during rest and running. Though this experiment explicitly tests the effects of running on auditory detection, the auditory system is also modulated during a wide variety of other body movements (Schneider et al., 2014; Singla et al.,

2017; Zatorre et al., 2007). Whether perceptual impairment occurs across different kinds of movements, and whether the magnitude of impairment is conserved across movements remain to be tested. Future experiments are also required to determine how movements impact the perception of other acoustic qualities (e.g. frequency, timing, etc.). Finally, the behavioral testing methods and results described in this section provide the basis for the circuit dissection and behavioral experiments undertaken in chapters 3 – 5.

6.2 Auditory cortex is part of the mouse auditory detection circuit

In the experiments described in chapter 3, I used a combination of behavioral, pharmacological and optogenetic methods to test the hypothesis that the auditory cortex plays a role in auditory detection in the mouse. Suppressing auditory cortical activity in a temporally specific and reversible manner significantly impaired the ability of mice to detect tones indicating that the mouse auditory cortex is involved in auditory detection. Control experiments suppressing visual cortical activity during auditory detection and sham experiments that involved shining blue light over intact skull indicated that neural or visual distractors could not account for this impairment respectively.

The role of the mouse auditory cortex in detecting simple auditory stimuli has remained unclear. Previous rodent studies using auditory cortical lesions and other

manipulations of auditory cortical activity have reported results that range from significant behavioral impairments to no sensory deficits (Kelly and Glazier, 1978; Gimenez et al., 2015; Kuchibhotla et al., 2016; Kato et al., 2015). It was therefore important to establish the role of the mouse auditory cortex in this particular auditory detection assay. To address this, I used pharmacological and optogenetic methods to reversibly suppress auditory cortical activity during auditory detection. These methods were adopted in order to minimize the possibility of reorganization in perceptual pathways that could potentially occur following permanent lesions of the auditory cortex (Talwar et al., 2001). While results from both manipulations suggested similar conclusions, i.e. suppression of auditory cortical activity impairs auditory detection; the magnitude of perceptual impairment was significantly greater during pharmacological silencing of auditory cortical activity (Figures 10, 13). One possibility is that muscimol ($GABA_A$ receptor agonist) induced suppression of auditory cortical activity acting non-selectively on all neurons expressing $GABA_A$ receptors is a more potent form of suppression compared to optogenetic suppression by activating local inhibitory interneurons within the auditory cortex. This is also evident from electrophysiological recordings of auditory cortical activity during these two manipulations. Whereas infusion of muscimol resulted in the complete silencing of both spontaneous and auditory evoked responses, optogenetically activating local inhibitory interneurons in

the auditory cortex resulted in the suppression, but not complete abolishment of responses to auditory stimuli (Figures 9, 11). These data suggest that the magnitude of behavioral impairment in these conditions is correlated with the magnitude of change in auditory cortical activity. The residual ability of mice to detect tones in the muscimol condition can be attributed to subcortical mechanisms (Figure 10).

Optogenetically suppressing neural activity in the visual cortex during auditory detection did not show significant impairment in detection levels (Figure 14). This suggests that mere perturbation of activity in the cortex could not account for the behavioral impairment observed during specifically suppressing the auditory cortex. Sham experiments that involved shining light over intact skull during auditory detection further confirmed that visual distractors could not account for the behavioral effects observed during optogenetic suppression of auditory cortical activity (Figure 15). Overall, these results provide evidence that auditory detection in the mouse is influenced by dynamic modulations of auditory cortical activity and motivate the circuit dissection experiments undertaken in chapter 4.

6.3 Secondary motor cortical projections to the auditory cortex can account for the movement-related changes in auditory detection

In the experiments described in chapter 4, I tested the hypothesis that movement-related changes in auditory perception are mediated by axonal projections from the secondary motor cortex to the auditory cortex. Optogenetically activating secondary motor cortical terminals within the auditory cortex of resting mice impaired auditory detection in a manner comparable to running. Repeating the same experiment while mice were running also decreased detection levels from baseline resting performance similar to running.

Previous work has indicated that mechanisms local to the cortex are responsible for more than half of the movement-related suppression in the auditory cortex. Optogenetic activation of M2 axon terminals in the auditory cortex changes membrane potential dynamics of auditory cortical neurons in a manner similar to movement and also suppresses action potential firing rates by engaging local inhibitory neurons within the auditory cortex, providing a cortical circuit mechanism for movement-related suppression in the auditory cortex (Schneider et al., 2014). These results taken together with the behavioral results from chapter 2 and 3, suggest that M2 projections to the auditory cortex can underlie movement-related changes in auditory detection.

Optogenetically activating M2 axon terminals within the auditory cortex during rest impaired auditory detection in mice. The magnitude of this impairment was comparable to the magnitude of the perceptual deficit observed during running (Figure 17). One possibility is that presumably a larger proportion of M2 axons are activated during optogenetic stimulation of M2 terminals in the auditory cortex compared to those that are active during normal movements. This could potentially drive similar overall levels of suppression of auditory cortical activity during both running and M2 terminal stimulation, ultimately resulting in similar detection levels in both conditions. Future electrophysiological and behavioral experiments that compare the magnitude of auditory cortical suppression during running and optogenetic stimulation of M2 terminals in the auditory cortex are required to confirm this possibility. These experiments would involve systematically titrating laser powers during optogenetic activation of M2 terminals in the auditory cortex to measure if the suppression of auditory cortical activity and the resulting impairment of auditory detection are correlated with the magnitude of optogenetic stimulation.

Optogenetically activating M2 neurons during running also resulted in the impairment of auditory detection compared to rest but did not further degrade performance compared to running (Figure 18). As described above, this would be possible if auditory cortical suppression during optogenetic activation of M2 axon

terminals during running is similar to the overall suppression of auditory cortical responses during running compared to rest. Future electrophysiological and behavioral experiments similar to those described above are again required to confirm this possibility.

Shining light on secondary motor cortical terminals expressing GFP in the auditory cortex did not impair detection (Figure 19) indicating that non-specific effects of shining blue light cannot account for the M2 mediated perceptual impairment. Sham experiments that involved shining blue light over intact skull during auditory detection also did not impair perception (Figure 20). In fact, mice showed enhanced detection on sham trials compared to baseline performance. This suggests that, in the absence of any stimulation, mice used light as a cue to augment their performance. Since the perceptual enhancement on sham trials is in the opposite direction to the behavioral impairment during M2 terminal stimulation, these results indicate that visual distractors cannot account for the diminished tone detection performance during optogenetic stimulation of M2 terminals in the auditory cortex.

In these experiments, though I specifically tested whether optogenetically activating M2 terminals within the auditory cortex impairs auditory perception, it is also interesting to ask what might happen during the converse experiment i.e. does suppressing M2 activity during running, improve auditory detection? One potential

complication in doing these experiments is that M2 plays a premotor role in mice and sends projections to several downstream motor regions. Suppressing M2 activity during movements might therefore interfere with the actual movements themselves. While one possibility is to use intersectional strategies targeting M2 neurons that specifically project to the auditory cortex, results from previous anatomical tracing experiments have indicated that M2 neurons projecting to the auditory cortex also send collaterals to other motor downstream regions (Nelson et al., 2013). It is therefore difficult to isolate M2 cell bodies that project to the auditory cortex alone, making this experiment technically challenging to perform. One potential strategy for future experiments in this direction would be to use chemogenetic methods such as expressing inhibitory Designer Receptors Exclusively Activated by Designer Drugs (DREADD) in M2, while locally injecting CNO in the auditory cortex to selectively suppress M2 terminals in the auditory cortex during auditory detection.

Future directions also entail testing the role of other movement-related inputs to the auditory cortex. These include projections from other regions such as the primary motor cortex (M1), cingulate cortex and the basal forebrain. Specifically, the basal forebrain has been shown to play a role in driving experience-dependent auditory cortical plasticity and providing movement-related modulation in auditory and other sensory cortices (Nelson and Mooney, 2016; Polack et al., 2013; Froemke et al., 2013).

Studying how these inputs modulate perceptual changes during movements could potentially shed light on the circuit mechanisms underlying changes in auditory perception driven by sensorimotor experience.

6.4 Movement-related changes in auditory detection are behaviorally adaptive

In the experiments described in chapter 5, I tested the hypothesis that movement-related changes in auditory perception are shaped by sensorimotor experience resulting in behaviorally adaptive perceptual differences between reafferent and exafferent sound stimuli. Mice trained on a two-frequency auditory detection task, initially displayed movement-related perceptual deficits at both tone frequencies. Following several days of movement-coupled acoustic experience with one of the tone frequencies, mice no longer showed a movement-related perceptual deficit for the non-coupled tone frequency. Data from preliminary experiments further suggest that secondary motor cortical to auditory cortical projections could account for this sensorimotor experience-dependent change in auditory perception.

To understand how movement-related suppression in the auditory cortex is shaped by sensorimotor experience to suppress responses to self-generated (reafferent) sounds, David Schneider developed an acoustic virtual reality (aVR) system in which a mouse heard a fixed-frequency tone that was predictively coupled with its locomotor

movements (Figure 21). Several days of temporally coupled locomotor – auditory experience in this aVR system resulted in the formation of a movement-dependent filter that selectively suppressed auditory cortical responses to reafferent sounds during running (Figure 22). Furthermore, the formation of this auditory cortical filter was accompanied by enhanced functional connections between M2 neurons and auditory cortical interneurons responsive to the reafferent frequency providing a plausible cortical circuit mechanism by which aVR experience suppresses tone-evoked responses in excitatory auditory cortical cells in a frequency- and movement-dependent manner (Figure 25).

To test whether this experience-dependent movement filter is also behaviorally adaptive, I trained mice on a two-frequency auditory detection task. Following initial training, mice showed a perceptual deficit during running for both tone frequencies (Figure 27). Notably, aVR experience ameliorated this perceptual deficit for the non-reafferent frequency, but not the reafferent tone frequency (Figure 28). While this is behaviorally adaptive, in that mice can better detect unexpected sounds compared to expected self-generated sounds during movements, this result poses two questions: First, if auditory cortical responses to non-reafferent tones remain suppressed during locomotion following aVR experience, why does perceptual performance improve? Second, if auditory cortical responses to the reafferent tone become even more

suppressed following aVR experience, why doesn't movement-related performance at the reafferent frequency decrease?

With regard to the first question, one explanation is that the physiology data reported in Figure 22 are agnostic of layer and cell type (e.g. excitatory or inhibitory neurons). Therefore, we may not expect all of the neurons included in Figure 22 to reflect changes in behavior. In fact, a host of recent studies using 2-photon calcium imaging in L2/3 neurons of several cortical regions have shown strong correlations between behavior and physiological recordings made specifically from L2/3 excitatory neurons (Komiyama et al., 2010; Makimo and Komiyama, 2015; Peron et al., 2015; Harvey et al., 2012, Nature; Kuchibhotla et al., 2016). Therefore, a better parallel might be to compare the behavioral data with the 2-photon calcium data presented in Figure 24, which were restricted to excitatory neurons (i.e. CaMKII+) in L2/3 of the auditory cortex. These data show a roughly uniform suppression of sound-evoked responses in naive mice and show a bidirectional change following aVR experience: The movement-related suppression of expected tones becomes stronger, while locomotion-related suppression to the unexpected sound disappears. The latter findings correspond nicely with the behavioral improvement observed in Figure 28. With regard to the second question, it is important to note that although on average, neural responses to the expected reafferent tone are almost completely abolished, a smaller subpopulation of A1

neurons (~23% of those that were responsive during rest) retained a response to the expected tone during locomotion, potentially allowing the auditory system to maintain sensitivity to the reafferent frequency (Hawkins and Ahmad, 2016; Schneider, Sundararajan and Mooney, 2018; Eliades and Wang, 2003).

While preliminary results suggest that the secondary motor cortical to auditory cortical projections play a role mediating this behaviorally adaptive perceptual change (Figure 30), future experiments combining behavioral and optogenetic methods are required to confirm this. Future work will also be key to determine how these experience dependent corollary discharge signals are useful in the context of ethologically relevant natural behaviors such as vocal communication.

6.4 Final conclusions

Interactions between the motor and auditory systems are critical for normal hearing and optimal social behavior, most notably in facilitating speech perception and production in humans. Dysfunction in the motor to auditory cortical circuitry has been thought to underlie symptoms of neuropsychiatric disorders such as auditory hallucinations in schizophrenic patients (Ford et al., 2001; Ford and Mathalon, 2004; Hicock et al., 2011; Feinberg and Guazzelli, 1999). An understanding of the behavioral

significance of this circuitry is therefore critical to understand how this circuitry enables normal auditory perception during both rest and movements.

Here, I implemented a simple psychophysical task to show that, like humans, mice are worse at detecting sounds while moving compared to resting. I further demonstrated that this task depended on auditory cortical activity and secondary motor cortical modulation of auditory cortical activity can account for the movement-related impairment in auditory detection. Finally, movement-related change in auditory perception is behaviorally adaptive, allowing for the better detection of unexpected sounds compared to self-generated sounds during movements. These experiments provide the first evidence of how corollary discharge signals in the auditory cortex are linked to auditory perception. Ultimately, the motor – auditory cortical circuit studied here can flexibly encode the relationship between a movement and the sound it produces, helping to maintain sensitivity to novel sounds in the environment while also monitoring predictable self-generated sounds.

Appendix A: Experimental Methods

All experimental protocols were approved by Duke University Institutional Animal Care and Use Committee. Male and female mice were purchased from Jackson Labs and were housed and bred in an onsite vivarium. During all experiments, mice were kept on a reverse day-night cycle (12 h day, 12 h night).

Behavioral setup for auditory detection

We built a non-motorized treadmill from a 6-inch Plexiglas disk (Delvies Plastic) that was coated with a thin silicone sheet (Durometer, Marian Chicago). A hole was drilled in the center of the disk, which was used for mounting the disk to the post of a rotary encoder (U.S. Digital) such that each revolution of the spinning disk translated to one complete revolution of the encoder. Output from the rotary encoder was monitored with a data acquisition card (National Instruments) connected to a computer (Dell) running custom Matlab software (Mathworks, PsychToolBox) and sampled at ~30 Hz. The computer was also connected to a sound card (RME Fireface UCX) capable of presenting sounds with sampling rates up to 192 kHz. The output of the soundcard was routed to an ultrasonic speaker (Tucker Davis Technologies) located lateral to the mouse, 6 inches from the mouse's right ear. We recorded the noise produced by the mouse's footsteps on the treadmill and the sound of the rotating treadmill itself during

running by placing an ultrasonic microphone close (~1 cm) to the mouse's ear. We measured <1dB increase (estimated by taking the rms value of 5 sec segments of recordings) in the noise produced when the mouse was running on the treadmill compared to rest. Mice were positioned on top of the treadmill and were held in place using two clamps (Altos Photonics) that secured the arms of the headpost. The position of the mouse atop the spinning disk allowed for running and resting behaviors that appeared natural and mice did not appear to be in a state of discomfort. Mice were acclimated to the treadmill for 2 to 3 days, during which time they naturally and spontaneously transitioned between periods of running and resting. Treadmill-acclimated mice were then water restricted for 24 hours prior to training. Licking behavior was measured with a custom-built infrared detector located between the mouse's mouth and the water delivery spout and was sampled with a data acquisition card (NI) connected to a computer (Dell) running custom software (Matlab), which controlled the training and testing phases of the psychophysics tasks.

Headpost Implantation

Prior to behavioral training, male and female mice (C57, VGAT::ChR2, PV::Cre) were anesthetized under isoflurane (1-2% in O_2) and placed in a stereotaxic holder (Leica). Skin was removed from the top of the skull and an annular or Y-shaped

titanium headpost with two bars extending laterally in both directions was attached to the skull using a transparent adhesive (Metabond). For electrophysiological, pharmacological or optogenetic experiments, small tattoos were placed on the skull surface over the right and left auditory cortex using stereotaxic coordinates before headpost implantation. Mice were returned to their home cage and allowed ~1-3 days to recover before beginning behavioral training.

Pharmacological manipulation during psychophysics

Male and female mice were anesthetized under isoflurane (1-2% in O_2) and implanted with a Y-shaped titanium headpost with two bars extending rostro-laterally in both directions. Mice were returned to their home cage and allowed to recover for ~1-3 days before training on the auditory detection task. Once mice were proficient with the task, they were anesthetized again under isoflurane and craniotomies were opened bilaterally over the auditory cortex. To confirm the localization of craniotomies to the auditory cortex, we performed single electrode (Carbostar-1, Kation Scientific) recordings in anaesthetized mice to ensure auditory responses to 8kHz tone pips at multiple locations within the brain region exposed by craniotomy. Exposed craniotomies were covered with a silicone elastomer (Kwik-Sil) and mice were allowed to recover in their home cage for a day before behavioral testing. Saline or muscimol (2ug/ul, 150nL)

was bilaterally pressure injected using a Nanoject system into the auditory cortex on alternate days. Mice were allowed to return to their home cage for 30 minutes after injection. At the end of this period, the mice were reintroduced to the testing chamber where they performed the behavioral task.

Optogenetic stimulation during psychophysics

For experiments involving the optogenetic stimulation of auditory cortical activity during auditory detection, mice were anesthetized with isoflurane and a custom Y-shaped titanium headpost was attached to the skull with Metabond. Mice were then trained on the single-tone detection task using operant methods. Once mice were proficient with the task, craniotomies were opened bilaterally and the location of the auditory cortex was confirmed by recording single electrode responses (Carbostar-1, Kation Scientific) to 8kHz tones at multiple locations within the brain region exposed by craniotomy. Circular glass coverslips (3mm diameter) were implanted bilaterally over the auditory cortex and sealed in place with Metabond. Mice were returned to their home cage and allowed to recover for ~1-2 days before behavioral testing.

For simultaneous psychophysics and optogenetic manipulation experiments in the visual cortex, circular glass coverslips were bilaterally implanted over visual cortex (identified using stereotaxic coordinates) at the same time as an annular headpost was

attached to the skull of VGAT ChR2 mice. Following ~1-3 days of recovery, mice were acclimated to the treadmill setup and trained on the auditory detection task using operant conditioning methods.

Electrophysiology during pharmacological/optogenetic manipulations

Mice were first implanted with a Y-shaped titanium headpost and acclimated to the treadmill setup. Before electrophysiology, mice were briefly anesthetized, and craniotomies were made to expose the auditory cortex. A small craniotomy was made over the right sensory cortex and a silver pellet was positioned atop the cortical surface and cemented in place (Metabond) for use as a ground electrode. Exposed auditory cortex craniotomies were covered with a silicone elastomer (Kwik-Sil) and the mouse was allowed to recover in its home cage.

During electrophysiological recording, a 32-channel electrode (Neuronexus, 4 x 8 configuration) was implanted into the auditory cortex. The electrode was connected to a digitizing headstage (Intan) and electrode signals were acquired, monitored in real-time, and stored for subsequent offline analysis (OpenEphys). The electrode was allowed to settle for ~30 minutes, after which time 8kHz tones were presented at inter-tone-intervals of 1-2s. A digital tag marking the onset time and frequency of each tone was output from a separate channel of the soundcard simultaneously with each tone and this

digital tag was acquired simultaneously with the electrophysiology data on the same data acquisition device (Intan). Electrode signals were filtered (300 to 5000 Hz) and artifacts were removed by subtracting the mean signal averaged across all 32 channels. Action potentials from individual neurons were sorted offline for each electrode independently based on visualization of the action potential waveform and principal component analysis of the waveform using custom Matlab software (PostHawk, D.M.S.). Tone-evoked action potential responses were calculated for each neuron and the response of every neuron that was responsive to 8kHz was averaged to calculate population peri-stimulus-time histograms (PSTHs). During electrophysiology with simultaneous pharmacological manipulations, saline or muscimol (2ug/ul, 150nL) was pressure injected using a nanoject ~30 minutes prior to recording tone responses. During electrophysiology combined with optogenetic manipulations, in addition to the multi-electrode array, an optical fiber coupled to a blue laser (420nm, Shanghai) was directed at the auditory cortical surface. Laser pulses (25 Hz, 50% duty cycle, 15–30mW) were presented on 50% of sound trials. Laser stimulation began 200 msec prior to tone presentation and continued for 1.2 sec. PSTHs were calculated for the laser and non-laser trials.

Viral Injections

Male and female C57 mice were anesthetized under isoflurane and placed in a stereotaxic holder (Leica). For expression of Channelrhodopsin or eGFP, the skull over M2 of C57 mice was exposed. Craniotomies were made bilaterally over M2 using stereotaxic coordinates, and approximately 300nL of AAV.1.hSyn.ChR2.EYFP.WPRE, AAV.1.CB7.eGFP.WPRE was pressure injected over the course of 20 minutes on each side. Following injections, craniotomies were filled with melted bone wax and headposts were implanted for behavioral training.

Acoustic virtual reality

This setup was designed by Dr. David Schneider to yoke a series of fixed-frequency tone pips (25 msec with 5 msec cosine ramp onset and offset) to a mouse's running on a treadmill. To create the aVR system, we built a non-motorized treadmill from a 6-inch Plexiglas disk (Delvies Plastic) that was coated with a thin silicone sheet (Durometer, Marian Chicago). A hole was drilled in the center of the disk, which was used for mounting the disk to the post of a rotary encoder (U.S. Digital) such that each revolution of the spinning disk translated to one complete revolution of the encoder. Output from the rotary encoder was monitored with a data acquisition card (National Instruments) connected to a computer (Dell) running custom Matlab software

(Mathworks, PsychToolBox) and sampled at ~30 Hz. The computer was also connected to a sound card (RME Fireface UCX) capable of presenting sounds with sampling rates up to 192 kHz. The output of the soundcard was routed to an ultrasonic speaker (Tucker Davis Technologies) located lateral to the mouse, 6 inches from the mouse's right ear. During every sampling period, we calculated two values: (1) the time since the previous tone presentation and (2) the desired interval between the previous tone and the upcoming tone (i.e. inter-tone-interval). To calculate the inter-tone-interval, we computed a filtered version of the mouse's velocity, which was the median of the last 5 velocity samples. Upon every sampling period, the desired inter-tone-interval was updated to be proportional to the reciprocal of the current median-filtered velocity, multiplied by a scaling constant. The scaling constant was set such that the rate of tone presentations closely matched the foot step rate, which was calculated from videos of mice running on the treadmill at various speeds. If during a given sampling period the time since the previous tone pip exceeded the desired inter-tone-interval, a tone was presented and the time since the previous tone presentation was reset to zero. At speeds greater than 30 cm/sec, the inter-tone-interval saturated at 100 msec to ensure spacing between tones. For the anti-coupled version of aVR, sounds were not presented while mice were running but the total number of tones that should have been presented, and the calculated intervals between them were stored in memory. Following at least 1 sec of

rest and throughout subsequent resting periods, tones were played back to the mouse with inter-tone-intervals drawn from the intervals that the mouse would have heard while running until the number of resting tones equaled the number of tones that mouse should have heard while running. For the metronome aVR, sounds were presented only during running and at a fixed rate (~2/sec) that was non-modulated by running speed. Mice were placed on the treadmill and were free to transition between periods of running and rest, which typically occurred several times during each aVR acclimation session.

References

Alexander, G. E., Newman, J. D., & Symmes, D. (1976). Convergence of prefrontal and acoustic inputs upon neurons in the superior temporal gyrus of the awake squirrel monkey. *Brain Res, 116*(2), 334-338.

Bauer, J. J., Mittal, J., Larson, C. R., & Hain, T. C. (2006). Vocal responses to unanticipated perturbations in voice loudness feedback: an automatic mechanism for stabilizing voice amplitude. *J Acoust Soc Am, 119*(4), 2363-2371.

Belin, P., Zatorre, R. J., Lafaille, P., Ahad, P., & Pike, B. (2000). Voice-selective areas in human auditory cortex. *Nature, 403*(6767), 309-312. doi:10.1038/35002078

Bizley, J. K., Walker, K. M., Silverman, B. W., King, A. J., & Schnupp, J. W. (2009). Interdependent encoding of pitch, timbre, and spatial location in auditory cortex. *J Neurosci, 29*(7), 2064-2075. doi:10.1523/JNEUROSCI.4755-08.2009

Borg, E., & Zakrisson, J. E. (1975). The activity of the stapedius muscle in man during vocalization. *Acta Otolaryngol, 79*(5-6), 325-333.

Bouchard, K. E., Mesgarani, N., Johnson, K., & Chang, E. F. (2013). Functional organization of human sensorimotor cortex for speech articulation. *Nature, 495*(7441), 327-332. doi:10.1038/nature11911

Budinger, E., Heil, P., Hess, A., & Scheich, H. (2006). Multisensory processing via early cortical stages: Connections of the primary auditory cortical field with other sensory systems. *Neuroscience, 143*(4), 1065-1083. doi:10.1016/j.neuroscience.2006.08.035

Burnett, T. A., Freedland, M. B., Larson, C. R., & Hain, T. C. (1998). Voice F0 responses to manipulations in pitch feedback. *J Acoust Soc Am, 103*(6), 3153-3161.

Carmel PW, S. A. (1963). Acoustic and nonacoustic factors modifying middle-ear muscle activity in walking cats. *J Neurophysiol, 26*, 598-616.

Crapse, T. B., & Sommer, M. A. (2008). Corollary discharge across the animal kingdom. *Nat Rev Neurosci, 9*(8), 587-600. doi:10.1038/nrn2457

Curio, G., Neuloh, G., Numminen, J., Jousmaki, V., & Hari, R. (2000). Speaking modifies voice-evoked activity in the human auditory cortex. *Hum Brain Mapp, 9*(4), 183-191.

David, S. V. (2018). Incorporating behavioral and sensory context into spectro-temporal models of auditory encoding. *Hear Res, 360*, 107-123. doi:10.1016/j.heares.2017.12.021

Dykstra, A. R., Koh, C. K., Braida, L. D., & Tramo, M. J. (2012). Dissociation of detection and discrimination of pure tones following bilateral lesions of auditory cortex. *PLoS One, 7*(9), e44602. doi:10.1371/journal.pone.0044602

Edeline, J. M., & Weinberger, N. M. (1991). Thalamic short-term plasticity in the auditory system: associative returning of receptive fields in the ventral medial geniculate body. *Behav Neurosci, 105*(5), 618-639.

Eliades, S. J., & Tsunada, J. (2018). Auditory cortical activity drives feedback-dependent vocal control in marmosets. *Nat Commun, 9*(1), 2540. doi:10.1038/s41467-018-04961-8

Eliades, S. J., & Wang, X. (2003). Sensory-motor interaction in the primate auditory cortex during self-initiated vocalizations. *J Neurophysiol, 89*(4), 2194-2207. doi:10.1152/jn.00627.2002

Eliades, S. J., & Wang, X. (2008). Neural substrates of vocalization feedback monitoring in primate auditory cortex. *Nature, 453*(7198), 1102-1106. doi:10.1038/nature06910

Ford, J. M., & Mathalon, D. H. (2004). Electrophysiological evidence of corollary discharge dysfunction in schizophrenia during talking and thinking. *J Psychiatr Res, 38*(1), 37-46.

Ford, J. M., Mathalon, D. H., Heinks, T., Kalba, S., Faustman, W. O., & Roth, W. T. (2001). Neurophysiological evidence of corollary discharge dysfunction in schizophrenia. *Am J Psychiatry, 158*(12), 2069-2071. doi:10.1176/appi.ajp.158.12.2069

Francis, N. A., Winkowski, D. E., Sheikhattar, A., Armengol, K., Babadi, B., & Kanold, P. O. (2018). Small Networks Encode Decision-Making in Primary Auditory Cortex. *Neuron, 97*(4), 885-897 e886. doi:10.1016/j.neuron.2018.01.019

Fritz, J., Shamma, S., Elhilali, M., & Klein, D. (2003). Rapid task-related plasticity of spectrotemporal receptive fields in primary auditory cortex. *Nat Neurosci, 6*(11), 1216-1223. doi:10.1038/nn1141

Froemke, R. C., Carcea, I., Barker, A. J., Yuan, K., Seybold, B. A., Martins, A. R., . . . Schreiner, C. E. (2013). Long-term modification of cortical synapses improves sensory perception. *Nat Neurosci, 16*(1), 79-88. doi:10.1038/nn.3274

Froemke, R. C., Merzenich, M. M., & Schreiner, C. E. (2007). A synaptic memory trace for cortical receptive field plasticity. *Nature, 450*(7168), 425-429. doi:10.1038/nature06289

Gao, E., & Suga, N. (2000). Experience-dependent plasticity in the auditory cortex and the inferior colliculus of bats: role of the corticofugal system. *Proc Natl Acad Sci U S A, 97*(14), 8081-8086. doi:10.1073/pnas.97.14.8081

Gimenez, T. L., Lorenc, M., & Jaramillo, S. (2015). Adaptive categorization of sound frequency does not require the auditory cortex in rats. *J Neurophysiol, 114*(2), 1137-1145. doi:10.1152/jn.00124.2015

Guo, W., Clause, A. R., Barth-Maron, A., & Polley, D. B. (2017). A Corticothalamic Circuit for Dynamic Switching between Feature Detection and Discrimination. *Neuron, 95*(1), 180-194 e185. doi:10.1016/j.neuron.2017.05.019

Harvey, C. D., Coen, P., & Tank, D. W. (2012). Choice-specific sequences in parietal cortex during a virtual-navigation decision task. *Nature, 484*(7392), 62-68. doi:10.1038/nature10918

Hattori, R., Kuchibhotla, K. V., Froemke, R. C., & Komiyama, T. (2017). Functions and dysfunctions of neocortical inhibitory neuron subtypes. *Nat Neurosci, 20*(9), 1199-1208. doi:10.1038/nn.4619

Hawkins, J., & Ahmad, S. (2016). Why Neurons Have Thousands of Synapses, a Theory of Sequence Memory in Neocortex. *Front Neural Circuits, 10*, 23. doi:10.3389/fncir.2016.00023

Heffner, H. E., & Heffner, R. S. (1986). Effect of unilateral and bilateral auditory cortex lesions on the discrimination of vocalizations by Japanese macaques. *J Neurophysiol, 56*(3), 683-701.

Heffner, H. E., & Heffner, R. S. (1990a). Effect of bilateral auditory cortex lesions on absolute thresholds in Japanese macaques. *J Neurophysiol, 64*(1), 191-205.

Heffner, H. E., & Heffner, R. S. (1990b). Effect of bilateral auditory cortex lesions on sound localization in Japanese macaques. *J Neurophysiol, 64*(3), 915-931.

Hickok, G., & Poeppel, D. (2007). The cortical organization of speech processing. *Nat Rev Neurosci, 8*(5), 393-402. doi:10.1038/nrn2113

Houde, J. F., & Jordan, M. I. (1998). Sensorimotor adaptation in speech production. *Science, 279*(5354), 1213-1216.

Houde, J. F., Nagarajan, S. S., Sekihara, K., & Merzenich, M. M. (2002). Modulation of the auditory cortex during speech: an MEG study. *J Cogn Neurosci, 14*(8), 1125-1138. doi:10.1162/089892902760807140

Hunter, K. P., & Willott, J. F. (1993). Effects of bilateral lesions of auditory cortex in mice on the acoustic startle response. *Physiol Behav, 54*(6), 1133-1139.

Ison, J. R., Allen, P. D., & O'Neill, W. E. (2007). Age-related hearing loss in C57BL/6J mice has both frequency-specific and non-frequency-specific components that produce a hyperacusis-like exaggeration of the acoustic startle reflex. *J Assoc Res Otolaryngol, 8*(4), 539-550. doi:10.1007/s10162-007-0098-3

Johnsrude, I. S., Penhune, V. B., & Zatorre, R. J. (2000). Functional specificity in the right human auditory cortex for perceiving pitch direction. *Brain, 123 (Pt 1)*, 155-163.

Kato, H. K., Gillet, S. N., & Isaacson, J. S. (2015). Flexible Sensory Representations in Auditory Cortex Driven by Behavioral Relevance. *Neuron, 88*(5), 1027-1039. doi:10.1016/j.neuron.2015.10.024

Kelly, J. B., & Glazier, S. J. (1978). Auditory cortex lesions and discrimination of spatial location by the rat. *Brain Res, 145*(2), 315-321.

Kilgard, M. P., & Merzenich, M. M. (1998). Plasticity of temporal information processing in the primary auditory cortex. *Nat Neurosci, 1*(8), 727-731. doi:10.1038/3729

King, A. J., Teki, S., & Willmore, B. D. B. (2018). Recent advances in understanding the auditory cortex. *F1000Res, 7*. doi:10.12688/f1000research.15580.1

Komiyama, T., Sato, T. R., O'Connor, D. H., Zhang, Y. X., Huber, D., Hooks, B. M., . . . Svoboda, K. (2010). Learning-related fine-scale specificity imaged in motor cortex circuits of behaving mice. *Nature, 464*(7292), 1182-1186. doi:10.1038/nature08897

Kuchibhotla, K. V., Gill, J. V., Lindsay, G. W., Papadoyannis, E. S., Field, R. E., Sten, T. A., . . . Froemke, R. C. (2017). Parallel processing by cortical inhibition enables context-dependent behavior. *Nat Neurosci, 20*(1), 62-71. doi:10.1038/nn.4436

Leichnetz, G. R., & Astruc, J. (1975). Efferent connections of the orbitofrontal cortex in the marmoset (Saguinus oedipus). *Brain Res, 84*(2), 169-180.

Makino, H., & Komiyama, T. (2015). Learning enhances the relative impact of top-down processing in the visual cortex. *Nat Neurosci, 18*(8), 1116-1122. doi:10.1038/nn.4061

Martikainen, M. H., Kaneko, K., & Hari, R. (2005). Suppressed responses to self-triggered sounds in the human auditory cortex. *Cereb Cortex, 15*(3), 299-302. doi:10.1093/cercor/bhh131

McGinley, M. J., David, S. V., & McCormick, D. A. (2015). Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection. *Neuron, 87*(1), 179-192. doi:10.1016/j.neuron.2015.05.038

Mottonen, R., & Watkins, K. E. (2009). Motor representations of articulators contribute to categorical perception of speech sounds. *J Neurosci, 29*(31), 9819-9825. doi:10.1523/JNEUROSCI.6018-08.2009

Mukerji, S., Windsor, A. M., & Lee, D. J. (2010). Auditory brainstem circuits that mediate the middle ear muscle reflex. *Trends Amplif, 14*(3), 170-191. doi:10.1177/1084713810381771

Muller-Preuss, P., & Ploog, D. (1981). Inhibition of auditory cortical neurons during phonation. *Brain Res, 215*(1-2), 61-76.

Nelson, A., & Mooney, R. (2016). The Basal Forebrain and Motor Cortex Provide Convergent yet Distinct Movement-Related Inputs to the Auditory Cortex. *Neuron, 90*(3), 635-648. doi:10.1016/j.neuron.2016.03.031

Nelson, A., Schneider, D. M., Takatoh, J., Sakurai, K., Wang, F., & Mooney, R. (2013). A circuit for motor cortical modulation of auditory cortical activity. *J Neurosci, 33*(36), 14342-14353. doi:10.1523/JNEUROSCI.2275-13.2013

Niell, C. M., & Stryker, M. P. (2010). Modulation of visual responses by behavioral state in mouse visual cortex. *Neuron, 65*(4), 472-479. doi:10.1016/j.neuron.2010.01.033

Niwa, M., Johnson, J. S., O'Connor, K. N., & Sutter, M. L. (2012). Activity related to perceptual judgment and action in primary auditory cortex. *J Neurosci, 32*(9), 3193-3210. doi:10.1523/JNEUROSCI.0767-11.2012

Otazu, G. H., Tai, L. H., Yang, Y., & Zador, A. M. (2009). Engaging in an auditory task suppresses responses in auditory cortex. *Nat Neurosci, 12*(5), 646-654. doi:10.1038/nn.2306

Peretz, I., Kolinsky, R., Tramo, M., Labrecque, R., Hublet, C., Demeurisse, G., & Belleville, S. (1994). Functional dissociations following bilateral lesions of auditory cortex. *Brain, 117 (Pt 6)*, 1283-1301.

Peron, S. P., Freeman, J., Iyer, V., Guo, C., & Svoboda, K. (2015). A Cellular Resolution Map of Barrel Cortex Activity during Tactile Behavior. *Neuron, 86*(3), 783-799. doi:10.1016/j.neuron.2015.03.027

Polack, P. O., Friedman, J., & Golshani, P. (2013). Cellular mechanisms of brain state-dependent gain modulation in visual cortex. *Nat Neurosci, 16*(9), 1331-1339. doi:10.1038/nn.3464

Polley, D. B., Steinberg, E. E., & Merzenich, M. M. (2006). Perceptual learning directs auditory cortical map reorganization through top-down influences. *J Neurosci, 26*(18), 4970-4982. doi:10.1523/JNEUROSCI.3771-05.2006

Poulet, J. F., & Hedwig, B. (2002). A corollary discharge maintains auditory sensitivity during sound production. *Nature, 418*(6900), 872-876. doi:10.1038/nature00919

Recanzone, G. H., Schreiner, C. E., & Merzenich, M. M. (1993). Plasticity in the frequency representation of primary auditory cortex following discrimination training in adult owl monkeys. *J Neurosci, 13*(1), 87-103.

Reznik, D., Henkin, Y., Schadel, N., & Mukamel, R. (2014). Lateralized enhancement of auditory cortex activity and increased sensitivity to self-generated sounds. *Nat Commun, 5*, 4059. doi:10.1038/ncomms5059

Rummell, B. P., Klee, J. L., & Sigurdsson, T. (2016). Attenuation of Responses to Self-Generated Sounds in Auditory Cortical Neurons. *J Neurosci, 36*(47), 12010-12026. doi:10.1523/JNEUROSCI.1564-16.2016

Rutkowski, R. G., & Weinberger, N. M. (2005). Encoding of learned importance of sound by magnitude of representational area in primary auditory cortex. *Proc Natl Acad Sci U S A, 102*(38), 13664-13669. doi:10.1073/pnas.0506838102

Schneider, D. M., & Mooney, R. (2018). How Movement Modulates Hearing. *Annu Rev Neurosci, 41*, 553-572. doi:10.1146/annurev-neuro-072116-031215

Schneider, D. M., Nelson, A., & Mooney, R. (2014). A synaptic and circuit basis for corollary discharge in the auditory cortex. *Nature, 513*(7517), 189-194. doi:10.1038/nature13724

Schneider, D. M., Sundararajan, J., & Mooney, R. (2018). A cortical filter that learns to suppress the acoustic consequences of movement. *Nature, 561*(7723), 391-395. doi:10.1038/s41586-018-0520-5

Schreiner, C. E., & Malone, B. J. (2015). Representation of loudness in the auditory cortex. *Handb Clin Neurol, 129*, 73-84. doi:10.1016/B978-0-444-62630-1.00004-4

Sheppard, J. P., Raposo, D., & Churchland, A. K. (2013). Dynamic weighting of multisensory stimuli shapes decision-making in rats and humans. *J Vis, 13*(6). doi:10.1167/13.6.4

Singla, S., Dempsey, C., Warren, R., Enikolopov, A. G., & Sawtell, N. B. (2017). A cerebellum-like circuit in the auditory system cancels responses to self-generated sounds. *Nat Neurosci, 20*(7), 943-950. doi:10.1038/nn.4567

Sommer, M. A., & Wurtz, R. H. (2002). A pathway in primate brain for internal monitoring of movements. *Science, 296*(5572), 1480-1482. doi:10.1126/science.1069590

Sperry, R. W. (1950). Neural basis of the spontaneous optokinetic response produced by visual inversion. *J Comp Physiol Psychol, 43*(6), 482-489.

Strominger, N. L., Oesterreich, R. E., & Neff, W. D. (1980). Sequential auditory and visual discriminations after temporal lobe ablation in monkeys. *Physiol Behav, 24*(6), 1149-1156.

Suga, N. (2008). Role of corticofugal feedback in hearing. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol, 194*(2), 169-183. doi:10.1007/s00359-007-0274-2

Suga, N., & Shimozawa, T. (1974). Site of neural attenuation of responses to self-vocalized sounds in echolocating bats. *Science, 183*(130), 1211-1213.

Talwar, S. K., Musial, P. G., & Gerstein, G. L. (2001). Role of mammalian auditory cortex in the perception of elementary sound properties. *J Neurophysiol, 85*(6), 2350-2358.

Thompson, G. C., & Cortez, A. M. (1983). The inability of squirrel monkeys to localize sound after unilateral ablation of auditory cortex. *Behav Brain Res, 8*(2), 211-216.

Tramo, M. J., Shah, G. D., & Braida, L. D. (2002). Functional role of auditory cortex in frequency processing and pitch perception. *J Neurophysiol, 87*(1), 122-139. doi:10.1152/jn.00104.1999

Ulanovsky, N., Las, L., & Nelken, I. (2003). Processing of low-probability sounds by cortical neurons. *Nat Neurosci, 6*(4), 391-398. doi:10.1038/nn1032

Weiss, C., Herwig, A., & Schutz-Bosbach, S. (2011). The self in action effects: selective attenuation of self-generated sounds. *Cognition, 121*(2), 207-218. doi:10.1016/j.cognition.2011.06.011

Williamson, R. S., Hancock, K. E., Shinn-Cunningham, B. G., & Polley, D. B. (2015). Locomotion and Task Demands Differentially Modulate Thalamic Audiovisual Processing during Active Search. *Curr Biol, 25*(14), 1885-1891. doi:10.1016/j.cub.2015.05.045

Williamson, R. S., & Polley, D. B. (2019). Parallel pathways for sound processing and functional connectivity among layer 5 and 6 auditory corticofugal neurons. *Elife, 8*. doi:10.7554/eLife.42974

Zatorre, R. J. (1988). Pitch perception of complex tones and human temporal-lobe function. *J Acoust Soc Am, 84*(2), 566-572.

Zatorre, R. J., Belin, P., & Penhune, V. B. (2002). Structure and function of auditory cortex: music and speech. *Trends Cogn Sci, 6*(1), 37-46.

Zatorre, R. J., Chen, J. L., & Penhune, V. B. (2007). When the brain plays music: auditory-motor interactions in music perception and production. *Nat Rev Neurosci, 8*(7), 547-558. doi:10.1038/nrn2152

Zhou, M., Liang, F., Xiong, X. R., Li, L., Li, H., Xiao, Z., . . . Zhang, L. I. (2014). Scaling down of balanced excitation and inhibition by active behavioral states in auditory cortex. *Nat Neurosci, 17*(6), 841-850. doi:10.1038/nn.3701

Biography

Janani Sundararajan graduated with a B. Eng. in Biomedical Engineering from National University of Singapore in May 2010. She has published the following articles from her undergraduate and pre-doctoral work: “Co-variation of tonality in the music and speech of different cultures”, “Hepatocyte function within a stacked double sandwich culture plate cylindrical bioreactor for bioartificial liver system”, “Expression of emotion in Eastern and Western music mirrors vocalization”, “Network connections that evolve to circumvent the inverse optics problem”, and “How biological vision succeeds in the physical world”. Janani Sundararajan’s doctoral work has been published in the article: “A cortical filter that learns to suppress the acoustic consequences of movement.” She was a recipient of the Holland Trice Graduate Fellowship in graduate school.