Sensing Movement: Encoding of Self and External Motion by Auditory Cortical Neuronal Ensembles

by

Carlos Arturo Vivaldo

A dissertation submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy
(Psychology)
in the University of Michigan
2024

Doctoral Committee:

Professor Gideon Rothschild, Chair
Professor Taraz Lee
Professor Martin Sarter
Professor Elise Savier
Professor Natalie Tronson

Carlos Arturo Vivaldo

vivaldo@umich.edu

ORCID iD : 0000-0001-7797-2674

Dedication

To my mom, Cecilia, my love, Summer, my family, my boy, Odin, and all my friends.

Acknowledgements

I want to take this page to reflect on the lack of acknowledgment, historic and systematic exclusion, racism, people of color and minority groups experience in this department, The University of Michigan, and Academia as a whole.

Preface

Before beginning my dissertation, I met a patient whose story and life shaped my academic trajectory completely and set the foundation of this dissertation. Patient X, as many do, fled Cuba to the US by sailing across the Atlantic. On this journey, his boat took on so much water during a storm that it almost sank. Luckily, he survived, moved to New York, and lived a happy life. When I met Patient X, he was fine, but as the years went on and his neurodegeneration progressed, he would get frightened by the sound of water. It triggered this memory. How could a person with severe dementia, who can't remember their own family, recall something so vividly? How could the sound of water trigger a behavioral response? What about the stage of his neurodegeneration allowed this memory to become aberrant? I was fascinated by how a sound could trigger a memory and behavior. Moreover, how did this circuitry change with neurodegeneration that now a neutral stimulus became an uncontrollable motor response?

Much to my surprise when I first began to try and understand Patient X, how a sound caused a motor behavioral response was still not fully understood. At the time, there was an understanding that auditory sounds could cause a behavioral response but that under moving conditions they would take a backseat to other sensory information. Patient X, however, would get frightened whether he was walking, sitting, or doing any household task. So how a sound could cause a behavior during changing locomotive states was still unclear. This dissertation begins by studying exactly this, how does a sound trigger a behavioral response, across locomotive states, what brain region is necessary such behavior and what neural mechanisms are

used to do so. As I will show in this dissertation, sounds can cause a behavioral response across locomotive states, due to neural activity in the primary cortex that integrates movement and sound information together to produce audiomotor behaviors. This work has been published in the Public Library of Science, Biology (Vivaldo, CA., Lee, J., Shorkey, M., Keerthy, A., Rothschild, G. Auditory cortex ensembles jointly encode sound and locomotion speed to support sound perception during movement. PLOS Biology, 21(8). https://doi.org/10.1371/journal.pbio.3002277).

While studying the phenomenon of how sounds were processed across locomotive states, I discovered a glaring gap in the literature, sounds themselves could move. While the ability to localize and respond to stationary sounds had been studied extensively, the ability to track the movement of a sound had very little to no work. As a compliment to the first part of this dissertation which studies how self-locomotion of influences auditory perception, in the second part of I examine how external movement influences auditory perception. Relating back to Patient X, it didn't matter if the sound of water came from a stationary faucet or from the falling rain drops outside, a sound would cause a behavioral response. Understanding how sound processing occurs under both self-locomotion and external movement is necessary for me to be able to fully understand how these processes change with neurodegeneration. Thus, this dissertation contributes to the basic understanding of auditory perception needed to be able to ask translational questions.

Table of Contents

List of Figures

List of Abbreviations

AC ..... Auditory cortex

CN ..... Cochlear nucleus

V1 ..... Visual cortex

Hz ..... Hertz

AVCN ..... Anteroventral cochlear nucleus

DCN ..... Dorsal cochlear nucleus

PVCN ..... Posteroventral cochlear nucleus

IC ..... Inferior colliculus

MGB ..... Medial geniculate body

M2 ..... Secondary motor cortex

fMRI ..... Functional magnetic resonance imaging

GECI ..... Genetically encoded calcium indicators

GFP ..... Green fluorescent protein

MUS ..... Muscimol

PBS ..... Phosphate buffer solution

2P ..... Two-photon calcium imaging

PV ..... Parvalbumin neurons

VIP ..... Vasoactive intestinal peptide neurons

PLI ..... Predictive lick index

GLM..... Generalized linear model

BBN .....Broadband Noise

Abstract

In the world, humans and animals continuously receive sensory information, decode from it information about the environment and use that information to guide survival. For each sensory modality, continuous information can be produced by either the self, or an external source, and both are equally important for survival. Recent technological advances have allowed neuroscientists the ability to understand how neural populations encode continuous sensory information and how to localize the functional cortical region producing the behaviors. Across sensory modalities, self-generated locomotion, a ubiquitous and simple form of continuous sensory input, has been shown to have a direct effect on sensory processing and behavior. In the visual and somatosensory cortices, self-generated locomotion information is encoded by neural populations in each respective sensory cortex, but that has yet to be addressed in auditory neural populations. This dissertation addresses this gap by combining behavioral learning, cortical inactivation, two-photon calcium imaging, electrophysiology, and computational modeling to demonstrate that auditory neural populations encode self-generated locomotion and that it is necessary for learned adaptive behaviors. This finding, along with previous studies, suggests that the ability to integrate continuous sensory information and locomotive state is a fundamental property across sensory modalities, and necessary for quick adaptive behavioral processing and learning. While the ability to continuously integrate self-locomotion and sensory information has been studied, very little to no work has attempted to understand how and if sensory neural populations encode and integrate the movement of external sensory objects. In the visual and auditory modalities, studies have replicated external movement by using a VR system to play a moving video or increasing the sound intensity to mimic something approaching. However, in the real world, humans and animals move, and so do predators, prey, and mates. Thus, the ability to continuously track, or monitor the movement of an external object, is necessary for survival. This dissertation combines behavioral learning and two-photon calcium imaging to demonstrate that animals can be trained to understand the movement of external object, and auditory neural populations encode information about the movement of external sources. Beyond simply

processing auditory information, primary auditory cortex, continuously monitors and integrates self-generated locomotion and external object motion information to guide behaviors.

Chapter 1 Introduction

1.1 General Overview

Continuous processing and integration of sensory and non-sensory information is crucial for survival and adaptive behavior. As humans and other animal species continuously perceive and behave in the world, one of the simplest and most crucial pieces of non-sensory information that can influence neural activity and behavior is, locomotion, or movement. While the neural mechanism of continuous sensory processing under immobile or stationary conditions have been extensively studied, recent studies have shifted to understanding how locomotion information is co-encoded, or integrated with sensory information to guide behavior (Bigelow et al.,2019, McGinley et al., 2015; Khoury et al., 2023; ; Schneider, 2020; Ayaz et al., 2013; Ayaz et al., 2019; Campbell & Giocomo, 2018; Saleem et al., 2013; Saleem et al., 2018; McGinley et al., 2015b) .

On a day-to-day basis, humans and animals integrate sensory and locomotion information to guide behavior. For example, if you're a human or animal and you hear a loud sound, you might get up or stop and look. Each of these behaviors would depend on if the human or animal was sitting down, or actively moving. It would require the processing and integration of sound, and self-locomotive state. Work examining the integration of sensory information and self-locomotive state shows a heterogenous effect of locomotion on sensory processing, enhancing some sensory neural populations while suppressing others simultaneously. For example, self-locomotion enhances responses of neurons in primary visual cortex (V1) but suppresses neurons in primary auditory cortex (AC) (Dadarlat & Stryker, 2017; Niell & Stryker, 2010; Vinck et al., 2015b; Audette et al., 2022; Clayton et al., 2021; Rummell et al., 2016; Schneider et al., 2018; Sigurdsson, 2019). Whether this suppression in auditory neural activity by self-locomotion also reflects a behavioral suppression has yet to be addressed. Because humans and other animals can readily react to sounds during locomotive states (Cuppone et al., 2018; Redd & Bamberg, 2012; Tajadura-Jiménez et al., 2015; Turchet et al., 2015; Turchet et al., 2018; Turchet et al., 2013;

Cornwell et al., 2020; Rodger et al., 2014; Schauer & Mauritz, 2003; Karpati et al., 2015; Ravignani & Cooke, 2016, Falk et al., 2014; Ghose et al., 2006; Moss & Surlykke, 2001; Triblehorn & Yager, 2005, Fox, 1984), I hypothesize and demonstrate that self-locomotion does not suppress behavioral performance.

Just as the self-locomotion of the human or animal can inform sensory processing, so can locomotion or movement of objects in the environment. For example, in the real world, as sounds move closer or father away, they produce sounds that get louder or dimmer in intensity, respectively. These gradual changes inform the human or the animal of the nature of the external object and allow for an appropriate behavioral response. Either move if an object is approaching or do nothing if an object is receding. While some studies have mimicked continuous external movement, if animals and how humans process and integrate this type information to drive behavior has yet to be fully addressed (Li et al., 2021; Marques et al., 2018; Douglas et al., 2006; Hoy et al., 2016; Niell & Stryker, 2008; Metin et al., 1988; Stiman et al., 2016; Ahissar et al., 1992; Konishi, 2003; Town et al., 2017; Gao et al., 2020)

In this dissertation, I aim to address how the continuous processing and integration of auditory and movement information is crucial for adaptive behavior. Specifically, I aim to show that both self-locomotion and external-object movement can be used to guide behavior and that auditory sensory neural populations process and integrate this with sound information.

To guide the reader, I will first introduce how sound processing occurs along the auditory pathway and the role of AC. Next, I will examine how continuous sensory and self-locomotion information is encoded in sensory neural populations. I will then specifically examine how self-locomotion information is processed and integrated in the auditory cortex. I will continue by summarizing work done to examine continuous processing of external movement by sensory neural populations. Because much of this work requires awake and behaving animals, I will introduce the reader to the techniques used in this study that allow for neural activity to be monitored under behaving conditions. And lastly, I will introduce the chapters of this dissertation and how they contribute to the understanding of how auditory information and movement information integrate for survival and adaptive behavior.

1.2 Sound processing along the auditory pathway

When humans and animals perceive a sound, a series of events occur that allow the percept of a sound to emerge. How a human or animal will respond to this sound percept is dependent on the identity of the sound but also on how and where a sound is processed. Here I provide a summary of sound processing along the auditory pathway and explain the role of AC in this dissertation.

A common saying goes, "if a tree falls in the woods and no one is around, does it make a sound?" The simple answer is no. When a tree falls it creates soundwaves, or pressure changes of alternating compression and rarefaction, in the air. The rate at which a sound wave alternates between compression and rarefaction is known as the frequency and is measured in Hertz (Hz), or cycles of compression and rarefaction per second. Humans and animals have specialized sensory organs called ears that collect these soundwaves and funnel them through the ear canal. At the end of the ear canal lies the tympanic membrane, or eardrum, a thin piece of tissue that moves in response to the arriving pressure changes, or soundwaves. The movement of the tympanic membrane in turn causes the movement of the malleus, incus, and stapes. These small bones are responsible for amplifying the soundwave and propagating frequency information to the Cochlea via the vestibular window (Peterson et al., 2022).

The Cochlea is instrumental in being able to perceive sounds as it is the site of transduction between mechanical energy from soundwaves to neural electrical energy. The Cochlea is a spiral, fluid-filled structure, with three distinct sections, the scala tympani, scala vestibule, and scala media. When the vestibular window is stimulated by the stapes, it creates waves in the fluid-filled cochlea which travel within the scala tympani to the scala vestibula and cause movement of these sections. In between these two sections is the scala media housing the basilar membrane and the Organ of Corti which contain specialized mechanoreceptors called, hair cells. Within the scala media also lies the tectorial membrane, which moves in reaction to the movement of the scala tympani or scala vestibula. As the tectorial membrane moves, the tips of the hair cells, or stereocilia, get pushed in different directions, resulting in the opening, or closing of potassium channels and the activation or deactivation of the hair cell, respectively (Rhode, 1984; Peterson et al., 2022). At the tip of each stereocilia are tip links, or complex extracellular filaments thought to establish tension and thereby set the resting tension needed to move the hair cell. In addition, the complexity of the tip links provides a mechanism to control

for adaption as hair cells can have different tensions to open or close the transducing potassium channels (Zhao & Muller, 2015).

When the vestibular window moves, it propagates mechanical information about the incoming sound waves measured as frequency. Within the cochlea, this information is organized along a tonotopic gradient, or there is a spatial and anatomical organization of how information is transduced from low frequencies to high frequencies. At the base of the cochlea, nearest the vestibular window, stereocilia are much shorter and stiffer and respond to higher frequencies, whereas at the apex, stereocilia are longer and more flexible, responding to low-frequency sounds. Potentially mediated by the complexity of tip links on stereocilia (Zhoa & Muller, 2015). This organization allows spiny ganglion axons from the cochlear nucleus (CN), which innervate hair cells and form auditory nerves, the ability to be able to transmit information about the sound along the tonotopic map (Rhode, 1984; Sanes et al., 1989; Peterson et al., 2022). Pioneering electrophysiological work done on cochlear nuclei from cats and then replicated in rats demonstrated that cochlear nuclei response profiles were similar across species but also arranged in a tonotopic organization, with lower frequency units found along the lateral surface (Rose et al., 1959; Moller, 1968).

As information leaves the cochlea along the auditory nerves, sound waves have been converted into an electrical neural signal and are now in the central nervous system. The CN is a subcortical structure in the brainstem responsible for the initial processing of sound information. As auditory nerve fibers enter the brainstem they branch off and innervate distinct divisions of the CN while maintaining their tonotopic arrangements in each branch (Oertel, 1991). The anterior branch of the auditory nerve innervates the anteroventral cochlear nucleus (AVCN) and is involved in sound localization and forms the ventral stream. The posterior branches of the auditory nerves innervate the dorsal cochlear nucleus (DCN) and the posteroventral cochlear nucleus (PVCN) and are involved in complex stimulus analysis, forming the dorsal stream (Pickles, 2015).

When sound information arrives in the AVCN, spherical and globular bushy cells work in tangent to refine the temporal properties of the incoming sounds. Work done by (Osen & Roth, 1969) histologically characterized bushy cells as distinct neuron types in the AVCN. In a follow-up study, bushy cells were electrophysiologically recorded and stimulated and displayed responses to short tone onsets better than individual auditory nerve inputs. Additionally, bushy

cells could perform one large action potential, followed up by smaller potentials milliseconds apart, during tone presentation and silence, thus being able to continuously transmit temporally accurate sound information (Smith & Rhode, 1987). Bushy cells are thought to be able to refine temporal accuracy so that this information can be used to perform neural sound localization computations. AVCN projections go to two distinct regions, the medial superior olivary (MSO) and lateral superior olivary (LSO) nuclei. It is important to note that AVCN projections to MSO and LSO are both ipsilateral and contralateral. As sounds approach a human or animal, they will reach each ear at slightly different times, due to the spatial distance of each ear creating a slight time delay. This spatial difference creates a difference in the distance a sound wave must travel to reach each ear. The longer a soundwave travels the weaker its intensity will be and thus there will be a slight level difference in intensity between each ear to the same sound. Having processed the sounds arriving at each ear independently, projections from the AVCN to both ipsilateral and contralateral MSO and LSO will carry information about the same sound but with slightly different time and level differences. AVCN projects to the MSO neurons in a monosynaptic manner, where ipsilateral projections innervate distal dendritic arbors and contralateral projections contact the medial part of dendritic arbors. Due to the combination of differences in neural path lengths, distal and proximal dendrites, and inhibitory synapses on the soma, MSO cells preferentially respond to sounds in the contralateral ear first (Golding & Oertel, 2012). In juxtaposition, LSO neurons receive excitatory monosynaptic projects from ipsilateral AVCN bushy cells. At the same time, contralateral AVCN projections go through the medial nucleus of the trapezoid body (MNTB) which then projects to the LSO via inhibitory glycinergic neurotransmitters (Wu & Kelly, 1991). This results in LSO neurons being more responsive to sounds originating in the ipsilateral ear first. These differences can thus inform whether a sound is localized to the right or left, or along the azimuth, depending on which ear receives auditory information first and if it is slightly louder (Pickles, 2015).

Along the dorsal stream, DCN and PVCN nuclei process sound complexity. When a soundwave is comprised of a single frequency it is called a pure tone, and this is encoded in the tonotopic organization of the cochlea. However, in nature sounds, for example water or speech, are comprised of many different simultaneous frequencies and are called complex sounds or tones. Thus, the ability to be able to encode for and integrate many frequencies is key to being able to determine a sound's identity. Along the dorsal stream, complex neural responses can be

achieved by both inhibitory and excitatory mechanisms which aid in the processing and perception of complex sounds. In the DCN, large proportions of interneurons aid in processing sound complexity by inhibiting excitatory neurons (Young et al., 1992). By increasing inhibition, interneurons can be refined for the specific frequencies of a sound. Additionally, by having local feedback loops, interneurons can inhibit themselves which creates complex patterns of neural responses to sounds. In one study, DCN neurons were shown to be responsive to complex broadband tones, despite their inhibitory responses to pure tones (Young & Brownwell, 1976). In the PVCN there are specialized cells called octopus cells that receive large numbers of auditory nerve afferents. In addition to receiving a wide range of afferents, octopus cells, respond only when large numbers of small excitatory post-synaptic potentials occur allowing for the integration of many tones with a high degree of temporal synchrony (McGinley & Oertel, 2006). This ability to respond to fluctuations in sounds is necessary to process complex sounds (Pickles, 2015).

The ventral and dorsal streams converge upon the inferior colliculus (IC) and is considered crucial in integrating simple acoustic characteristics into sound objects. The IC receives processed information in a tonotopic organization for sound complexity, sound localization, and sound timing from previous processing centers. Using un-anesthetized rabbits implanted with microelectrodes researchers demonstrated that IC neurons robustly encoded sounds along the azimuth modulated by sound intensity (Kuwada et al., 2011). By using previously processed information, neurons in the central core of the IC can respond to sounds in directions along the azimuth and differences in locations along this plane. Additionally, neurons in the external nucleus of the IC have been shown to have novel stimulus detection, or stimulus-specific adaptation as regions of the IC habituate rapidly to ongoing stimuli but respond more strongly to onset of new stimuli (Pickles, 2015). IC neurons recorded using a single barrel glass micropipette showed that a tone burst evoked a response with a longer latency when it is presented repetitively than when it is embedded in a train of tone bursts with various different frequencies (Lumani & Zhang, 2010). While there is more complexity to the IC, this succinct summary highlights that the IC serves as an integration center of dorsal and ventral auditory information along the auditory pathway. Projections from the IC enter the thalamus, innervating the medial geniculate body (MGB).

The MGB, is known as a thalamic relay center, as it receives information from many sensory modalities, and projects to many different neural structures. Thus, this region is known for multisensory processing and integration and is not of focus for this dissertation. In the context of auditory processing, auditory information in the MGB is conserved in a tonotopic map in the ventral division of the nuclei and this projects to the primary auditory cortex (Pickles, 2015).

The primary auditory cortex (AC) serves as the final neural structure along the auditory pathway responsible for auditory processing. In the auditory cortex, information is organized along a tonotopic map. However, embedded in this larger organization is a local heterogeneity of neural responses. Thus, while sound frequencies are on average encoded along a tonotopic map, individual neurons within small regions can respond to a wide range of frequencies (Rothschild et al., 2010). This dual encoding of sounds is thought to aid in higher-order sound processing, such that in the primary auditory cortex sounds can be processed along parallel and simultaneous maps (Nelken et al., 2008). Beyond processing along a tonotopic map, auditory information is processed along cortical layers. According to the canonical cortical microcircuit, auditory information from the MGB enters the AC in layer 4 and ascends to layers 2/3. After auditory processing neural information from layers 2/3 projects to neurons in layers 5/6 for projection to other neural areas (Weible et al., 2020). Interestingly using in-vivo two-photon calcium imaging of layers 2/3 and layer 4 showed that AC neurons were more homogenously organized in frequency in layer 4 as compared to neurons in layer 2 which show a local heterogeneity (Winkowski & Kanold, 2013). Thus, the tonotopic organization of auditory information is transferred to layer 4 from the MGB, and intracortical projections to layer 2/3 give rise to local heterogeneity embedded within a global tonotopy (Kanold et al., 2014). As this dissertation is focused on understanding how auditory information is processed along the auditory pathway, I specifically focus on AC neurons in L2/3 as they are the last processing center before auditory information is relayed to neurons in Layers 5/6 for efferent projections out of the auditory cortex. In the bat auditory cortex, AC neurons can respond to their best frequencies, or the sound that matches their tonotopic organization, while also responding to transient less optimal stimuli (Wang et al., 2005). Having received not only auditory information but affective information from the MGB, AC serves as a center for experience-dependent plasticity. When pairing auditory stimuli with aversive or appetitive stimuli, auditory cortical responses change with the association of the two to reflect learning of the contingencies (Xiong et al., 2009). Additionally,

AC neuronal populations will tune, or change their response profiles, to better encode for a salient stimulus. Over repeated exposure, more neurons will respond to a salient frequency or sound reflecting an enhancement of cortical representation (Xiong et al., 2009). As this dissertation will focus on auditory processing in locomotive states, the auditory cortex has been implicated in state-dependent processing. By comparing anesthetized and awake states researchers showed that auditory information is encoded with higher fidelity in awake-active states compared to immobile-anesthetized states. Suggesting cortical state influences auditory processing (Pachitariu et al., 2015). As this dissertation aims to show how auditory processing under self and external active states influence behavior and learning the primary auditory cortex is a key region to explore as it involves both state and experience-dependent learning and processing and higher-order processing.

1.3 Continuous processing and integration of sensory and self-locomotion information

In both humans and other animals, the ability to continuously process sensory information is crucial for survival, and the integration of locomotion information enhances this probability. Specifically, I will review work examining how self-locomotion, is encoded, and integrated by sensory neural populations to show that across sensory modalities locomotion information is continuously processed and integrated by sensory neural populations to refine adaptive behaviors.

In the world, humans and animals move and navigate their environment. This ability to change from an immobile state to an active moving state is called locomotion. While most of our understanding of sensory processing has been studied under non-behaving or immobile conditions (Pachitariu et al., 2015), some of the most critical and daily sensory processing occurs under awake and locomotive states. Research on self-locomotion demonstrates a heterogeneous effect on sensory neural populations. Studies in the visual, barrel, and somatosensory cortices show that during self-locomotion, sensory neural populations are heterogeneously modulated, but overall enhanced by locomotion (Ayaz et al., 2013, Saleem et al., 2013; Vinck et al., 2015; Ayaz et al., 2019; Schneider, 2020). In the AC, a heterogeneous but net inhibitory effect of locomotion on sound evoked activity emerges (Audette et al., 2022; Clayton et al., 2021; Rummell et al., 2016; Schneider et al., 2018; Sigurdsson, 2019; Bigelow et al., 2019).

In the visual modality researchers implanted multisite electrodes into the primary visual cortex of head-fixed awake mice that were allowed to run or stay still as visual stimuli were presented on a virtual reality screen (Ayaz et al., 2013, Saleem et al., 2013). In one study, researchers found that the population-level firing activity of visual neurons was significantly higher when animals were running, compared to stationary (Saleem et al., 2013). Following this up, another study using the same behavioral setup replicated the finding that visual neurons were enhanced during locomotion and added that individual neurons encoded for the speed of the animal (Ayaz et al., 2013). Using the firing rate of single-unit activity, researchers showed that some neurons proportionally increased their firing rate to the speed of the animal, other neurons proportionally suppressed their activity, while some showed a bimodal effect by increasing their activity during low speeds and suppressing this effect after reaching top speeds (Ayaz et al., 2013). Using a linear decoder to computationally examine neural data researchers were able to reliably decode the speed of the animal based on neural data collected during self-generated locomotion (Ayaz et al.,2013). Overall, studies show that in the visual cortex self-locomotion modulates and encodes neural activity at both the single cell and population level, with an overall enhancement of activity.

In the barrel cortex, or somatosensory modality, self-locomotion has also been shown to have a local-heterogenous, yet overall population-level enhancement of neural activity (Ayaz et al., 2019). In a study, awake head-fixed mice were allowed to freely initiate locomotion bouts under whisker-touching or no-touching conditions. Using two-photon calcium imaging, researchers recorded the neural activity of layer 2/3-barrel cortical neurons under states of locomotion and locomotion with somatosensation to examine how self-generated movement modulated neural activity. The results of this study showed that a large proportion of barrel cortical neurons were enhanced by locomotion when it co-occurred with whisking. While there was an enhancement of neural activity caused by self-locomotion alone, neurons were most activated when running co-occurred with whisking. This increase was much larger than when whisking occurred in stationary conditions (Ayaz et al., 2019). Thus, this study suggests that locomotion enhances neural activity in the barrel cortex but is most modulatory when it occurs simultaneously with somatosensation.

Because locomotion affects all sensory systems simultaneously, sensory neural populations must all be modulated while also being able to maintain or perform behavioral

functions. Given the nature of locomotion information to have a heterogeneous effect on neural activity across sensory modalities, this dissertation will focus on how locomotion information modulates the auditory modality. The auditory modality is an excellent model to study how locomotion information can modulate neural activity while maintaining behavioral function as I will explain in the following section, during locomotion auditory neural activity is suppressed. Yet we know we don't stop listening when we walk. In this dissertation, I examine the auditory cortex as a key candidate brain region for processing incoming sounds during locomotion due to its well-established role in context-, behavior-, and decision-making- dependent sound processing (Cohen et al., 2011; David et al., 2012; Fritz et al., 2010; Jaramillo & Zador, 2011; Kuchibhotla et al., 2017; McGinley et al., 2015b; Nelken, 2014; Rodgers & DeWeese, 2014; Saderi et al., 2021; Ulanovsky et al., 2003; Xiong et al., 2015; Znamenskiy & Zador, 2013). This dissertation aims to address, how does auditory cortex continuously process and integrate sensory information to aid in behavior, when locomotion is suppressing its activity.

1.4 Effects of Self-generated locomotion on auditory cortical processing

During self-generated locomotion, as I will explain below, auditory cortical activity is suppressed relative to other sensory modalities. However, some of the most crucial and ordinary auditory behaviors can only occur in a locomotive state. For example, a runner will move at the sound of a car horn, just as an animal would change course at the sound of a mate. A runner will increase their running speed when listening to music, with faster tempos and louder intensities enhancing this effect (Edworthy & Warring, 2007). In a study that examined the functional magnetic resonance imaging (fMRI) activity of people learning to play piano, an audiomotor task, there was a shift in activity from motor to auditory regions as one moved from novice to expert (Hasegawa et al., 2004). In a similar but more controlled study, subjects were asked to play a musical sequence on a piano keyboard or listen to the same sequence during an fMRI. Interestingly temporal regions, associated with auditory processing, were enhanced during the active audiomotor task (Resnik et al. 2014). Altogether these examples and studies demonstrate that auditory processing and locomotion occur simultaneously and can drive or modulate behavior.

Locomotion-induced suppression of auditory cortical activity was demonstrated in a study where head-fixed mice were implanted with sharp microelectrodes into the auditory cortex

while producing locomotion on a rotating plate. In this study sounds were played when the mouse was in either a running or immobile state to examine how locomotion modulated sound-evoked activity. The results of this study showed a significant suppression of sound-evoked activity during locomotion, compared to immobility (Schneider et al., 2014). By infusing a retrograde tracer into the auditory cortex, previous work has shown that during locomotion, motor projections primarily from the secondary motor cortex (M2) innervate local auditory excitatory and interneurons. Furthermore, by optogenetically stimulating these projects researchers were able to replicate the suppressive effects of locomotion on sound-evoked activity (Nelson et al., 2013). While motor projections to the AC innervate both excitatory and inhibitory neurons, whole cell voltage clamp recordings of these neurons during optogenetic stimulation, showed that inhibitory currents were almost four-fold greater than excitatory currents. (Nelson et al., 2013). Additionally, using multi-electrode arrays to record spiking activity of local neuronal populations, researchers showed that firing rates of parvalbumin (PV) and vasoactive intestinal peptide (VIP) interneurons were enhanced with locomotion and excitatory firing rates were suppressed (Schneider et al., 2014; Bigelow et al., 2019). Interestingly, VIP neurons in layer 4 were suppressed by locomotion for both spontaneous and sound-evoked activity, suggesting that the net suppressive effect of locomotion in L2/3 is not mediated by VIP networks (Yavorska & Wehr, 2021). This suggests a feedforward inhibition mechanism where motor projections activate more interneurons than excitatory neurons in the auditory cortex and create an overall suppressive effect of sound-evoked activity. In a more recent study, researchers implanted head-fixed mice with electrode arrays while allowing self-generated locomotion and found that while overall population-level sound-evoked activity was suppressed, small proportions of neurons could be enhanced with locomotion (Bigelow et al., 2019). Thus, locomotion has a heterogenous effect on sound-evoked activity, with an overall net population-level suppression mediated by feedforward inhibition from local intraneuronal circuits.

A proposed explanation for a suppressive effect of locomotion on auditory processing, supported by the finding that responses in the primary visual cortex are generally enhanced during locomotion (Ayaz et al., 2013, Saleem et al., 2013;Dadarlat & Stryker, 2017; Niell & Stryker, 2010; Vinck et al., 2015b), is that locomotion reflects a neural resource allocation shift from auditory to visual (Schneider et al., 2014; Zhou et al., 2014). According to this model, reduced sound responses during locomotion reflect a functional attenuation of AC, possibly

involving reliance on subcortical regions for sound processing in this state. In line with this idea is that locomotion induces suppression of auditory cortical activity from a corollary discharge hypothesis. As research has shown when animals move, motor projects innervate auditory regions, providing locomotion-induced changes (Nelson et al., 2013). This motor information is useful in the context of self-generated locomotion to reafferent sounds, or self-producing sounds. During movement, reafferent sounds are produced in by one's behavior such as footsteps hitting the ground, or mouths moving to produce speech or song. Because the sounds are constant and expected, suppressing auditory activity to these sounds allows for enhanced detection of external stimuli, or quick modulation of motor activity (Audette et al., 2022; Clayton et al., 2021; Rummell et al., 2016; Schneider et al., 2018; Sigurdsson, 2019). In a behavioral experiment animal were trained to press a lever that elicited a sound, thus animals learned to associate a lever press with a reafferent cue. Auditory cortical activity from freely moving mice implanted with microelectrodes was recorded as animals pressed the lever. Randomly the same sound was played when the animal did not generate the cue by pressing the lever. Auditory cortical activity to the exafferent sound was significantly greater than activity generated by the reafferent sound (Rummell et al., 2016). In a similar design, mice were trained to press a lever that generated a reafferent sound followed by a reward. In this study, a different sound was played during the lever press and similarly, the probe, or novel stimulus showed less suppression of activity and less licking, or a dampening of behavioral responsiveness (Audette et al., 2022). Interestingly overall sound evoked activity was suppressed during the movement of the lever, but the novelty of the sound reduced the suppressive effects. Despite being able to explain why a suppressive effect of auditory cortical activity occurs in locomotion the evolutionary and functional benefit of both a resource allocation shift and corollary discharge cannot explain how sound information can guide behavior during a locomotive state (Bigelow et al., 2019; Schneider, 2020).

Alternatively, it has been suggested that rather than a resource allocation shift from the auditory to the visual modality, locomotion may induce a shift toward spatial information processing and coordination of information processing across modalities (Bigelow et al., 2019; Ghosh et al. 2022). According to this hypothesis, the AC may play a critical role in sound processing during locomotion, but it may include encoding of locomotion-related non-acoustic information, which, if unaccounted for, could appear as simple suppression. This hypothesis is supported by the robust encoding of locomotion-related signals and their integration with cue-

evoked responses in other sensory cortical regions (Ayaz et al., 2013; Ayaz et al., 2019; Campbell & Giocomo, 2018; Saleem et al., 2013; Saleem et al., 2018).

Overall, the inhibitory effect of self-locomotion on auditory neural populations reflects a modulation of neural activity but has not shown that auditory and locomotion information are continuously processed and integrated to aid in behavior.

1.5 Continuous processing and integration of sensory and external-movement information

In most sensory modalities, the integration and processing of movement information has focused on how information about the human or animal is used to aid in adaptive behaviors. Locomotion information modulates sensory neural activity based on the individual; however, external movement information can also influence neural activity. Furthermore, movement information gathered about the external object is crucial to survival. For example, a human or animal hears a faint sound that progressively gets louder and faster. This would signal a fast-approaching object. If this sound is a growl, it's a threat and run, but if the sound is a sibling's call, then prepare for a hug. Combining sound information with movement information about the external object allows the human or animal to predict and react appropriately to the environment or track a sensory object. Of the sensory modalities, not many would have a direct evolutionary need or ability to track sensory objects, however visual and auditory processing do. Surprisingly, the few studies that have attempted to address how movement information about external objects influences neural activity and behavior have failed to move an object as it would occur naturally.

In the visual modality when an object moves along the frontal plane, perceptually it will change size to the human or animal. If the object gets bigger, it signals that it is getting closer, or smaller, and the object is further. By using this principle, studies have used virtual reality to create the illusion of movement along a virtual track by changing the size of objects on a fixed-stationary screen (Ayaz et al., 2013; Campbell et al., 2018; Saleem et al., 2013; Saleem et al., 2018; Goa et al., 2020). Specifically, by head-fixing mice and placing them on a running wheel in a closed-loop system, as animals generated self-locomotion the external screen would change to reflect walking along a track. This closed-loop system was yoked to the animals' speed such that faster locomotion would cause faster changes in the virtual track. In one study, the animal and the virtual reality were inversely paired such that faster running caused slower virtual changes and vice versa. Using this paradigm while simultaneously recording from neurons in the

visual cortex, researchers demonstrated that neurons in the primary visual cortex encoded for self-locomotion speed, visual locomotion speed, and some neurons responded to the integration of self and external movement speed (Saleem et al., 2013). As external objects move, their position changes and that can be used to guide behavior. By placing distinct markers along the virtual reality track, researchers trained animals to expect a reward at a certain location. As animals moved two visual markers 40 cm in length were used to determine their location and relative position to the reward zone which followed 2 repeats of each visual stimuli. Using two-photon calcium imaging, researchers demonstrated that visual cortical neurons responded to the visual cues in a spatially dependent manner, such that some neurons responded to the same visual cue twice, whereas others responded to the same visual cue depending on if it was the first or second repeat. Thus, visual cortical neurons encoded the stimuli and the movement of the external cues to determine if it was spatially the beginning of the track or further along the track (Saleem et al., 2018). Beyond moving along the frontal plane, objects can also move along the azimuth, or horizontal plane to determine directionality. Using a virtual reality screen, animals were shown random dot kinetograms, which moved visual stimuli in either a nasal or temporal direction. Using the direction of motion of the visual stimuli, animals were trained to lick to either a left or right lick port depending on if the objects moved nasally or temporally, respectively. Using 2P calcium imaging, neurons in the visual cortex showed robust encoding of direction-specific visual stimuli with distinct subpopulations of neurons encoding for nasal or temporal direction (Marques et al 2018). Thus, visual neurons can distinguish the movement of an object as it moves direction, and this information can be used to guide behavior.

In the auditory modality, the ability to track changes in location and direction has been shown. Ferrets were head-fixed and trained to listen for sounds as they moved from left to right, and vice versa, along a series of speakers 30 degrees apart. If sounds moved from right to left, marked by a reference sound followed by a target sound moving along the azimuth, animals were trained to lick to a left reward port, and to a right reward port if the sounds traveled in the reverse order. By implanting electrodes into AC during behavior, auditory cortical neuronal activity showed that neurons robustly encoded the spatial location of the target sound (Wood et al., 2019). Thus, animals could learn and encode for the changes in the spatial location of objects as they move along the azimuth. In a pioneering study, macaques were implanted with microelectrodes into the auditory cortex and placed in front of a speaker attached to a base that

moved along the azimuth. In this study, auditory neurons responded to sounds at distinct spatial locations. A subset of neurons also showed changes in auditory spiking activity as the object continuously moved from one direction to the next (Ahissar et al., 1992). Thus, not only could auditory cortical neurons encode for location, but the external motion of the object was modulating neural activity. While the ability to encode for spatial location and movement has been demonstrated along the azimuth, the ability to detect sounds as they approach and recede is key to survival. In the studies above as sounds move along the azimuth, interaural time and intensity differences aid in the ability to process and behave to a changing sound. However, when objects move along the frontal plane, these differences are negated as sounds will arrive at both ears simultaneously. More importantly, when sounds move along the frontal plane, they are getting closer to the self, which has more implications for survival than sounds that remain at a distance but change location. As an auditory object moves along the frontal plane, the perceptual loudness of the object can be used to infer its movement such that approaching sound objects means getting louder, and leaving objects means getting quieter. In a study to test this phenomenon, researchers used looming sounds to replicate objects approaching or receding. In this study freely moving animals were placed in an open field and exposed to looming noises, or fast changes in sound intensity, which could either be in a crescendo, or quickly getting louder, or a decrescendo, or quickly getting quieter. By using these sounds scientists were able to elicit defensive behaviors from a mouse in the crescendo conditions, with no changes to behavior occurring in the decrescendo conditions. When using Muscimol (MUS) a GABA agonist to disrupt neural activity, animals no longer displayed defensive behaviors to looming sounds. This study suggests that the AC is necessary to produce defensive behaviors or respond to sounds that signal an approaching object (Li et al, 2021). Overall, the ability to detect changes in location, movement, and directionality has been shown in both the auditory modality, but under conditions that do not reflect how external objects move in the environment and under movements that would be most important for survival.

1.6 Introduction to Methodology

Over the past three decades, technological advancements, have revolutionized the way neuroscience can explore the brain and behavior. With the invention of two-photon calcium imaging, and countless cortically modulating agents, neuroscientists have been able to examine

neural phenomena in awake and behaving animals without damage to brain structures. Some of the earliest and most pioneering work in neuroscience and the study of locomotor effects on sensory processing occurred using techniques that required the brain to be extracted from the animal, recording equipment to be inserted through layers of cortical tissue, or animals to be in heavily anesthetized states (Nelson et al., 2013; Schneider et al., 2014). However, to truly understand how the brain and behavior interact, being able to explore neural activity inside awake and behaving animals is key.

Two-photon Calcium Imaging was first invented in 1990 at Cornell University, as an upgrade to the traditional confocal microscope (Heath, 2018). In a confocal microscope, a continuous beam of single photons is used to pierce all cortical layers until they react with a fluorophore, causing the emission of a single fluorescent photon. In a two-photon microscope, pulses of photons are used to pierce the cortical layers until two photons interact with a fluorophore at the same time, to cause the emission of a single fluorescent photon. By pulsing photons, as opposed to a continuous beam, two-photon calcium imaging can use wavelengths at half the energy, as the sum of the two photons will have the same energy needed to excite a fluorophore. By using less energy per photon there is less background noise and increased precision in imaging the brain and neural activity. A continuous beam of single photons will interact with all layers that it crosses until it reaches a fluorophore of interest, whereas the pulsing of two photons will not have enough power to do so, only when they collide at the intended region of interest (Heath, 2018; Svoboda & Yasuda, 2006). For studying awake and behaving animals this comes in handy as animals move their heads, groom, and walk when recording. Being able to reduce the amount of noise coming from imaging sessions is critical to being able to precisely record brain activity from a specific region. In this case, being able to record more precisely during these active states allows neuroscientists the ability to understand how these processes are encoded in the brain.

Two-photon microscopy goes hand in hand with the use of genetically encoded calcium indicators (GECIs). GECIs are genetically engineered compounds that can be used to track changes in large populations of cell bodies or record subcellular processes in the axons, dendrites, or synapses (Dana et al., 2019). Specifically in this dissertation, I use GCamp6f, which is a GECI that has a green fluorescent protein (GFP) based sensor to monitor neural activity from excitatory pyramidal cells (Chen et al., 2013). When an action potential occurs in a neuron,

calcium channels open allowing the rise of intracellular calcium concentrations which in turn aid in neurotransmitter release. The more action potentials, the larger the intracellular calcium concentration and neurotransmitter release a neuron experiences. GCamp6f when present in a neuron will bind to the intracellular calcium influx caused by an action potential, and the more calcium the more binding that occurs. When two photons simultaneously collide with a neuron tagged with Gcamp6f that has calcium bound to it, the sum of the energy of each photon will be enough to cause a green fluorophore will be emitted. Thus, neural activity can be measured as the number of green fluorophores being emitted by a neuron based on the intracellular calcium concentration triggered by an action potential (Chen et al., 2013).

Although this technique allows for near-perfect spatial precision in being able to only record the intended region and neural population of interest, it is not perfect. GECIs can be introduced into neural populations by cortical infusions, or genetically modified mouse lines. Via cortical infusions, a GCamp6f virus is injected into the region of interest, which still requires damaging some cortical layers. This dissertation used genetically modified GCamp6F homo/heterozygous mouse lines that express this GECI in all excitatory cortical pyramidal cells to avoid damaging cortical structures. However, to be able to image neural activity, a craniotomy must still be performed. In a craniotomy, a piece of the skull is removed and replaced with a piece of glass that allows the two-photon microscope to pulse photons into the brain. Thus, while no cortical layers are damaged, the animal has a foreign piece of glass attached to the skull. Additionally, because the neural activity is measured as a function of calcium influx and binding to GCamp6F and its subsequent fluorescent emission, there is a time delay of 2 seconds to peak fluorescence and return to baseline with activity emerging as early as 20-200ms after stimulus onset (Dana et al., 2014); Chen et al., 2013). While no technique can be perfect, two-photon microscopy is currently one of the most advanced and precise techniques that allow neural activity to be measured in awake and behaving animals (Heath, 2018).

As noted above, being able to record neural activity from awake and behaving animals is crucial to understanding the interactions between the brain and behavior, and with recent technological advances like two-photon microscopy, this is becoming more common. Because animals can more readily behave during neural recordings the need to localize the origin of a behavior to a neural population has become more common as well (Heath, 2018; Svoboda & Yasuda, 2006). To do this requires two steps, designing behavioral paradigms that train animals

on a behavior of interest, and inactivating a local population to understand if it is involved in the production of said behavior.

In this dissertation, I will present two novel behavioral paradigms that train animals to perform a behavior. To create these behavior paradigms, the increased affordability and accessibility to microcontrollers, microprocessors, custom hardware, and open-source programming languages have been key. For this dissertation, I use an Arduino microcontroller to be able to integrate a speaker, a solenoid-based reward-delivery system, motion detectors, and a linear actuator to create custom behavioral rigs. By combining Arduino circuits with multichannel processors used in two-photon microscopy I can record neural data while animals are behaving in these custom-built behavioral rigs. Using Arduino Programming Language and MATLAB I can build custom software that controls and coordinates the hardware to be able to train and record from animals in these novel behavioral rigs. Specific details about each behavioral rig and paradigm will be explained in the following chapters.

Once a behavioral rig has been built and a behavioral paradigm established, inactivating a specific region of interest is next. In this dissertation, I use a cannula infusion system paired with Muscimol to inactivate the auditory cortex following behavioral training. Before training, animals are implanted with bilateral cannulas into the auditory cortex that pierces the cortex and stops at the point of interest. The cannula is a small metal that is inserted into the cortex and left there for the entirety of training. When trained and ready, a pharmacological agent can be delivered to the region of interest. To inactivate the auditory cortex, I use Muscimol which is a GABA agonist. When Muscimol is delivered into a neural population it binds to GABA receptors on inhibitory neurons in the brain altering normal cortical dynamics, by enhancing inhibitory activity (Majchrzak & Di Scala, 200). If animals can still perform a behavior under Muscimol, then this region is not involved in the production of behavior. However, if animals can no longer perform a behavior under Muscimol, then it stands to reason that this neural population is involved in the production of a learned behavior.

Overall, the advancement of technologies has vastly improved what is capable in neuroscience. From advanced recording techniques like two-photon microscopy to more accessible hardware and software, the ability to record awake and behaving animals has become a new frontier. I will now explain the chapters of my dissertation which take advantage of these new technologies.

1.7 Introduction to current experiments

The experiments and results described here aim to fill in a gap in the literature and to provide further understanding of how sensory and non-sensory information are continuously processed and integrated to aid in behavior. Using the auditory modality, I aim to show that non-sensory information can be used to guide behaviors and that this information is encoded and integrated with sensory information. Specifically, this dissertation aims to test if self-locomotion and external-object locomotion information can be used to guide behaviors and how this information is encoded in neural populations. First, I test the hypothesis that a net-suppressive effect of self-locomotion on auditory sound-evoked responses does not impair active auditory behavior and learning but reflects an alternate neural computation. Next, I test the hypothesis that external sound source object movement can be used to guide adaptive behaviors. Lastly, I examine if and how external sound source object movement is processed and encoded by auditory cortical neuronal ensembles.

1.7.1 Chapter 2: Auditory cortex ensembles jointly encode sound and locomotion speed to support sound perception during movement

AC may play a critical role in sound processing during locomotion, but that it may include encoding of locomotion-related non-sensory information, which, if unaccounted for, could appear as simple suppression. Here we combined AC inactivation of mice performing a sound-guided reward-predictive behavior during locomotion, two-photon calcium imaging in head-fixed mice, and electrophysiological recordings in freely-moving rats to test the hypothesis that auditory cortical ensembles are not simply suppressed during locomotion but rather explicitly encode it and incorporate it with sound information into an integrated audiomotor neural code.

1.7.2 Chapter 3: The Doppler; a behavioral system to train external object tracking behavior

Given that studies examining how external objects are processed have only mimicked aspects of sensation. Recording with a more naturally and ethologically behaving object would allow auditory and visual neuroscientist the ability to more accurately understand if animals use the non-sensory information of external objects to guide their behavior. Here I design and

develop “The Doppler,” a behavioral training and recording system that allows an auditory sound source to move continuously in front of an animal at varying speeds and sounds. Using The Doppler, I show that mice can be trained to integrate sound information with non-sensory information about a moving object, to demonstrate rodents can predict upcoming rewards based on the movement of an object. Furthermore, by using a series of control experiments, I demonstrate that mice understand and use the movement of objects to guide behavior and not changes in time, sound intensity, or location. When external objects move in the environment the slight changes in sensory information can be enough to derive non-sensory information about them. Did the object stay put or move? Is it getting closer or further? If it is moving at what speed? By combining two-photon calcium imaging in head-fixed mice on the Doppler, I test the hypothesis that auditory cortical neuronal ensembles continuously process and incorporate sound and external object movement information into an integrated neural code. Specifically, I show that auditory cortical neuronal ensembles continuously process for and integrate information about the relative distance, locomotive state, direction, and speed of an externally moving sound object.

1.8 References

1. Ahissar, M., Ahissar, E., Bergman, H., & Vaadia, E. (1992). Encoding of sound-source location and movement: Activity of single neurons and interactions between adjacent neurons in the monkey auditory cortex. Journal of Neurophysiology, 67(1), 203–215. https://doi.org/10.1152/jn.1992.67.1.203
2. Ayaz A, Saleem AB, Scholvinck ML, Carandini M. Locomotion controls spatial integration in mouse visual cortex. Curr Biol. 2013; 23(10):890–4. Epub 2013/05/15. https://doi.org/10.1016/j.cub.2013.04. 012 PMID: 23664971; PubMed Central PMCID: PMC3661981.
3. Ayaz A, Stauble A, Hamada M, Wulf MA, Saleem AB, Helmchen F. Layer-specific integration of locomotion and sensory information in mouse barrel cortex. Nat Commun. 2019; 10(1):2585. Epub 2019/ 06/15. https://doi.org/10.1038/s41467-019-10564-8 PMID: 31197148; PubMed Central PMCID:PMC6565743
4. Audette NJ, Zhou W, La Chioma A, Schneider DM. Precise movement-based predictions in the mouse auditory cortex. Curr Biol. 2022; 32(22):4925–40 e6. Epub 20221024. https://doi.org/10.1016/j.cub.2022.09.064 PMID: 36283411; PubMed Central PMCID: PMC9691550.
5. Bennett, C., Arroyo, S., & Hestrin, S. (2013). Subthreshold mechanisms underlying state-dependent modulation of visual responses. Neuron, 80(2), 350–357. https://doi.org/10.1016/j.neuron.2013.08.007
6. Bigelow J, Morrill RJ, Dekloe J, Hasenstaub AR. Movement and VIP Interneuron Activation Differentially Modulate Encoding in Mouse Auditory Cortex. eNeuro. 2019; 6(5). Epub 20190918. https://doi. org/10.1523/ENEURO.0164-19.2019 PMID: 31481397; PubMed Central PMCID: PMC6751373.
7. Campbell MG, Giocomo LM. Self-motion processing in visual and entorhinal cortices: inputs, integration, and implications for position coding. J Neurophysiol. 2018; 120(4):2091–106. Epub 2018/08/09.https://doi.org/10.1152/jn.00686.2017 PMID: 30089025; PubMed Central PMCID: PMC6230811.

8. Chen, T.-W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., Schreiter, E. R., Kerr, R. A., Orger, M. B., Jayaraman, V., Looger, L. L., Svoboda, K., & Kim, D. S. (2013). Ultrasensitive fluorescent proteins for imaging neuronal activity. Nature, 499(7458), 295–300. https://doi.org/10.1038/nature12354
9. Clayton KK, Williamson RS, Hancock KE, Tasaka GI, Mizrahi A, Hackett TA, et al. Auditory Corticothalamic Neurons Are Recruited by Motor Preparatory Inputs. Curr Biol. 2021; 31(2):310–21 e5. Epub 20201105. https://doi.org/10.1016/j.cub.2020.10.027 PMID: 33157020; PubMed Central PMCID: PMC7855066.
10. Cohen L, Rothschild G, Mizrahi A. Multisensory integration of natural odors and sounds in the auditorycortex. Neuron. 2011; 72(2):357–369. https://doi.org/10.1016/j.neuron.2011.08.019 PMID: 22017993.
11. Cornwell T, Woodward J, Wu MM, Jackson B, Souza P, Siegel J, et al. Walking With Ears: Altered Auditory Feedback Impacts Gait Step Length in Older Adults. Frontiers in Sports and Active. Living. 2020; 2(38). https://doi.org/10.3389/fspor.2020.00038 PMID: 33345030
12. Cuppone AV, Cappagli G, Gori M. Audio Feedback Associated With Body Movement Enhances Audio and Somatosensory Spatial Representation. Front Integr Neurosci. 2018; 12(37). https://doi.org/10.3389/fnint.2018.00037 PMID: 30233334
13. Dadarlat MC, Stryker MP. Locomotion Enhances Neural Encoding of Visual Stimuli in Mouse V1. JNeurosci. 2017; 37(14):3764–3775. https://doi.org/10.1523/JNEUROSCI.2728-16.2017 PMID: 28264980; PubMed Central PMCID: PMC5394894.
14. Dana, H., Chen, T.-W., Hu, A., Shields, B. C., Guo, C., Looger, L. L., Kim, D. S., & Svoboda, K. (2014). Thy1-GCAMP6 transgenic mice for neuronal population imaging in vivo. PLoS ONE, 9(9). https://doi.org/10.1371/journal.pone.0108697
15. Dana, H., Sun, Y., Mohar, B., Hulse, B. K., Kerlin, A. M., Hasseman, J. P., Tsegaye, G., Tsang, A., Wong, A., Patel, R., Macklin, J. J., Chen, Y., Konnerth, A., Jayaraman, V., Looger, L. L., Schreiter, E. R., Svoboda, K., & Kim, D. S. (2019). High-performance calcium sensors for imaging activity in neuronal populations and microcompartments. Nature Methods, 16(7), 649–657. https://doi.org/10.1038/s41592-019-0435-6

16. David SV, Fritz JB, Shamma SA. Task reward structure shapes rapid receptive field plasticity in auditory cortex. Proc Natl Acad Sci U S A. 2012; 109(6):2144–9. Epub 20120123. https://doi.org/10.1073/ pnas.1117717109 PMID: 22308415; PubMed Central PMCID: PMC3277538.
17. Douglas, R. M., Neve, A., Quittenbaum, J. P., Alam, N. M., & Prusky, G. T. (2006). Perception of visual motion coherence by rats and mice. Vision Research, 46(18), 2842–2847. https://doi.org/10.1016/j.visres.2006.02.025
18. Edworthy, J., & Waring, H. (2006). The effects of music tempo and loudness level on treadmill exercise. Ergonomics, 49(15), 1597–1610. https://doi.org/10.1080/00140130600899104
19. Falk B, Jakobsen L, Surlykke A, Moss CF. Bats coordinate sonar and flight behavior as they forage in open and cluttered environments. J Exp Biol. 2014; 217(Pt 24):4356–4364. https://doi.org/10.1242/ jeb.114132 PMID: 25394632; PubMed Central PMCID: PMC4375838.
20. Fisher, S. P., Cui, N., McKillop, L. E., Gemignani, J., Bannerman, D. M., Oliver, P. L., Peirson, S. N., & Vyazovskiy, V. V. (2016). Stereotypic wheel running decreases cortical activity in mice. Nature Communications, 7(1). https://doi.org/10.1038/ncomms13138
21. Flossmann, T., & Rochefort, N. L. (2021). Spatial navigation signals in rodent visual cortex. Current Opinion in Neurobiology, 67, 163–173. https://doi.org/10.1016/j.conb.2020.11.004
22. Fox MW. The whistling hunters: field studies of the Asiatic wild dog (Cuon alpinus). Albany: State University of New York Press; 1984. viii, p. 150.
23. Fritz JB, David SV, Radtke-Schuller S, Yin P, Shamma SA. Adaptive, behaviorally gated, persistent encoding of task-relevant auditory information in ferret frontal cortex. Nat Neurosci. 2010; 13(8):1011–9. Epub 20100711. https://doi.org/10.1038/nn.2598 PMID: 20622871; PubMed Central PMCID:PMC2921886.
24. Gao S, Webb J, Mridha Z, Banta A, Kemere C, McGinley M. Novel Virtual Reality System for Auditory Tasks in Head-fixed Mice. Annu Int Conf IEEE Eng Med Biol Soc. 2020; 2020:2925–2928. https://doi.org/10.1109/EMBC44109.2020.9176536 PMID: 33018619.

25. Ghose K, Horiuchi TK, Krishnaprasad PS, Moss CF. Echolocating bats use a nearly time-optimal strategy to intercept prey. PLoS Biol. 2006; 4(5):865–73. ARTN e108 https://doi.org/10
26. Ghosh, M., Yang, F.-C., Rice, S. P., Hetrick, V., Gonzalez, A. L., Siu, D., Brennan, E. K. W., John, T. T., Ahrens, A. M., & Ahmed, O. J. (2022). Running speed and REM sleep control two distinct modes of rapid interhemispheric communication. Cell Reports, 40(1), 111028. https://doi.org/10.1016/j.celrep.2022.111028
27. Golding, N. L., & Oertel, D. (2012). Synaptic integration in dendrites: Exceptional need for speed. The Journal of Physiology, 590(22), 5563–5569. https://doi.org/10.1113/jphysiol.2012.229328
28. Hasegawa, T., Matsuki, K.-I., Ueno, T., Maeda, Y., Matsue, Y., Konishi, Y., & Sadato, N. (2004). Learned audio-visual cross-modal associations in observed piano playing activate the left Planum Temporale. an fmri study. Cognitive Brain Research, 20(3), 510–518. https://doi.org/10.1016/j.cogbrainres.2004.04.005
29. Heath, J. (Ed.). (2018). Multiphoton Microscopy (1st ed.). John Wiley & Sons.
30. Hoy, J. L., Yavorska, I., Wehr, M., & Niell, C. M. (2016). Vision drives accurate approach behavior during prey capture in laboratory mice. Current Biology, 26(22), 3046–3052. https://doi.org/10.1016/j.cub.2016.09.009
31. Jaramillo S, Zador AM. The auditory cortex mediates the perceptual effects of acoustic temporal expectation. Nat Neurosci. 2011; 14(2):246–U340. https://doi.org/10.1038/nn.2688 WOS:000286595400023. PMID: 21170056
32. Kanold, P. O., Nelken, I., & Polley, D. B. (2014). Local versus global scales of organization in Auditory Cortex. Trends in Neurosciences, 37(9), 502–510. https://doi.org/10.1016/j.tins.2014.06.003
33. Karpati FJ, Giacosa C, Foster NEV, Penhune VB, Hyde KL. Dance and the brain: a review. Ann NyAcad Sci. 2015; 1337:140–146. https://doi.org/10.1111/nyas.12632 WOS:000351580900020. PMID:25773628
34. Khoury CF, Fala NG, Runyan CA. Arousal and Locomotion Differently Modulate Activity of Somatostatin Neurons across Cortex. eNeuro. 2023; 10(5). Epub 20230525.

https://doi.org/10.1523/ ENEURO.0136-23.2023 PMID: 37169583; PubMed Central PMCID: PMC10216262.

35. Konishi, M. (2003). Coding of Auditory Space. Annual Review of Neuroscience, 26(1), 31–55. https://doi.org/10.1146/annurev.neuro.26.041002.131123
36. Kuchibhotla KV, Gill JV, Lindsay GW, Papadoyannis ES, Field RE, Sten TAH, et al. Parallel processing by cortical inhibition enables context-dependent behavior. Nat Neurosci. 2017; 20(1):62–71. https://doi.org/10.1038/nn.4436 WOS:000391085500013. PMID: 27798631
37. Kuwada, S., Bishop, B., Alex, C., Condit, D. W., & Kim, D. O. (2011). Spatial tuning to sound-source azimuth in the inferior colliculus of unanesthetized rabbit. Journal of neurophysiology, 106(5), 2698–2708. https://doi.org/10.1152/jn.00532.2011
38. Li, Z., Wei, J.-X., Zhang, G.-W., Huang, J. J., Zingg, B., Wang, X., Tao, H. W., & Zhang, L. I. (2021). Corticostriatal control of defense behavior in mice induced by auditory looming cues. Nature Communications, 12(1). https://doi.org/10.1038/s41467-021-21248-7
39. Lumani, A., & Zhang, H. (2010). Responses of neurons in the rat's dorsal cortex of the inferior colliculus to monaural tone bursts. Brain research, 1351, 115–129. https://doi.org/10.1016/j.brainres.2010.06.066
40. Marques, T., Summers, M. T., Fioreze, G., Fridman, M., Dias, R. F., Feller, M. B., & Petreanu, L. (2018). A role for mouse primary visual cortex in motion perception. Current Biology, 28(11). https://doi.org/10.1016/j.cub.2018.04.012
41. Majchrzak, M., & Di Scala, G. (2000). GABA and Muscimol as reversible inactivation tools in learning and memory. Neural Plasticity, 7(1–2), 19–29. https://doi.org/10.1155/np.2000.19
42. McGinley MJ, David SV, McCormick DA. Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection. Neuron. 2015; 87(1):179–192. https://doi.org/10.1016/j.neuron.2015.05.038 WOS:000360977400017. PMID: 26074005
43. McGinley, M. J., Vinck, M., Reimer, J., Batista-Brito, R., Zagha, E., Cadwell, C. R., Tolias, A. S., Cardin, J. A., & McCormick, D. A. (2015). Waking state: Rapid variations modulate neural and behavioral responses. Neuron, 87(6), 1143–1161. https://doi.org/10.1016/j.neuron.2015.09.012

44. McGinley, M. J., & Oertel, D. (2006). Rate thresholds determine the precision of temporal integration in principal cells of the ventral cochlear nucleus. Hearing research, 216, 52-63.
45. Mineault, P. J., Tring, E., Trachtenberg, J. T., & Ringach, D. L. (2016). Enhanced spatial resolution during locomotion and heightened attention in mouse primary visual cortex. The Journal of Neuroscience, 36(24), 6382–6392. https://doi.org/10.1523/jneurosci.0430-16.2016
46. Metin, C., Godement, P., & Imbert, M. (1988). The primary visual cortex in the mouse: Receptive field properties and functional organization. Experimental Brain Research, 69(3). https://doi.org/10.1007/bf00247312
47. Moller, A. R. (1969). Unit responses in the cochlear nucleus of the rat to pure tones. Acta Physiologica Scandinavica, 75(4), 530-541.
48. Moss CF, Surlykke A. Auditory scene analysis by echolocation in bats. J Acoust Soc Am. 2001; 110 (4):2207–2226. https://doi.org/10.1121/1.1398051 WOS:000171526400054. PMID: 11681397
49. Nelken I. Stimulus-specific adaptation and deviance detection in the auditory system: experiments and models. Biol Cybern. 2014; 108(5):655–63. Epub 20140130. https://doi.org/10.1007/s00422-014-0585-7 PMID: 24477619.
50. Niell CM, Stryker MP. Modulation of visual responses by behavioral state in mouse visual cortex. Neuron. 2010; 65(4):472–479. https://doi.org/10.1016/j.neuron.2010.01.033 PMID: 20188652; PubMed Central PMCID: PMC3184003.
51. Niell, C. M., & Stryker, M. P. (2008). Highly selective receptive fields in mouse visual cortex. The Journal of Neuroscience, 28(30), 7520–7536. https://doi.org/10.1523/jneurosci.0623-08.2008
52. Osen, K. K., & Roth, K. (1969). Histochemical localization of cholinesterases in the cochlear nuclei of the cat, with notes on the origin of acetylcholinesterase-positive afferents and the superior olive. Brain Research, 16(1), 165–185. https://doi.org/10.1016/0006-8993(69)90092-4

53. Oertel, D. (1991). The role of intrinsic neuronal properties in the encoding of auditory information in the cochlear nuclei. Current Opinion in Neurobiology, 1(2), 221–228. https://doi.org/10.1016/0959-4388(91)90082-i
54. Peterson DC, Reddy V, Launico MV, et al. Neuroanatomy, Auditory Pathway. [Updated 2023 Oct 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532311/
55. Pickles, J. O. (2015). Auditory pathways. The Human Auditory System - Fundamental Organization and Clinical Disorders, 3–25. https://doi.org/10.1016/b978-0-444-62630-1.00001-9
56. Polack, P.-O., Friedman, J., & Golshani, P. (2013). Cellular mechanisms of brain state–dependent gain modulation in visual cortex. Nature Neuroscience, 16(9), 1331–1339. https://doi.org/10.1038/nn.3464
57. Peterson DC, Reddy V, Launico MV, et al. Neuroanatomy, Auditory Pathway. [Updated 2023 Oct 24]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK532311/
58. Ravignani A, Cooke PF. The evolutionary biology of dance without frills. Curr Biol. 2016; 26(19):R878–R879. https://doi.org/10.1016/j.cub.2016.07.076 WOS:000385690800005. PMID: 27728787
59. Redd CB, Bamberg SJM. A Wireless Sensory Feedback Device for Real-Time Gait Feedback and Training. Ieee-Asme T Mech. 2012; 17(3):425–433. https://doi.org/10.1109/Tmech.2012.2189014WOS:000303502000004.
60. Reimer J, McGinley MJ, Liu Y, Rodenkirch C, Wang Q, McCormick DA, et al. Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nat Commun. 2016; 7:13289. Epub 20161108. https://doi.org/10.1038/ncomms13289 PMID: 27824036; PubMed Central PMCID:PMC5105162.
61. Rhode, W. S. (1984). Cochlear mechanics. Annual review of physiology, 46(1), 231-246.
62. Rodgers CC, DeWeese MR. Neural Correlates of Task Switching in Prefrontal Cortex and Primary Auditory Cortex in a Novel Stimulus Selection Task for Rodents. Neuron. 2014; 82(5):1157–1170. https://doi.org/10.1016/j.neuron.2014.04.031 WOS:000337359800020. PMID: 24908492

63. Rose, J. E., Galambos, R., & Hughes, J. R. (1959). Microelectrode studies of the cochlear nuclei of the cat. Bulletin of the Johns Hopkins Hospital, 104(5), 211–251.
64. Rummell BP, Klee JL, Sigurdsson T. Attenuation of Responses to Self-Generated Sounds in Auditory Cortical Neurons. J Neurosci. 2016; 36(47):12010–12026. https://doi.org/10.1523/JNEUROSCI.1564-16.2016 PMID: 27881785; PubMed Central PMCID: PMC6604922.
65. Saleem AB, Ayaz A, Jeffery KJ, Harris KD, Carandini M. Integration of visual motion and locomotion in mouse visual cortex. Nat Neurosci. 2013; 16(12):1864–9. Epub 2013/11/05. https://doi.org/10.1038/ nn.3567 PMID: 24185423; PubMed Central PMCID: PMC3926520.
66. Saleem AB, Diamanti EM, Fournier J, Harris KD, Carandini M. Coherent encoding of subjective spatial position in visual cortex and hippocampus. Nature. 2018; 562(7725):124–7. Epub 2018/09/12. https:// doi.org/10.1038/s41586-018-0516-1 PMID: 30202092; PubMed Central PMCID: PMC6309439.
67. Saderi D, Schwartz ZP, Heller CR, Pennington JR, David SV. Dissociation of task engagement and arousal effects in auditory cortex and midbrain. Elife. 2021; 10. Epub 20210211. https://doi.org/10. 7554/eLife.60153 PMID: 33570493; PubMed Central PMCID: PMC7909948
68. Sanes, D. H., Merickel, M., & Rubel, E. W. (1989). Evidence for an alteration of the tonotopic map in the gerbil cochlea during development. Journal of Comparative Neurology, 279(3), 436–444. https://doi.org/10.1002/cne.902790308
69. Schneider DM, Sundararajan J, Mooney R. A cortical filter that learns to suppress the acoustic consequences of movement. Nature. 2018; 561(7723):391–5. Epub 20180912. https://doi.org/10.1038/ s41586-018-0520-5 PMID: 30209396; PubMed Central PMCID: PMC6203933.
70. Schneider, D. M. (2020). Reflections of action in sensory cortex. Current Opinion in Neurobiology, 64, 53–59. https://doi.org/10.1016/j.conb.2020.02.004
71. Schauer M, Mauritz K-H. Musical motor feedback (MMF) in walking hemiparetic stroke patients: randomized trials of gait improvement. Clin Rehabil. 2003; 17(7):713–722. https://doi.org/10.1191/0269215503cr668oa PMID: 14606736.

72. Shimaoka, D., Harris, K. D., & Carandini, M. (2018). Effects of arousal on mouse sensory cortex depend on modality. Cell Reports, 22(12), 3160–3167. https://doi.org/10.1016/j.celrep.2018.02.092
73. Smith, P. H., & Rhode, W. S. (1987). Characterization of hrp-labeled globular bushy cells in the cat anteroventral cochlear nucleus. Journal of Comparative Neurology, 266(3), 360–375. https://doi.org/10.1002/cne.902660305
74. Sigurdsson T. Filtering of self-generated sounds by auditory cortical neurons. Acta Physiol. 2019; 227.WOS:000487978200047.
75. Sofroniew, N. J., Vlasov, Y. A., Hires, S. A., Freeman, J., & Svoboda, K. (2015). Neural coding in barrel cortex during whisker-guided locomotion. eLife, 4. https://doi.org/10.7554/elife.12559
76. Stirman, J. N., Townsend, L. B., & Smith, S. L. (2016). A touchscreen based global motion perception task for Mice. Vision Research, 127, 74–83. https://doi.org/10.1016/j.visres.2016.07.006
77. Svoboda, K., & Yasuda, R. (2006). Principles of two-photon excitation microscopy and its applications to neuroscience. Neuron, 50(6), 823–839. https://doi.org/10.1016/j.neuron.2006.05.019
78. Tajadura-Jime´nez A, Basia M, Deroy O, Fairhurst M, Marquardt N, Bianchi-Berthouze N. As Light as your Footsteps: Altering Walking Sounds to Change Perceived Body Weight, Emotional State and Gait. CHI ’15 Proceedings of the 33rd Annual ACM Conference on Human Factors in Computing Systems. 2015:2943–52. https://doi.org/10.1145/2702123.2702374
79. Town SM, Brimijoin WO, Bizley JK. Egocentric and allocentric representations in auditory cortex.PLoS Biol. 2017; 15(6):e2001878. Epub 20170615. https://doi.org/10.1371/journal.pbio.2001878 PMID: 28617796; PubMed Central PMCID: PMC5472254.
80. Triblehorn JD, Yager DD. Timing of praying mantis evasive responses during simulated bat attack sequences. J Exp Biol. 2005; 208(Pt 10):1867–1876. https://doi.org/10.1242/jeb.01565 PMID:15879067.

81. Turchet L, Camponogara I, Cesari P. Interactive footstep sounds modulate the perceptual-motor aftereffect of treadmill walking. Exp Brain Res. 2015; 233(1):205–214. https://doi.org/10.1007/s00221-014-4104-9 WOS:000348048100020. PMID: 25234404
82. Turchet L, Camponogara I, Nardello F, Zamparo P, Cesari P. Interactive footsteps sounds modulate the sense of effort without affecting the kinematics and metabolic parameters during treadmill-walking. Appl Acoust. 2018; 129:379–385. https://doi.org/10.1016/j.apacoust.2017.08.021 WOS:000413060300038.
83. Turchet L, Serafin S, Cesari P. Walking Pace Affected by Interactive Sounds Simulating Stepping on Different Terrains. Acm T Appl Percept. 2013; 10(4). Artn 23 https://doi.org/10.1145/2536764. 2536770 WOS:000329136700006.
84. Ulanovsky N, Las L, Nelken I. Processing of low-probability sounds by cortical neurons. Nat Neurosci. 2003; 6(4):391–398. https://doi.org/10.1038/nn1032 WOS:000181833100017. PMID: 12652303
85. Vinck M, Batista-Brito R, Knoblich U, Cardin JA. Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. Neuron. 2015; 86(3):740–754. https://doi.org/10.1016/j. neuron.2015.03.028 PMID: 25892300; PubMed Central PMCID: PMC4425590.
86. Wang, X., Lu, T., Snider, R. et al. Sustained firing in auditory cortex evoked by preferred stimuli. Nature 435, 341–346 (2005). https://doi.org/10.1038/nature03565
87. Winkowski, D. E., & Kanold, P. O. (2013). Laminar transformation of frequency organization in Auditory Cortex. The Journal of Neuroscience, 33(4), 1498–1508. https://doi.org/10.1523/jneurosci.3101-12.2013
88. Yackle, K., Schwarz, L. A., Kam, K., Sorokin, J. M., Huguenard, J. R., Feldman, J. L., Luo, L., & Krasnow, M. A. (2017). Breathing control center neurons that promote arousal in mice. Science, 355(6332), 1411–1415. https://doi.org/10.1126/science.aai7984
89. Yavorska, I., & Wehr, M. (2021). Effects of locomotion in auditory cortex are not mediated by the VIP Network. Frontiers in Neural Circuits, 15. https://doi.org/10.3389/fncir.2021.618881
90. Young, E.D. & Brownell, W.E. 1976 Responses to tones and noise of single cells in dorsal cochlear nucleus of unanesthetized cats. J. Neurophysiol. 39, 282-300

91. Young Eric D. , Spirou George A. , Rice John J. and Voigt Herbert F. 1992Neural organization and responses to complex stimuli in the dorsal cochlear nucleusPhil. Trans. R. Soc. Lond. B336407–413 http://doi.org/10.1098/rstb.1992.0076
92. Weible, A. P., Yavorska, I., Kayal, D., Duckler, U., & Wehr, M. (2020). A layer 3→5 circuit in auditory cortex that contributes to pre-pulse inhibition of the acoustic startle response. Frontiers in Neural Circuits, 14. https://doi.org/10.3389/fncir.2020.553208
93. Wu, S. H., & Kelly, J. B. (1991). Physiological properties of neurons in the mouse superior olive: Membrane characteristics and postsynaptic responses studied in vitro. Journal of Neurophysiology, 65(2), 230–246. https://doi.org/10.1152/jn.1991.65.2.230
94. Zhao, B., & Müller, U. (2015). The elusive mechanotransduction machinery of hair cells. Current Opinion in Neurobiology, 34, 172–179. https://doi.org/10.1016/j.conb.2015.08.006
95. Zhou M, Liang F, Xiong XR, Li L, Li H, Xiao Z, et al. Scaling down of balanced excitation and inhibition by active behavioral states in auditory cortex. Nat Neurosci. 2014; 17(6):841–50. Epub 20140420. https://doi.org/10.1038/nn.3701 PMID: 24747575; PubMed Central PMCID: PMC4108079.
96. Xiong QJ, Znamenskiy P, Zador AM. Selective corticostriatal plasticity during acquisition of an auditory discrimination task. Nature. 2015; 521(7552):348. https://doi.org/10.1038/nature14225 WOS:000354816500055. PMID: 25731173
97. Znamenskiy P, Zador AM. Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. Nature. 2013; 497(7450):482. https://doi.org/10.1038/nature12077 WOS:000319254000046. PMID: 23636333

Chapter 2 Auditory Cortex Ensembles Jointly Encode Sound and Locomotion Speed to Support Sound Perception During Movement

The ability to process and act upon incoming sounds during locomotion is critical for survival and adaptive behavior. Despite the established role that the auditory cortex plays in behavior- and context-dependent sound processing, previous studies have found that auditory cortical activity is on average suppressed during locomotion as compared to immobility. While suppression of auditory cortical responses to self-generated sounds results from corollary discharge, which weakens responses to predictable sounds, the functional role of weaker responses to unpredictable external sounds during locomotion remains unclear. Whether suppression of external sound-evoked responses during locomotion reflects reduced involvement of the auditory cortex in sound processing, or whether it results from masking by an alternative neural computation in this state remains unresolved. Here, we tested the hypothesis that rather than simple inhibition, reduced sound-evoked responses during locomotion reflects a tradeoff with the emergence of explicit and reliable coding of locomotion velocity. To test this hypothesis, we first used neural inactivation in behaving mice and found that the auditory cortex plays a critical role in sound-guided behavior during locomotion. To investigate the nature of this processing, we used two-photon calcium imaging of local excitatory auditory cortical neural populations in awake mice. We found that locomotion had diverse influences on activity of different neurons, with a net suppression of baseline-subtracted sound-evoked responses and neural stimulus detection, consistent with previous studies. Importantly, we found that the net inhibitory effect of locomotion on baseline-subtracted sound-evoked responses was strongly shaped by elevated ongoing activity which compressed the response dynamic range, and that rather than reflecting enhanced “noise”, this ongoing activity reliably encoded the animal’s locomotion speed. Decoding analyses revealed that locomotion speed and sound are robustly co-encoded by auditory cortical ensemble activity. Finally, we found consistent patterns of joint coding of sound and locomotion speed in electrophysiologically recorded activity in freely moving rats. Together, our data suggest that rather than being suppressed by locomotion,

auditory cortical ensembles explicitly encode it alongside sound information to support sound perception during locomotion.

2.1 Introduction

Continuous processing of incoming sensory information is critical for survival and adaptive behavior. While the neural mechanisms of sensory processing have traditionally been studied in immobile subjects, some of the most critical behaviors in humans and other animal species, such as foraging for food, seeking a mate, and navigating to safety, occur during locomotion. To gain a coherent perception of the environment during locomotion and be able to rapidly trigger appropriate behavior, the brain must encode incoming external cues and integrate them with one's own motion. For example, humans integrate incoming sounds with locomotion during simple walking, as manifested by the modification of walking pace based on auditory feedback (Cuppone et al., 2018; Redd & Bamberg, 2012; Tajadura-Jiménez et al., 2015; Turchet et al., 2015; Turchet et al., 2018; Turchet et al., 2013). Moreover, auditory feedback has been shown to improve walking in aged patients and those with neurodegenerative disorders (Cornwell et al., 2020; Rodger et al., 2014; Schauer & Mauritz, 2003). Integration of sounds with self-motion has also been studied in the context of other behaviors such as dance (Karpati et al., 2015; Ravignani & Cooke, 2016) and sound-guided finger-tapping (Carr et al., 2016; Chen et al., 2008; Tierney & Kraus, 2013, 2016). In non-humans, perhaps the best known example is bat echolocation (Falk et al., 2014; Ghose et al., 2006; Moss & Surlykke, 2001), yet various forms of audiomotor integration have been studied in diverse animal species, including Praying mantids (Triblehorn & Yager, 2005), Dholes (Fox, 1984) and mice (Whitton et al., 2014). Thus, the ability to process incoming sounds during locomotion and integrate them with the locomotive state to guide appropriate behavior is fundamental in both humans and other animal species.

The auditory cortex (AC) is a key candidate brain region for processing incoming sounds during locomotion due to its well-established role in context-, behavior-, and decision-making-dependent sound processing (Cohen et al., 2011; David et al., 2012; Fritz et al., 2010; Jaramillo & Zador, 2011; Kuchibhotla et al., 2017; McGinley et al., 2015b; Nelken, 2014; Rodgers & DeWeese, 2014; Saderi et al., 2021; Ulanovsky et al., 2003; Xiong et al., 2015; Znamenskiy & Zador, 2013). Intriguingly, previous studies have found that locomotion has a generally suppressive effect on sound-evoked responses in the AC (Bigelow et al., 2019; Schneider et al.,

2014; Schneider et al., 2018; Zhou et al., 2014). Attenuation of responses to self-generated sounds produced during locomotion are well explained by corollary discharge, which acts to suppress responses to predictable sounds and enhance sensitivity to unpredictable sounds (Audette et al., 2022; Clayton et al., 2021; Rummell et al., 2016; Schneider et al., 2018; Sigurdsson, 2019)(though see (Reznik et al., 2021; Reznik et al., 2014; Reznik et al., 2015)). However, the functional benefit of the observed attenuation of AC responses to unpredictable external sounds during locomotion has remained illusive. A proposed explanation, supported by the finding that responses in the primary visual cortex are generally enhanced during locomotion (Dadarlat & Stryker, 2017; Niell & Stryker, 2010; Vinck et al., 2015b), is that locomotion reflects a neural resource allocation shift from audition to vision (Schneider et al., 2014; Zhou et al., 2014). According to this model, reduced sound responses during locomotion reflects a functional attenuation of AC, possibly involving reliance on subcortical regions for sound processing in this state. However, the evolutionary and functional benefit of this suggestion remains debated (Bigelow et al., 2019). Alternatively, it has been suggested that, rather than a resource allocation shift from the auditory to visual modality, locomotion may induce a shift towards spatial information processing across modalities (Bigelow et al., 2019). According to this hypothesis, the AC may play a critical role in sound processing during locomotion, but that it may include encoding of locomotion-related non-acoustic information, which, if unaccounted for, could appear as simple suppression. This hypothesis is supported by robust encoding of locomotion-related signals and their integration with cue-evoked responses in other sensory cortical regions (Ayaz et al., 2013; Ayaz et al., 2019; Campbell & Giocomo, 2018; Saleem et al., 2013; Saleem et al., 2018). However, this hypothesis has yet to be directly tested in the AC. Here we combined AC inactivation in mice performing sound-guided reward-predictive behavior during locomotion, two-photon calcium imaging in head-fixed mice and electrophysiological recordings in freely-moving rats to test the hypothesis that auditory cortical ensembles are not simply suppressed during locomotion but rather explicitly encode it and incorporate it with sound information into an integrated audiomotor neural code.

2.2 Auditory Cortical Activity is required for Sound Processing during Locomotion

Previous studies have shown that during immobility, the AC is not necessary for simple tone detection or discrimination, but is required for more demanding tasks such as discrimination of

complex sounds and sound source localization (Goldberg & Neff, 1961a; Harrington et al., 2001; Kato et al., 2015; Kavanagh & Kelly, 1987; Kelly & Glazier, 1978b; Nodal et al., 2012; Ohl et al., 1999a; Porter et al., 2011; Scharloc.Dp et al., 1965). To determine whether AC activity is required for sound-guided behavior during locomotion, we measured the influence of AC inactivation on sound-guided reward-predictive licking during locomotion in mice. Male and female mice were first implanted with bilateral cannula into the AC for subsequent drug delivery and allowed to recover for at least 5 days. Mice were then put on water restriction and were trained on an appetitive trace conditioning task during head fixation while standing on a rotatable plate that allowed the animals to stand or run at will. Using a closed-loop system that received the output of a rotary encoder at the base of the plate, training trials were selectively initiated during locomotion and consisted of the presentation of an 8 kHz tone followed by a drop of water reward, delivered 1 s after sound termination. Mice (n=8) were trained until they learned the sound-reward association as evidenced by an increase in lick rate following the sound and before reward delivery ("predictive licking", Fig. 1A).

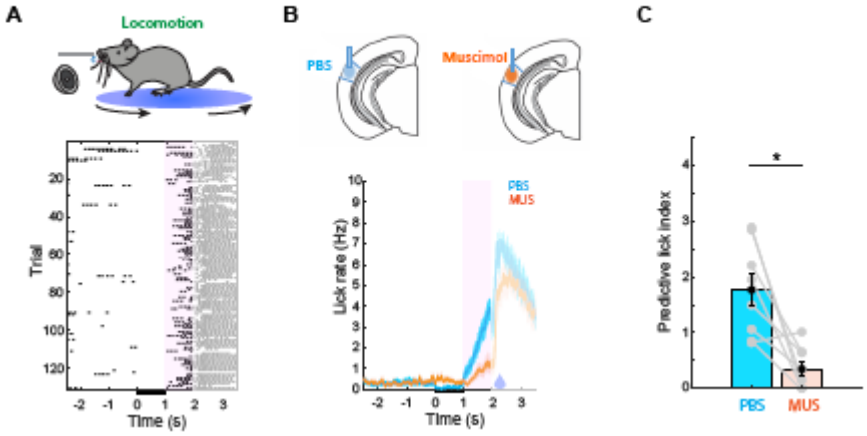


Figure 2.1 Auditory cortical activity is necessary for sound processing during locomotion. (A) Top: Illustration of the behavioral setup for sound-guided predictive licking in locomotion. Bottom: Peri-sound lick histograms of an example behavioral session from a trained animal performing the task. Licking in the pink shaded area following sound termination represents prediction of upcoming reward (delivered at 2 s). Licks following reward delivery are shaded as they do not require sound processing or reward prediction **(B)** Peri-sound lick histogram across animals performing the task when the AC was infused with either PBS or muscimol. Solid lines denote the mean and the shaded area represents s.e.m across animals. Predictive licking is reduced following AC inactivation using muscimol. **(C)** There was a significant reduction in predictive lick index following infusion of MUS (P=0.0156, signed rank test). Error bars represent mean±s.e.m across animals. Lines connecting gray circles represent data from the same animal in the different conditions.

To test whether AC activity is necessary for this behavior, we measured the influence of AC inactivation on sound-triggered reward-predictive licking. To this end, we measured behavioral performance in trained mice following infusion of the GABA receptor agonist muscimol (MUS),

or inert phosphate buffer solution (PBS) as a control, into the AC, in a within-subject design (Fig. 1B). We found that inactivation of the AC induced a significant and near-complete reduction in sound-triggered predictive licking during locomotion (Fig. 1B,C). Furthermore, while following PBS delivery mice exhibited sound-triggered reduction of running speed leading to the time of reward delivery, this effect was significantly weaker following MUS delivery (Suppl. Fig. 1). In a version of this task performed during immobility, AC inactivation induced a trend of an impairment, but it did not reach significance (Suppl. Fig. 2), consistent with previous studies (Goldberg & Neff, 1961b; Kato et al., 2015; Kavanagh & Kelly, 1988; Kelly & Glazier, 1978a; Ohl et al., 1999b).

The finding that during locomotion the AC plays an important role in sound-guided predictive licking and locomotion speed modulation suggests that its reduced sound-evoked responses during locomotion may reflect part of an alternative neural computation in this state. To test this possibility, we carried out optical recording of AC ensemble activity in mice.

2.3 A Heterogeneous and Overall Inhibitory Influence of Locomotion on Sound-evoked Responses of Local Excitatory L2/3 Neuronal Ensembles in the Auditory Cortex

To study the nature of information processing by local groups of L2/3 excitatory neurons ("neuronal ensembles") of the AC during locomotion, we carried out two-photon calcium imaging in head-fixed Thy1-GCaMP6f mice that were free to stand or run at will on a rotatable plate (Fig. 2A-C).

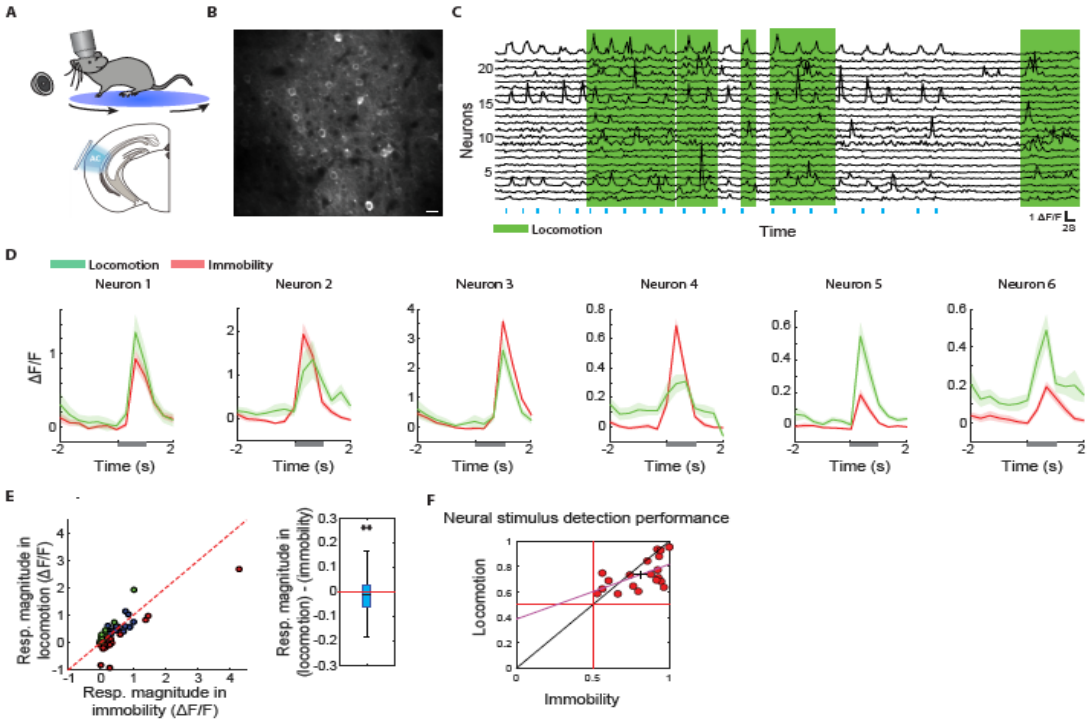


Figure 2.2 A heterogeneous and overall inhibitory influence of locomotion on sound-evoked responses of local excitatory L2/3 neuronal ensembles in the auditory cortex (A) Illustration of the experimental setup **(B)** Two-photon average micrograph of an example local neuronal ensemble in L2/3 of the auditory cortex. Scale bar: 10μm. **(C)** Relative change in fluorescence (ΔF/F) of 22 neurons from the micrograph in 'B' during an imaging session. Periods of locomotion are marked in green. **(D)** Sound-triggered peri-stimulus time histograms from 6 example neurons. Sound presentation trials in which the animal was immobile (red) and running (green) were grouped separately. Locomotion had diverse effects on sound-evoked responses of different neurons, including invariance (neurons 1+2), reduction (neurons 3+4) and enhancement (neurons 5+6) **(E)** Left: Sound-evoked responses in immobility and locomotion across all BBN-responsive neurons. Red and green dots represent neurons that individually exhibited a significantly stronger and weaker response during immobility, respectively. Blue dots represent neurons that did not exhibit a significant difference. Right: Box plot describing sound-evoked responses in locomotion minus immobility across all BBN-responsive neurons. The distribution was significantly lower than 0 (P=0.009, two-sided Wilcoxon signed-rank). For this and subsequent whisker plots, the central mark indicates the median, the bottom and top edges of the box indicate the 25th and 75th percentiles, respectively and the whiskers extend to the most extreme data points not considered outliers. **(F)** Ensemble-level neural stimulus detection performance in immobility and locomotion. Detection performance in immobility and locomotion was significantly correlated across ensembles (P=0.012, Pearson correlation). Detection in immobility was significantly higher than in locomotion (P=0.036, signed rank test).

We first examined how locomotion modulates the responses of neurons to broad-band noise (BBN) bursts in 985 AC neurons from 7 mice, of which 612 neurons had a sufficient number of responses in both immobility and locomotion to allow for comparison. In keeping with most previous studies, we started with examining baseline-subtracted responses, which are defined as the difference between the activity evoked by the sound and the activity immediately preceding the sound. Locomotion had a diverse influence on sound-evoked responses of individual neurons, including invariance, suppression and enhancement (Fig. 2D). Across all neurons that

exhibited significant BBN-evoked responses in immobility (194/612, 31.7%, of which sound-evoked response magnitudes of 23/194, 34/194 and 137/194 were individually significantly enhanced, suppressed, and not showing a significant difference, respectively), the population-average responses were significantly reduced during locomotion (Fig. 2E), consistent with previous studies (Bigelow et al., 2019; Schneider et al., 2014; Zhou et al., 2014). To test whether these findings were unique to responses to BBN, we examined how locomotion modulates responses to pure tones and complex sounds. These experiments revealed a similar influence of locomotion on sound evoked responses, namely a net population-average decrease that coexists with heterogeneous influences at the single-cell level (Suppl. Fig. 3).

Given our observation that sound-evoked responses of individual neurons showed heterogeneous modulation by locomotion, and that across animals the location of the imaging field within AC may slightly vary, we wondered whether some local groups of neurons were preferentially dedicated to sound processing during locomotion and others to sound processing in immobility. To test this, we used cross-validated classification models to quantify ensemble-level stimulus detection in immobility and locomotion (separately) for each ensemble. We posited that if sound detection in immobility and locomotion is supported by distinct ensembles, stimulus detection performance in immobility and locomotion would be negatively correlated across ensembles. Instead, we found that stimulus detection performance in locomotion and immobility were significantly positively correlated (Fig. 2F). Furthermore, this analysis showed that across ensembles, stimulus detection performance was mildly but significantly lower in locomotion as compared to immobility, consistent with the average weaker sound-evoked responses in locomotion (Fig. 2E). Thus, overlapping AC L2/3 ensembles encode sounds during immobility and locomotion, with a net weaker sound detection performance during locomotion.

2.4 Enhanced Ongoing-activity during Locomotion Reduces Baseline Subtracted Sound-evoked Response Magnitudes

We sought to further investigate the source of the net reduction in baseline-subtracted sound-evoked responses and noticed that many neurons exhibited increased ongoing activity during locomotion, which manifested as increased activity before stimulus onset (Fig. 2D).

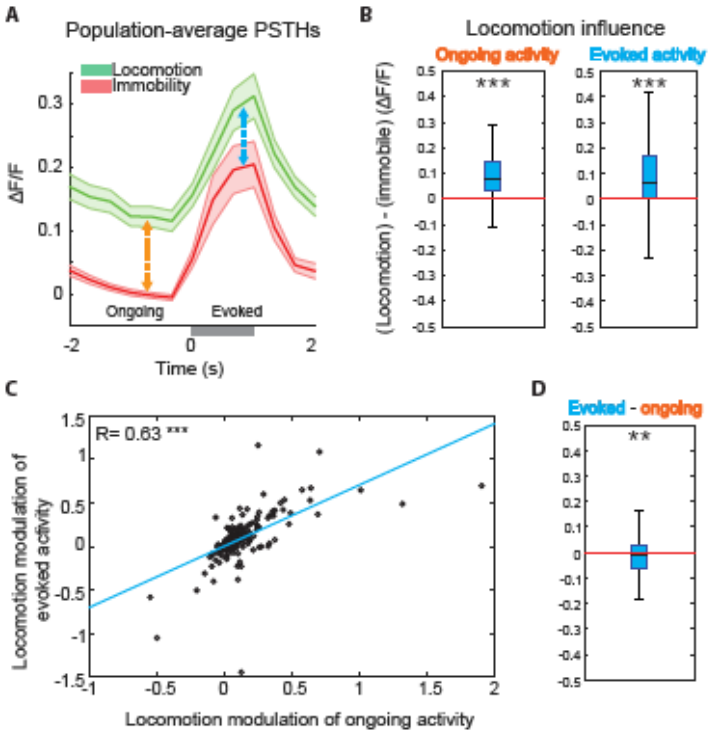


Figure 2.3 Enhanced ongoing activity during locomotion reduces baseline-subtracted sound-evoked responses (A) Population-level peri-stimulus time histogram across all BBN-responsive neurons during immobility (red) and locomotion (green). Solid lines and shaded areas indicate mean±SEM. **(B)** Locomotion increased ongoing activity of sound-responsive neurons (left, P= $2.9e^{-23}$, two-sided Wilcoxon signed-rank test), as well as of evoked activity (middle, P= $8e^{-13}$, two-sided Wilcoxon signed-rank test). **(C)** Locomotion influence on ongoing and evoked activity across neurons **(D)** The per-neuron difference between the locomotion influence on evoked and ongoing activity. The locomotion influence on evoked activity was significantly lower than that of ongoing activity, resulting in a net reduction in baseline-subtracted sound-evoked responses (P= 0.0094, two-sided Wilcoxon signed-rank test).

Increased baseline activity during locomotion could contribute to reduced sound responses by increasing the subtrahend in the baseline-subtracted sound-evoked response calculation. To test this possibility, we calculated the average sound-triggered peri-stimulus time histogram (PSTH) across the population of BBN-responsive neurons and found that it exhibits a significant elevation in ongoing, pre-stimulus activity during locomotion as compared to immobility (Fig. 3A, orange arrow, Fig. 3B, left panel). Increased ongoing activity during locomotion was also observed in the presence of a constant masking sound, suggesting it is at least partly independent of self-generated sounds (Suppl. Fig. 4). Locomotion also produced a significant increase in evoked activity during the stimulus time window (Fig. 3A, blue arrow, Fig. 3B, right panel), and the locomotion influences on ongoing and evoked activity were positively correlated across neuron (Fig. 3C). However, the locomotion-induced increase in activity in the evoked window was significantly smaller than the increase in ongoing activity, likely reflecting a saturation effect, resulting in a net negative influence of locomotion on baseline-subtracted sound evoked

activity (Fig. 3D). This suggests that increased ongoing activity during locomotion compresses the dynamic range of the baseline-subtracted response. When including sound-unresponsive cells, locomotion increased activity during the ongoing and sound time windows, but did not induce a significant reduction in baseline-subtracted sound-evoked responses, as expected (Suppl. Fig. 5).

These data demonstrate that the observed average reduction in baseline-subtracted sound responses during locomotion is at least partly due to increased ongoing, pre-sound activity. We therefore wondered whether this enhanced ongoing activity during locomotion, which is subtracted out in the standard calculation of sound response magnitude and impairs neural sound detection, may in fact reflect encoding of meaningful information for auditory cortical processing during behavior.

2.5 Enhanced Ongoing-activity Reliably Encodes Locomotion Speed

During locomotion, a key behavioral parameter which can shape how to process and act upon incoming sensory stimuli is locomotion speed (Campbell & Giocomo, 2018). In particular, robust speed coding in the hippocampus and medial entorhinal cortex are believed to be critical for cue-guided navigation (Dannenberg et al., 2020; Farrell et al., 2021; Geisler et al., 2007; Gois & Tort, 2018; Hinman et al., 2016). Moreover, hippocampal coding of space and locomotion is coordinated with the primary visual cortex (Haggerty & Ji, 2015; Saleem et al., 2018), where locomotion speed is robustly encoded and integrated with cue-evoked responses (Ayaz et al., 2013; Saleem et al., 2013). We therefore tested the hypothesis that the enhanced ongoing activity that we observed during locomotion encodes movement speed. To test this hypothesis, we first asked whether neural activity of individual neurons is significantly correlated with locomotion speed. We calculated the correlations between the continuous relative change in fluorescence of each neuron and the running speed of the mouse, utilizing a large subset of our imaged neurons (647/985) that were imaged while the continuous running speed of the animal was acquired. We found that activity of auditory cortical neurons could exhibit surprisingly high positive correlations with locomotion speed (Fig. 4A), and in fewer cases significant negative correlations with locomotion speed (Fig. 4B). Across the population, ongoing activity of 52% of neurons (335/647) showed significant positive correlation with locomotion speed, 24% of neurons (155/647) exhibited significant negative correlation with locomotion speed and 24% (157/647)

showed no significant correlation with locomotion speed (Fig. 4C). The distribution of correlations between neural activity and locomotion speed was skewed to the right (Fig. 4D, skewness=0.84), consistent with our finding of a population-level enhancement in baseline activity during locomotion.

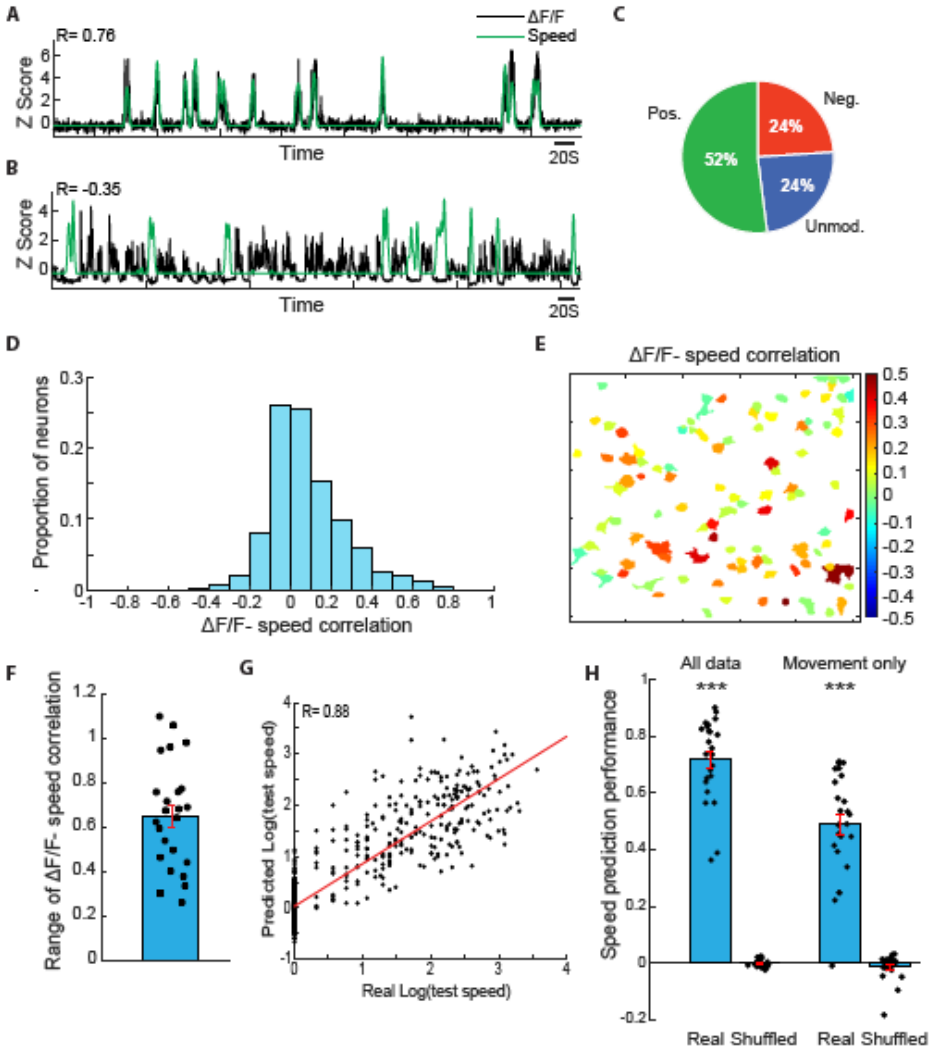


Figure 2.4 Auditory cortical L2/3 neurons and ensembles reliably encode locomotion speed. (A) Z-scored ΔF/F of an example neuron (black trace) overlaid on the Z-scored locomotion speed of the mouse (green trace) during an example imaging session. This neuron exhibited a correlation of R=0.76 with locomotion speed across the session. **(B)** An example from a different neuron, showing a negative correlation with locomotion speed of R=-0.35. **(C)** Proportions of AC L2/3 neurons showing significant positive, significant negative and non-significant correlation with locomotion speed **(D)** The distribution of ΔF/F-locomotion speed correlations across the population **(E)** An illustration of all neurons in an example imaging session (same as in Fig. 1F), color coded according to each neuron's ΔF/F-locomotion speed correlation value. Local ensembles exhibited a high degree of heterogeneity in correlation with locomotion speed. **(F)** The ensemble-level range in ΔF/F-locomotion speed correlation values across ensembles. **(G)** The predicted log(speeds) of an example test-set against the real log(speeds) of that test-set, showing a correlation of 0.88. **(H)** Speed prediction performance, measured as the correlation values between the predicted and real locomotion speeds across ensembles. Shuffled values were derived by randomly shuffling the predicted speed values. Left: all data included ($P=3e^{-9}$), right: movement-only ($P=1.9e^{-8}$)

Interestingly, we also found that within local excitatory auditory cortical ensembles in L2/3, individual neurons exhibited high diversity in correlations between neural activity and locomotion speed (Fig. 4E). Within local neuronal ensembles, the average range of correlations between locomotion speed and relative change in fluorescence of the different neurons was 0.65 (Fig. 4F). These findings suggest that despite the net excitatory effect, locomotion modulates ongoing activity of local excitatory neuronal populations in a spatially fine-tuned manner rather than acting as a global uniform modulator.

To further quantify the degree of information that auditory cortical ensembles convey about locomotion speed, we implemented a cross-validated generalized linear model (GLM) to test if locomotion speed can be decoded from ongoing ensemble activity. For each imaging session of a single neuronal ensemble, a GLM was trained on a random half of the imaging session data and tested on the other half, and this procedure was repeated 200 times for robust estimation. In the test phase, the GLM model that was constructed in the training phase predicted locomotion speed based on ensemble patterns of neural activity of the test set. We found that in many cases the predictions of the model were highly correlated with the actual speeds (Fig. 4G). The correlations between the predicted speed and real speed were large and highly significant, even when excluding all immobility periods (Fig. 4H). These findings suggest that ongoing locomotion speed is reliably encoded by the activity of local neuronal ensembles in the AC.

2.6 Integration of sound and locomotion information by excitatory neuronal ensembles in L2/3 of the auditory cortex

Taken together, our results suggest that excitatory neurons in L2/3 of the AC robustly encode both external sounds and locomotion speed. These findings raise the question of whether these two variables- external sounds and locomotion - are simultaneously represented and integrated within the local network level.

To test this, we first quantified whether the activity of individual neurons and local ensembles could predict both locomotion state (immobility/locomotion) and sound occurrence during locomotion (n=19 ensembles). To this end we implemented cross-validated support vector machine (SVM) analyses on each neuron's or ensemble's activity patterns and quantified the predictive power that it provided to discriminate between immobility and locomotion and between sound occurrence and no sound during locomotion (Fig. 5A). We found that while

individual neurons typically showed moderate prediction, with high prediction of at most one of these attributes, local ensembles could display high prediction of both locomotion state and stimulus occurrence (Fig. 5B-D). Indeed, while discrimination performance of ensembles was not better than that of their highest-predictive individual neuron for sound or state, sound-state discrimination average was significantly higher at the ensemble level than the best neuron level, demonstrating sound-state integration at the ensemble level (Fig. 5E). Furthermore, we found that local neuronal ensembles consisting of a few dozen neurons could exhibit both high-fidelity speed coding and stimulus detection in both immobility (Fig. 5F) and locomotion (Fig. 5G). Together, these data suggest that neuronal ensembles in L2/3 of the AC robustly co-encode locomotion speed alongside sound information during movement.

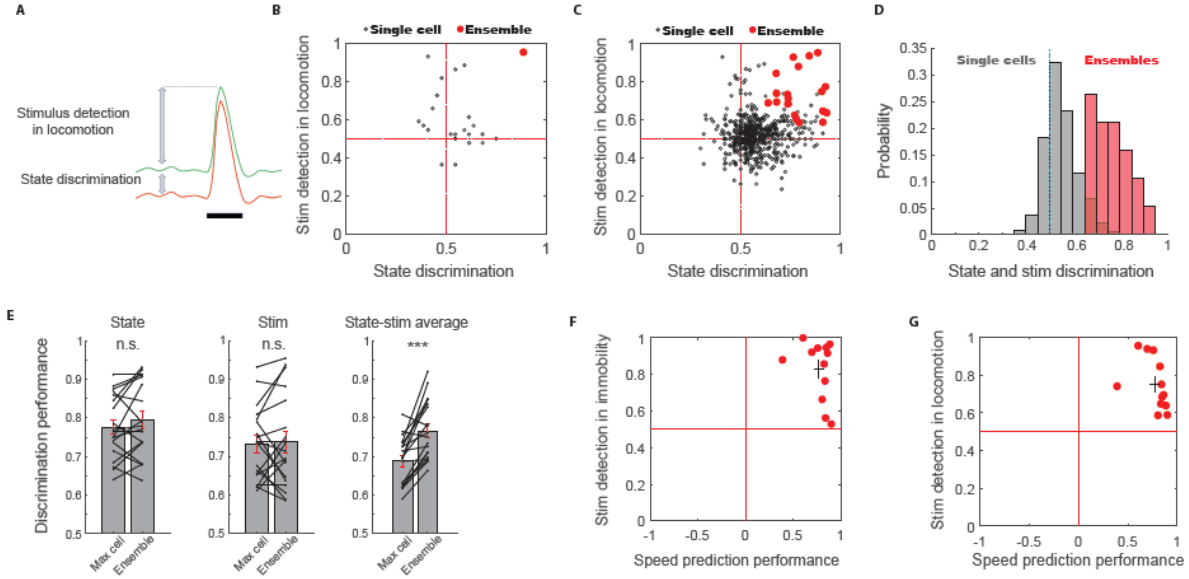


Figure 2.5 Integration of sound and locomotion information by excitatory neuronal ensemble in L2/3 of the auditory cortex. (A) Schematic illustration of the measures used for stimulus detection in locomotion and state discrimination. (B) Performance of stimulus detection in locomotion against state discrimination in an example ensemble (C) Performance of stimulus detection in locomotion against state discrimination across ensembles and single cells. (D) Histogram of average state and stim discrimination for single cells (gray) and ensembles (E) Comparison of discrimination performance of ensembles and their best-predictive neuron (per attribute), for State (left, P=0.21), Stim (middle, P=0.687) and State-stim average (left, P= 0.0004), signed rank test (F) Stimulus detection in immobility against speed prediction performance across ensembles. Black cross shows mean±STD of the two measures. (G) Stimulus detection in locomotion against speed prediction performance across ensembles. Black cross shows mean±STD of the two measures.

2.7 Integration of sound and locomotion in the freely moving rat

Finally, we wished to test whether our findings of joint coding of locomotion speed and sound in head-fixed animals generalize to freely-moving animals. To this end, we analyzed electrophysiological recordings from freely-moving rats that were implanted with tetrodes in the AC (Rothschild et al., 2017). Recordings were carried out as rats traversed a Y-shaped track for

food reward delivered at reward wells (Fig. 6A). In a pseudorandom ~25% of trials, following nose-poking in the Home well rats were presented with series of chirp-pair sounds, which signaled that subsequent reward is delivered in the Sound well. Rats trained on this task for up to 12 days and reached good performance within 5-6 days (Rothschild et al., 2017). We identified putative excitatory and fast spiking interneurons based on spike waveform and firing rates (Suppl. Fig. 6). We recorded a total of 248 putative excitatory neurons that had a sufficient number of responses in both immobility and locomotion to allow comparison. Of these, 21% (51/248) were significantly responsive to the target sound during immobility.

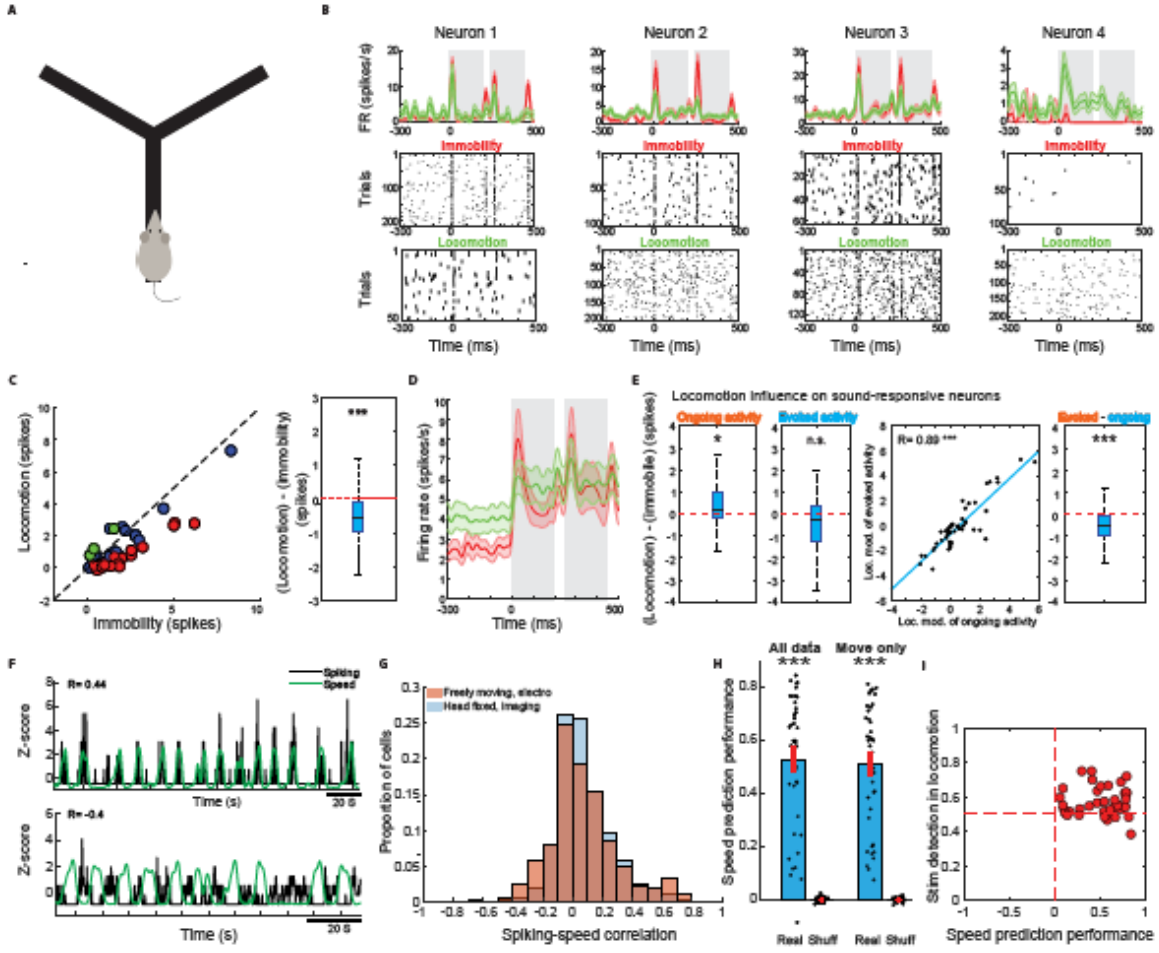


Figure 2.6 Integration of sound and locomotion in the freely moving rat (A) Illustration of the experimental setup for electrophysiological recordings in freely-moving rats **(B)** Sound-triggered peri-stimulus time histograms from 4 example neurons. Sound presentation trials in which the animal was immobile (red) and running (green) were grouped separately. Neurons showed diverse patterns of modulation of sound-evoked responses during locomotion **(C)** Left: Sound-evoked responses in immobility and locomotion across all target-sound responsive neurons. Red and green circles denote neurons that individually exhibited a significantly stronger and weaker response during immobility, respectively. Blue circles denote neurons that did not exhibit a significant difference. Right: The per-neuron difference in sound-evoked response between locomotion and immobility across all responsive neurons was significantly lower than 0 (P=3.4e-5, two-sided Wilcoxon signed-rank). For this and subsequent whisker plots, the central mark indicates the median, the bottom and top edges of the box indicate the 25th and 75th percentiles,

respectively and the whiskers extend to the most extreme data points not considered outliers. **(D)** Population-level peri-stimulus time histogram across all target-sound responsive neurons during immobility (red) and locomotion (green). Solid lines and shaded areas indicate mean±SEM. **(E)** Locomotion increased ongoing activity of sound-responsive neurons (left, P=0.0144, two-sided Wilcoxon signed-rank test). Locomotion did not significantly modulate evoked activity (second from left, P=0.2687, two-sided Wilcoxon signed-rank test). Locomotion influence on ongoing and evoked activity was correlated across neurons (second from right). The locomotion influence on evoked activity was significantly lower than that of ongoing activity, resulting in a net reduction in baseline-subtracted sound-evoked responses (right, P=3.4e-5, two-sided Wilcoxon signed-rank test). **(F)** Top: Z-scored spiking of an example neuron (black trace) overlaid on the Z-scored locomotion speed of the rat (green trace) during an example session. This neuron exhibited a correlation of R=0.44 with locomotion speed across the session. Bottom: An example from a different neuron, showing a negative correlation with locomotion speed of R=-0.4. **(G)** Distribution of spiking-locomotion speed correlation values (orange). The parallel distribution from the imaging data (Fig. 4D) is shown in light blue in the background as comparison. **(H)** Speed prediction performance, measured as the correlation values between the predicted and real locomotion speeds across ensembles. Shuffled values were derived by randomly shuffling the predicted speed values. **(I)** Stimulus detection in locomotion against speed prediction performance across ensembles.

We first examined the effect of locomotion on baseline-subtracted sound-evoked spiking responses in freely moving rats by separating responses of putative excitatory neurons that occurred during immobility and locomotion. While individual neurons exhibited diverse influence by locomotion, across the population of target sound-responsive neurons, sound-evoked responses were significantly weaker during locomotion as compared to immobility (Fig. 6B,C), consistent with our imaging data (Fig. 2E) and previous studies (Bigelow et al., 2019; Schneider et al., 2014). Putative fast spiking interneurons exhibited an even stronger suppression of sound evoked responses during locomotion (Suppl. Fig. 6C).

We thus sought to test whether this locomotion-related decrease in baseline-subtracted sound-evoked responses could in part be due to increased baseline firing during locomotion as our imaging data in mouse indicated. Indeed, we found that ongoing activity, measured as the spike rate preceding stimuli presentations, was significantly higher during locomotion as compared to immobility across sound-responsive putative excitatory neurons (Fig. 6D, Fig. 6E, left panel). Locomotion had no significant overall effect on spiking activity during the sound time window, which differed from the elevation observed in the imaging data, likely due to differences in the targeted cortical layers across these datasets. Nevertheless, as in the imaging data, we observed a larger increase in ongoing activity than evoked activity, resulting in a net reduction in baseline-subtracted sound-evoked responses (Fig. 6E). These data demonstrate that, consistent with our head-fixed mouse data, increased ongoing activity during locomotion contributes to weaker sound-evoked responses in the freely-moving rat as well.

To test whether increased ongoing activity during locomotion encodes locomotion speed in the freely-moving rat, we examined correlations between continuous spiking activity and

locomotion speed. We found similar results to the head-fixed mouse data, with the spiking activity of some neurons reliably tracking locomotion speed (Fig. 6F). Across the population of putative excitatory neurons, the distribution of correlations between ongoing activity and locomotion speed was skewed to the right and highly similar to the distribution of the head-fixed data (Fig. 6G). The spiking-speed correlation distribution of putative fast-spiking interneurons was significantly higher (Suppl. Fig. 6D). To further investigate the temporal relationship between locomotion speed and neural activity, we calculated the cross-correlation between these signals. We found that neural activity-speed cross correlation peaked at 0 second lag and decayed substantially even with a shift of 1 second, indicating that AC neural activity tracks locomotion speed with a rapid time constant (Suppl. Fig. 7). Consistently, despite having a substantially lower number of simultaneously recorded putative excitatory neurons as compared to the imaging data (mean±sem electro: 4.5±0.39, imaging: 29.3±4.6), ensemble-level spiking activity could reliably predict locomotion speed (Fig. 6H). Indeed, the number of neurons within an ensemble positively correlated with speed prediction performance (Suppl. Fig. 8). Finally, we carried out a similar decoding analysis to that implemented on the imaging data and found that despite the low number of neurons per ensemble, many ensembles jointly coded for locomotion speed and sound in locomotion (Fig. 6I). Interestingly, sound detection in locomotion showed a small but significant increase across days of training on this task while speed prediction did not significantly change across days (Suppl. Fig. 9). Together, these findings suggest that coding of locomotion speed and its integration with sound information is a robust feature of AC ensembles in the freely moving rat as well.

2.8 Discussion

In this study, we tested the hypothesis that the AC plays a key role in auditory processing during locomotion and investigated the neural computation that it performs in this state. Using AC inactivation in behaving mice, we found that AC activity is required for sound-guided behavior during locomotion. Using two-photon calcium imaging in L2/3 of AC of head-fixed mice, we found that locomotion had a diverse but overall inhibitory influence on sound-evoked responses of individual neurons, which resulted in a mild but significant reduction in ensemble-level stimulus detection. Across ensembles, stimulus detection in immobility and locomotion were positively correlated, suggesting that sound processing across these states is supported by

shared local neural populations in L2/3 of AC. Furthermore, we found that the net reduction in sound-evoked responses during locomotion are at least partly a result of increased ongoing neural activity, and importantly, that this ongoing activity robustly encoded the animal's running speed. Thus, lower sound-evoked responses during locomotion reflected a tradeoff with the emergence of locomotion speed coding. Decoding analyses revealed that local neuronal ensembles of a few dozen neurons could jointly code locomotion speed and sound with high fidelity. Finally, we found consistent patterns of co-encoding of sound and locomotion speed in electrophysiologically recorded freely-moving rats.

Previous studies have found that AC sound-evoked responses are on average weaker during locomotion as compared to immobility (Bigelow et al., 2019; Schneider et al., 2014; Schneider et al., 2018; Zhou et al., 2014), a finding we have replicated here in both head-fixed mice and freely-moving rats. Attenuation of responses to locomotion-associated self-generated sounds are well explained by corollary discharge, which acts to suppress responses to predictable sounds and enhance sensitivity to unpredictable sounds (Audette et al., 2022; Clayton et al., 2021; Rummell et al., 2016; Schneider et al., 2018; Sigurdsson, 2019)(though see (Reznik et al., 2021; Reznik et al., 2014; Reznik et al., 2015)). However, the functional benefit of the observed attenuation of AC responses to unpredictable external sounds during locomotion has remained illusive. This finding is particularly enigmatic given the critical need to be able to efficiently process external sounds and their associated meaning during locomotion for survival, and the well-established role that AC plays in behavior- and context- dependent sound processing (Cohen et al., 2011; David et al., 2012; Fritz et al., 2010; Jaramillo & Zador, 2011; Kuchibhotla et al., 2017; McGinley et al., 2015b; Nelken, 2014; Rodgers & DeWeese, 2014; Saderi et al., 2021; Ulanovsky et al., 2003; Xiong et al., 2015; Znamenskiy & Zador, 2013). A proposed explanation for this finding is that during locomotion neural computational resources shift from auditory to visual processing (Schneider et al., 2014; Zhou et al., 2014). According to this proposal, weaker AC responses during locomotion reflect a reduced involvement of AC in sound processing in this state, in parallel to an enhancement of visual processing supported by increased responses in the visual cortex (Dadarlat & Stryker, 2017; Niell & Stryker, 2010; Vinck et al., 2015b). According to this model, reduced sound responses during locomotion reflects a functional attenuation of AC, possibly suggesting reliance on subcortical regions for sound

processing in this state. However, the evolutionary and functional logic of this finding remains debated (Bigelow et al., 2019).

Our data strongly points at an alternative role of the AC in processing external sounds during locomotion. First, in contrast to the processing of simple sounds in immobility (Goldberg & Neff, 1961a; Harrington et al., 2001; Kato et al., 2015; Kavanagh & Kelly, 1987; Kelly & Glazier, 1978b; Nodal et al., 2012; Ohl et al., 1999a; Porter et al., 2011; Scharloc.Dp et al., 1965), our inactivation experiments show that sound processing during locomotion is strongly dependent on the AC (Fig. 1, Suppl. Figs. 1-2), arguing against a reduction of AC involvement in sound processing during locomotion. Second, both our imaging and electrophysiological data show that the locomotion-associated reduction in baseline-subtracted sound-evoked responses is at least partly a result of increased ongoing activity, which compresses the response dynamic range, rather than being a result of simple reduction in evoked firing rates (Fig. 3 and Fig. 6E). Critically, rather than reflecting enhanced "noise", the locomotion-associated elevated ongoing activity robustly encodes locomotion speed (Fig. 4 and Fig. 6G-H). While individual neurons showed heterogeneous locomotion-associated modulation of sound responses and ongoing activity, AC ensembles robustly jointly coded and integrated locomotion and sound (Fig. 5 and Fig. 6I). Thus, in contrast to the prevailing model, we propose that rather than being inhibited during locomotion, AC ensembles explicitly encode it, alongside sound information, to provide a sound-in-motion signal.

Why is locomotion speed encoded in the AC? For both humans and other animals, the same sound can carry different meaning and appropriate motor response depending on the precise locomotive state, requiring ongoing integration between sound and motion. For example, a person hearing a train passing a few feet in front of them may not show a behavioral response if the person is standing still, may slow down if they are walking slowly, or may jolt backwards if they are running. Similar audiomotor integration is required in rodents, for example when fleeing a predator or hunting prey using sound. While one possibility is that in these scenarios, the auditory pathway would only represent sound and the integration with action would occur at a downstream integrative brain region, our inactivation and neurophysiological data suggest that the auditory cortex itself may be that region. Moreover, recent findings suggest that robust representation of motor action and its integration with sensory information may be a common functional principle across sensory cortices. Specifically, although V1 responses are on average

enhanced during locomotion, a number of studies have found that the influence of locomotion on visual cortical processing is better explained by sensory-motor integration than a general increase in gain. For example, one study found that locomotion modulates visual spatial integration by preferentially enhancing responses to larger visual objects (Ayaz et al., 2013). An additional study found that V1 neurons are tuned to weighted combinations of locomotion speed and the speed of the incoming visual stimulus, giving rise to multimodal locomotion-visual representations in V1 (Saleem et al., 2013). Based on these and additional studies (Fiser et al., 2016; Keller et al., 2012; Saleem et al., 2018), it has been suggested that beyond simple modulation of response magnitude, a key function of V1 is to integrate visual and locomotion information in ways that inform action and navigation (Parker et al., 2020). Although the influence of locomotion on the magnitude of stimulus-evoked responses in the visual and auditory cortices appear distinct, our findings suggest that the neural coding scheme reflecting joint representation and integration of locomotion and sensory cues is dominant in the AC as well and may reflect a general cortical functional principle.

In both our head-fixed mouse and freely-moving rat data, locomotion speed coding emerged from individual neurons showing diverse but largely positive correlations between ongoing activity and locomotion speed (Fig. 4A-E). This finding differs from some previous studies, which found that ongoing activity in AC was strongly suppressed during (and even just before) locomotion, due to input from the motor cortex and activity of local interneurons (Schneider et al., 2014; Zhou et al., 2014), a finding that would result in an overall negative correlation between neural activity and locomotion speed. However, our findings are consistent with other studies that observed an overall increase in ongoing activity during locomotion (Bigelow et al., 2019; McGinley et al., 2015b). The discrepancy between these findings may be due to experimental differences that may result in targeting of functionally different neuronal subpopulations.

Locomotion is a complex behavior that is a result of coordinated motor activity, but it also involves changes in other factors such as arousal, motivation, attention and effort. This raises the question of whether the findings described here- a reduction in magnitude of sound-evoked responses in parallel to the emergence of speed coding- are specific to locomotion or may be an indirect consequence of one of these factors. A reduction in the magnitude of AC sound-evoked responses is not unique to locomotion, and has also been observed, for example,

as a result of increased behavioral engagement in the absence of locomotion (Otazu et al., 2009; Rummell et al., 2016), although the mechanisms may differ. However, we believe that locomotion speed coding by ongoing activity is unlikely to be a result of alternative factors. Our data shows that the spontaneous activity of many neurons tracks ongoing locomotion speed with high reliability even when excluding periods of immobility (Fig. 4H and Fig. 6H) and that neural activity tracks locomotion speed with a rapid time constant (Suppl. Fig. 7). In contrast, while arousal level, attention and motivation can all influence spontaneous activity in the auditory cortex (Lin et al., 2019; McGinley et al., 2015b; Reimer et al., 2016; Schwartz et al., 2020) and typically increase in the transition from immobility to locomotion, there is no evidence that they rapidly track ongoing changes in locomotion speed. Specifically, previous studies that recorded pupil size as an indicator of arousal found that pupil size is larger during periods of locomotion relative to immobility, but it does not fluctuate rapidly enough to track locomotion speed (Khoury et al., 2023; McGinley et al., 2015b; Mineault et al., 2016; Reimer et al., 2016; Vinck et al., 2015a). Furthermore, ongoing firing rates were found to be higher during locomotion than during immobility for the same level of pupil-measured arousal (McGinley et al., 2015a). Moreover, the similarity in our findings of locomotion speed coding in the mouse imaging data and the rat electrophysiology data, as well as the similarity in speed coding across the rat training days (Suppl. Fig. 9C), despite substantial differences in the motivational and attentional demands across these conditions, further reduce the likelihood that these factors substantially contribute to the findings. Finally, studies in other cortical areas also suggest distinct contributions of arousal and locomotion on cortical activity (Khoury et al., 2023; Vinck et al., 2015a). The contribution of physical effort is harder to dissociate from locomotion speed, as these behavioral attributes are strongly linked. To do this, future studies could use elevated surfaces or treadmills with varying degrees of resistance. An additional alternative is that the encoding of locomotion speed is linked to coding of sound source location (Harrington et al., 2008; King et al., 2007; Middlebrooks & Pettigrew, 1981; Recanzone et al., 2000; Town et al., 2017; Wood et al., 2019). As an animal runs faster through its environment, it experiences an increased level of change in sound source locations relative to itself, and this could be reflected in ongoing activity. In our mouse imaging data, animals were head fixed and thus sound source locations did not actually move relative to the animal, arguing against this possibility. However, we cannot rule out a contribution from secondary factors related to spatial hearing, such as increased spatial attention effort. To test this

possibility, future studies could modulate the relationship between running speed and attended sound source location, for example with an auditory virtual reality (Gao et al., 2020).

Finally, the degree of stimulus-specificity of the apparent response suppression during locomotion remains to be further explored. Indeed, locomotion-related attenuation of self-generated sounds is stimulus- and prediction-dependent (Audette et al., 2022; Clayton et al., 2021; Rummell et al., 2016; Schneider et al., 2018; Sigurdsson, 2019). In our imaging experiments we found that responses to BBN, pure tones and complex sounds were similarly suppressed during locomotion, though neither of these sounds carried immediate behavioral meaning to the animal and were presented at the same sound intensity. In our electrophysiological data, in which the target sound gained reward-location-predictive meaning across training, we found that locomotion-related responses strengthened (or were less attenuated) across days of training (Suppl. Fig. 9). This suggests that locomotion-related attenuation of sound responses in the auditory cortex may be salience-dependent. Moreover, sound intensity may strongly shape locomotion-related response modulation, with larger suppression of near-threshold sounds and lower suppression of mild sounds. Indeed, studies in humans have found that sound intensity and meaning influence the interaction between motor action and sound perception (Reznik & Mukamel, 2019). Future studies are required to further determine how locomotion modulates AC responses to sounds of varying attributes, such as intensity, behavioral relevance, valence and sound source location.

2.9 Methods

All procedures followed laboratory animal care guidelines approved by the University of Michigan Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines.

2.9.1 Animals

A total of 32 male and female Thy1-GCaMP6f mice (C57BL/6J-Tg(Thy1-GCaMP6f)GP5.17Dkim/J, JAX stock No: 025393) between the ages of 12-23 weeks were used in this study (15 in the behavioral experiments and 17 in the two-photon experiments). Mice were kept on a reverse light cycle and all imaging and behavioral sessions were performed in the dark cycle.

Data from 4 Long Evans male rats aged 4–5 months and weighing 450–550 g were also included in this study. Auditory cortical sleep data from these rats has been reported in an earlier study (Rothschild et al., 2017).

2.9.2 Mouse surgery

Mice were anesthetized with Ketamine-Xylazine or isoflurane and implanted with a custom lightweight (<1 gr.) titanium head bar. For two photon calcium imaging, the muscle overlying the right AC was removed and a 3 mm diameter glass cranial window was implanted over the right AC. For the cortical inactivation experiments, small bilateral craniotomies were drilled above the AC and either 2 mm or 3 mm length custom cannulas (Plastics One, MA) were lowered into the AC. Mice received postop antibiotic ointment and Carprofen and were allowed to recover for at least 5 days before any imaging or behavioral sessions.

2.9.3 Appetitive trace conditioning and AC inactivation

Mice were placed on water restriction 48 hours prior to behavioral training and received ad libitum access to food. During training and testing, mice were placed in a custom built behavioral training box, in which they were head fixed on top of a rotatable plate with an accessible water reward port. A custom Arduino-based system that received input from a rotary encoder at the base of the plate allowed presenting sounds from a speaker placed ~10cm in front of the animal in either immobility or locomotion.

Appetitive trace conditioning in immobility: Animals were trained to associate a 1 s 8 kHz tone with subsequent water reward delivered after a delay of 1s following sound termination. Sounds (followed by water rewards) were presented following a period of continuous immobility that randomly varied across trials between 5-10 s. If the animal ran, the immobile duration counter was reset. Animals advanced to the testing phase only after they displayed consistent post-sound reward-predictive licking in locomotion for 2 consecutive days. Animals were tested following bilateral infusion of 750 nL PBS solution into AC. 24 hours following PBS infusions animals were tested following bilateral infusions of 750 nL muscimol (1 μg/μl). Unrewarded catch trials (10% of trials) were used to validate sound-triggered licking.

Appetitive trace conditioning in locomotion: A different group of mice were trained on a similar task in which sounds were presented during locomotion. Specifically, mice were trained on a

task in which sounds (followed by water reward) were presented exclusively in locomotion after the animal had run a distance that randomly varied across trials between 25 and 55 20ths of a full rotation. If the animal paused for longer than 2 s then the trial was reset. Animals advanced to the testing phase only after they displayed consistent post-sound reward-predictive licking in locomotion for 2 consecutive days. Unrewarded catch trials (10% of trials) and immobility trials were used to validate sound-triggered and locomotion-specific licking, respectively. Similar to the immobility conditions, mice were first tested following infusion of 750 nL PBS solution into AC and 24 hours later following muscimol infusion.

To quantify the association between sound and subsequent water reward, we quantified the degree of increased licking in the 1 s window following sound termination and before reward delivery (0-1000 ms from sound offset) relative to the pre-sound baseline lick rate ((-1500) – (-500) ms from sound onset). To this end we defined a "predictive lick index" as the across-trials average difference between the number of licks in the post-sound window and that of the pre-sound window.

2.9.4 Two-photon imaging

During imaging sessions, mice were placed on a rotating plate while being head fixed under the microscope objective. Imaging was carried out while the head of the animal was straight, with the objective tilted using an orbital nosepiece to allow optical access to the AC. Mice were allowed to initiate movement at their leisure. Imaging was performed using an Ultima IV two-photon microscope (Bruker), a pulsed tunable laser (MaiTai eHP DeepSee by Spectra Physics) providing excitation light at 940nm and 16X or 40X water-immersion objectives (Nikon). Images (256X256 pixels) were acquired using galvanometric mirrors at ~3 Hz to optimize signal quality and cell separation. The microscope was placed in an enclosed chamber in a dark, quiet room. Neurons were imaged at depths of 150-350 μM, corresponding to cortical L2/3.

During imaging sessions, the mouse's behavior was video recorded using an infrared camera, which was synchronized offline with the imaging data acquisition. Locomotion and immobility were determined offline using semi-automatic movement-detection MATLAB code with manual thresholding and supervision. In addition, in most imaging sessions a rotary encoder was positioned at the base of the rotating plate allowing to acquire continuous locomotion speed. In a given daily imaging session, responses of the same neurons were imaged to multiple sound

protocols. Different neuronal ensembles in the same mice were typically imaged on separate days.

Auditory stimuli were delivered via an open-field magnetic speaker (MF1, Tucker Davis Technologies) at 75 dB. The broadband noise bursts protocol consisted of 45 repeats of 1 s white noise bursts with an interstimulus interval of 3 ± 1s. The sound-masking sessions consisted of continuous presentation of broad band noise at 80 dB. The pure tone protocol (Suppl. Fig. 1) consisted of three randomly shuffled pure tones (2 kHz, 4 kHz, 8 kHz), of 20 repeats, with a duration of 1s and an interstimulus interval of 3±1s. The complex sound protocol (Suppl. Fig. 1) consisted of four randomly shuffled complex sounds (cricket, sparrow, scratch, water), with 20 or 9 repeats per stimulus. Complex stimuli duration ranged from 0.2-0.5s, padded with 0.8-0.2s of silence to create 1s long stimuli frames and an insterstimulus interval of 3±1s.

2.9.5 Imaging data preprocessing and analysis

Daily imaging data of the same ensemble across multiple sound protocols was concatenated and then preprocessed using the open source Suite2P software (Pachitariu et al., 2017) for movement correction and neuronal ROI detection within the ensemble. Neural data, sound stimuli and locomotion speed signals were aligned.

Data analysis was performed using custom software written in Matlab (MathWorks).

Relative change in fluorescence (ΔF/F) across time (t) was calculated for each detected cell as (F(t)-median(F))/(median(F)), where F(t) is the mean brightness of the cell's pixels at time t.

For determination of BBN-responsiveness of individual neurons and quantification of activity in the pre-stimulus time window (Ongoing) and stimulus time window (Evoked), the mean ΔF/F was taken across 1-4 samples preceding stimulus onset (corresponding to ~-1.2 – 0 s), and 1-4 samples following stimulus onset (corresponding to ~0 – 1.2 s), respectively. A cell was determined as BBN-responsive if ΔF/F during the stimulus time window was significantly higher than during the pre-stimulus time window using a one-tailed paired t-test at $P<0.05$ across all immobile trials. Ongoing activity levels in immobility/locomotion in the presence of background masking noise was quantified as the average ΔF/F across all time points of immobility/locomotion in the session.

A difference in BBN-evoked response magnitude between immobility and locomotion was determined using an unpaired two-sided t-test (at $P<0.05$) of the response magnitudes during the

immobility and locomotion trials. Neurons with 8 or fewer responses in either state (immobility/locomotion) were excluded from immobility/locomotion comparisons. To determine a difference in the influence of locomotion on responses to tones and complex sounds, locomotion and immobility trials of each stimulus were compared separately.

Noise correlations between pairs of simultaneously imaged neurons were calculated as the Pearson correlation between their trial-by-trial baseline-subtracted sound responses. Cross-correlations between pairs of simultaneously imaged neurons were calculated as the cross-correlation between their continuous ΔF/F traces. Cross-correlations were normalized such that the autocorrelations at zero lag equal 1. Noise correlations and cross-correlations were calculated separately between pairs of neurons whose sound-evoked responses were (1) Both significantly enhanced during locomotion, (2) Both significantly suppressed during locomotion and (3) One neuron significantly enhanced and the other significantly suppressed during locomotion. A difference in the peak of cross-correlations between groups was tested by taking the maximum values of each cross-correlogram within a lag of ±0.66 and comparing these values across groups using a one-way ANOVA.

For calculating ΔF/F-locomotion speed correlations and speed prediction, the daily locomotion speed was smoothed using a 6-sample (~2 s) moving average filter. The ΔF/F-locomotion speed correlation was calculated as the Pearson correlation between the continuous ΔF/F trace of each neuron and the animal's locomotion speed.

Speed prediction was carried out using cross-validated generalized linear models on the day's ensemble continuous activity patterns and locomotion speed. For a given ensemble, the data included the daily continuous locomotion speed and ΔF/F traces of all cells. ΔF/F traces of each cell were smoothed using a 3-sample (~1 s) moving average filter. Locomotion speed in cm/s was log-transformed using $\log_{f_0}\llbracket(\text{speed}+1).\rrbracket$ In the model training phase, a random half of the daily sample points ("training set") of locomotion and corresponding ensemble ΔF/F values were used to train a generalized linear model. In the test phase, the model used the remaining ensemble ΔF/F values ("test set") to predict the corresponding (log) locomotion speeds. Prediction performance was quantified by the Spearman correlation between the predicted speeds and the real speeds. This procedure was repeated 200 times and the correlation values averaged across repeats to yield the final prediction performance. Repeats in which the test set included fewer than 10 non-zero speed values were excluded.

Stimulus detection during locomotion was quantified using cross-validated SVM analyses. Only ensembles with more than 10 cells and 12 trials in both immobility and locomotion were included in the decoding analyses. Data consisted of all ensemble activity patterns before BBN presentation (i.e., across-trials ensemble activity in the pre-stimulus time windows) and during BBN presentation (i.e., across-trials ensemble activity in the stimulus time windows) that occurred during locomotion. An SVM model was constructed on this data and a 10-fold cross validation was used to estimate the ability of the ensemble to discriminate between the pre-stimulus and stimulus ensemble activity patterns. Detection performance was defined as the across-fold average of percent correct predictions. To estimate significance of prediction, this procedure was performed 200 times on shuffled data identity and significant detection was determined if detection performance was higher than 95% of shuffles. Discrimination of locomotion state (immobility/locomotion) was carried out in a similar manner, but using (1) The ensemble activity patterns during the pre-stimulus time windows in immobility and (2) The pre-stimulus time windows in locomotion, as the data to be discriminated. Discrimination between the four combinations of sound occurrence and locomotion state was carried out similarly using a linear discriminant analysis, but using the ensemble activity patterns during (1) Pre-stimulus time windows in immobility (2) Pre-stimulus time windows in locomotion (3) Stimulus time windows in immobility (4) Stimulus time windows in locomotion, as the data to be discriminated. The same number of trials was included in the three models (stimulus detection during locomotion, state discrimination and stimulus+state discrimination) by removing excess trials in the data with more trials.

Analysis of the electrophysiology data was carried out similarly to the imaging data, but using spike counts instead of $\Delta F/F$ as the neural measure and using a stimulus response time window of 1-450 ms and a pre-stimulus time window of -450-0 ms relative to sound onset. Immobility was determined as speed <= 4 cm/s, Neurons with 10 or fewer responses in either state (immobility/locomotion) were excluded from immobility/locomotion comparisons. A minimum of 20 trials in both immobility and locomotion were required for inclusion in the decoding analyses. Spiking-speed correlations were calculated by binning spiking and speeds into 200 ms bins and calculating the Spearman correlation. Speed prediction of movement-only data excluded all data with movement slower than 0.65 cm/s. Discrimination between the 4 state/sound combinations were carried out using a pseudolinear discriminant analysis.

2.9.6 Rat pretraining, surgery and electrophysiological recordings

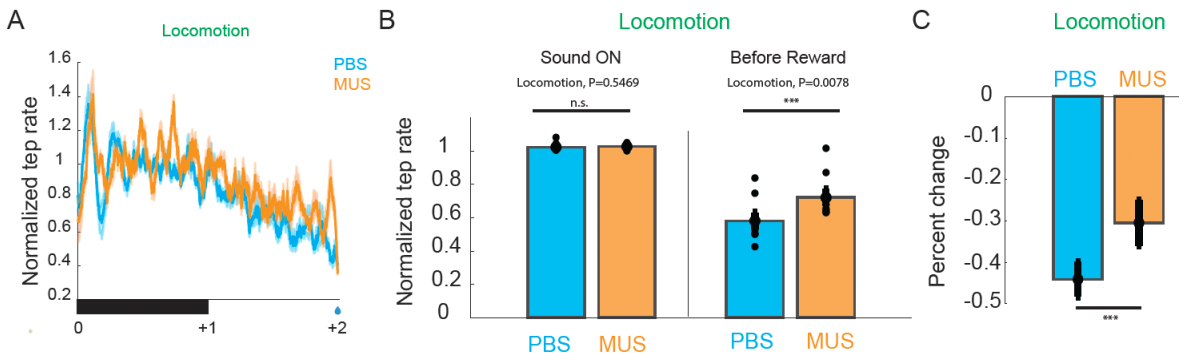
The rat behavioral and surgery procedures have been described previously (Znamenskiy & Zador, 2013). Briefly, after habituation to daily handling over several weeks, rats were pretrained to run on an E-shaped raised track for liquid food rewards (sweetened condensed milk). Rats were then implanted with a microdrive array with 21 independently moveable tetrodes (groups of four twisted 12.5 μm nichrome wires assembled in a bundle). Seven tetrodes were targeted to the left primary AC (−4.8 mm AP, 5.5 mm ML, 25° lateral from midline). Other tetrodes targeted left dorsal CA1 region of the hippocampus and left PFC, but these data are not included here. Over the course of two weeks following implantation, AC tetrodes were advanced gradually and responses to sound stimuli were used to validate approach to primary AC. Based on histological sections and the ventro-lateral angle of parallel tetrodes targeting the AC, the rat data likely includes neurons from a mixture of layers from 2-5

Data were collected using the NSpike data acquisition system (L.M. Frank and J. MacArthur, Harvard Instrumentation Design Laboratory). Spike data were sampled at 30 kHz, digitally filtered between 300 Hz and 6 kHz (two-pole Bessel for high and low pass) and threshold crossing events were saved to disk (40 samples at 30 kHz). Individual units (putative single neurons) were identified by clustering spikes using peak amplitude, principal components and spike width as variables (MatClust). Behavior sessions were recorded with an overhead monochrome CCD camera (30 fps) and the animal's position and speed were detected using an infrared light emitting diode array with a large and a small cluster of diodes attached to the preamps. For binary assignment of immobility and locomotion we used a standard 4 cm/s speed threshold.

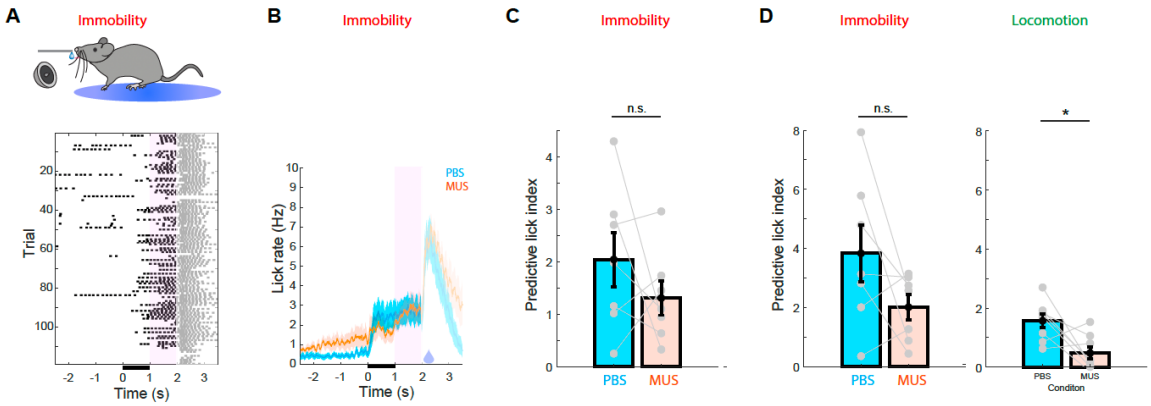
Approximately 14 d after implantation, animals were introduced to the Y-track and data gathering commenced. Animals were trained on the Y-track for 10–12 d in 3–4 20-min training sessions per day with interleaving 20- to 30-min sleep sessions in the rest box. Data for each neuron was pooled across daily sessions. During training sessions, sweetened condensed milk rewards were automatically delivered in food wells triggered by animal's nose-poke crossing of an IR beam. Rats initiated each trial by a nose-poke in the home well and receiving a reward. In ~75% of trials the next reward was delivered in the silent well if the rat nose-poked there. In a pseudorandom ~25% of trials (sound trials separated by 2–5 silent trials), 5 s after nose-poking in the home arm, a target sound series was emitted from a speaker, indicating that the next

reward would be delivered in the sound well if the rat next nose-poked there. The speaker was placed at the end of the sound arm in the first days of training and moved to the center junction after rats displayed consistent correct choices in more than ~70% of trials. The target sound was a pair of upward chirps, consisting of one 200-ms chirp with frequency modulated from 3 to 4 kHz, an interchirp interval of 50 ms, and a second 200-ms chirp with frequency modulated from 9 to 12 kHz. The series of target sounds was presented at 1 Hz and stopped after 12 s or once the rat made a correct or incorrect choice by a nose-poke in one of the wells. The sound-evoked responses analyzed in Fig. 6 include sounds in which the spatial orientation and distance between the animal and the speaker varied based on the animal's behavior. Thus, the data contains variability of effective intensity and spatial orientation of the sound source. Reward amount in the sound well was double the reward amount in the home or silent well. Following the Y-track training days, two rats continued to perform the same task on a W-shaped track for an additional 3 days.

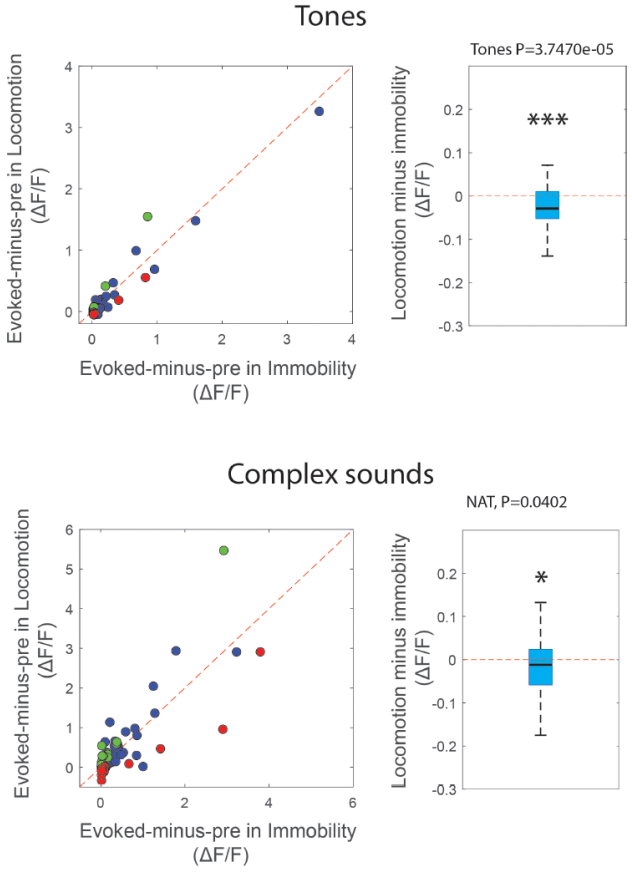
2.10 Supplemental Figures



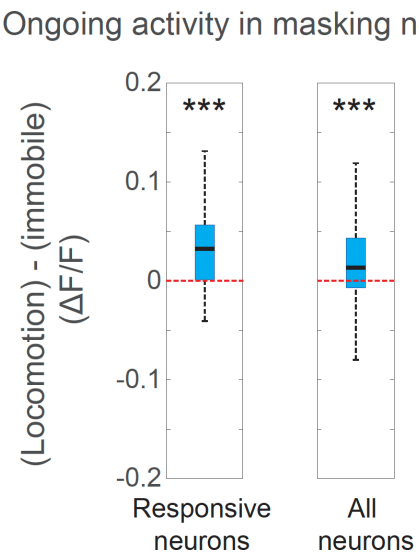
Supplemental Figure 1 AC inactivation alters locomotion speed following sound presentation (A) Sound-triggered PSTH showing changes in locomotor activity following sound presentation in locomotion in PBS (blue) and MUS (orange). **(B)** (Left) No significant difference in average step rate at sound onset for animals under PBS and MUS conditions ($P = 0.569$, two-sided Wilcoxon signed-rank test). (Right) Significant difference in the average step rate preceding reward delivery for animals under PBS and MUS conditions ($P = 0.0078$, two-sided Wilcoxon signed-rank test). **(C)** Relative decrease in locomotion speed following sound onset and preceding reward delivery is significantly greater in PBS compared to MUS conditions ($P = 0.0078$, two-sided Wilcoxon signed-rank test).



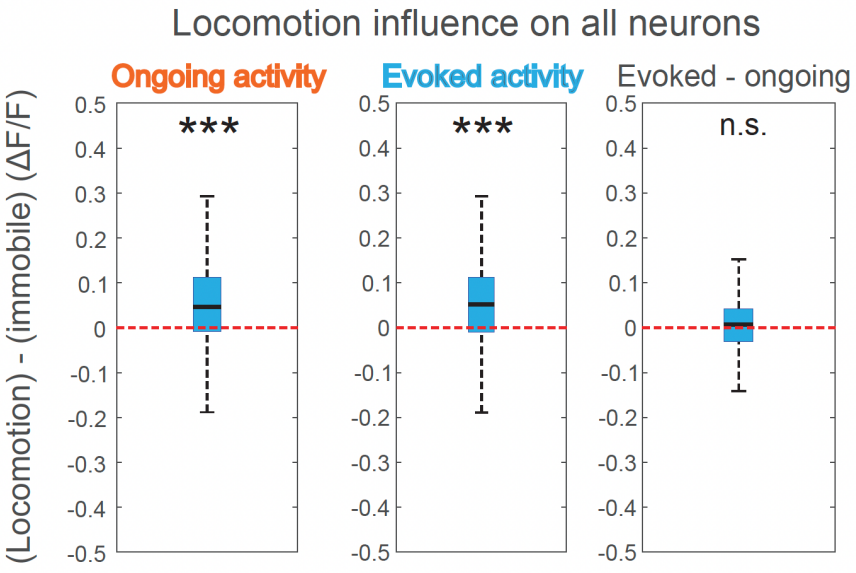
Supplemental Figure 2 Auditory cortical inactivation does not induce a significant impairment in sound-triggered reward-predictive licking in immobility (A–C) Similar to Figure 2.1A–1C for immobility conditions. C: P = 0.46875, signed-rank test. **(D)** Calculation of predictive lick index in immobility and locomotion using an alternative lick window of 0–2 s. Immobility: P = 0.15625; Locomotion: P = 0.0391, signed-rank test.



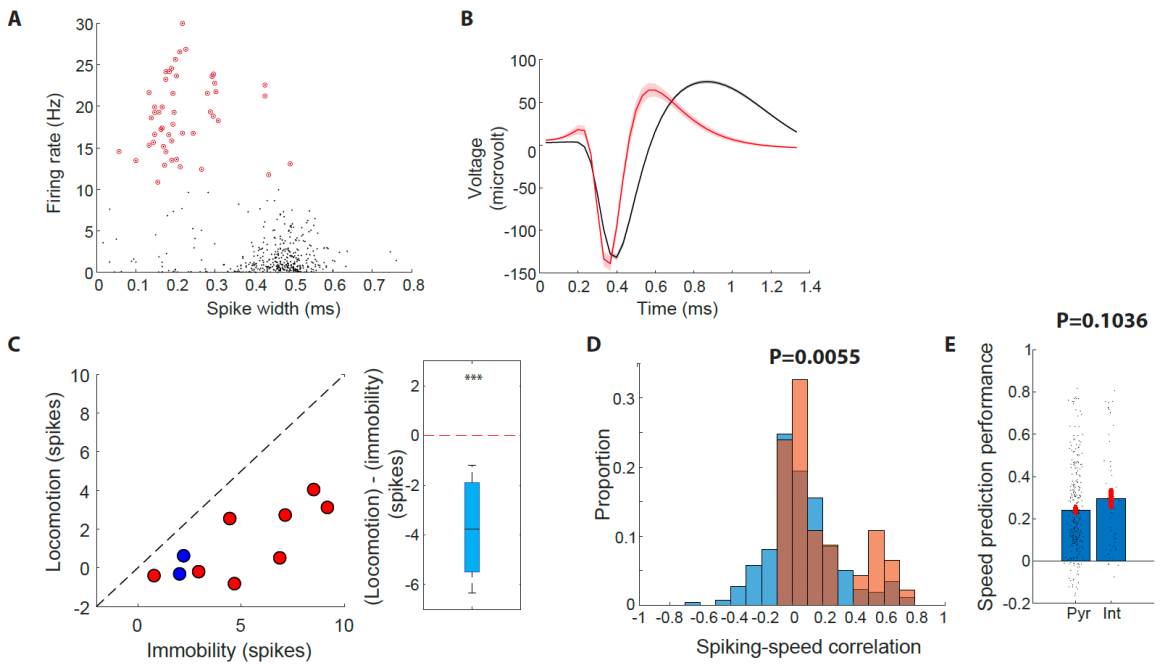
Supplemental Figure 3 Locomotion influence on AC responses to tones and complex sounds Baseline-subtracted sound-evoked responses in immobility and locomotion for tones (top) and complex sounds (bottom). Graphical conventions same as Figure 2.2E. While individual responses showed diversity in locomotion-related influence, population-level responses to both tones and complex sounds were significantly reduced during locomotion (Tones: P = 3.7 × 10−5, Complex sounds: P = 0.0402, two-sided Wilcoxon signed-rank test).



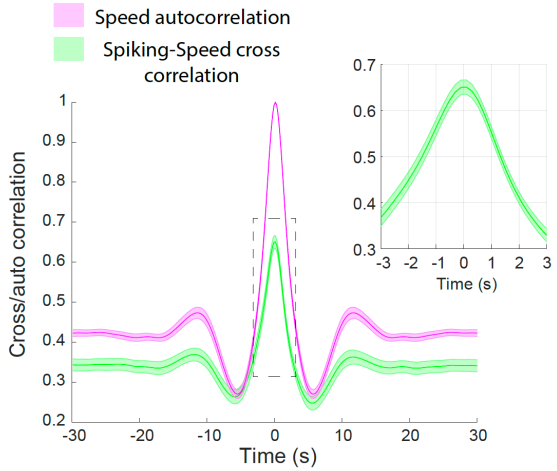
Supplemental Figure 4 Influence of locomotion on ongoing activity under masking noise conditions.



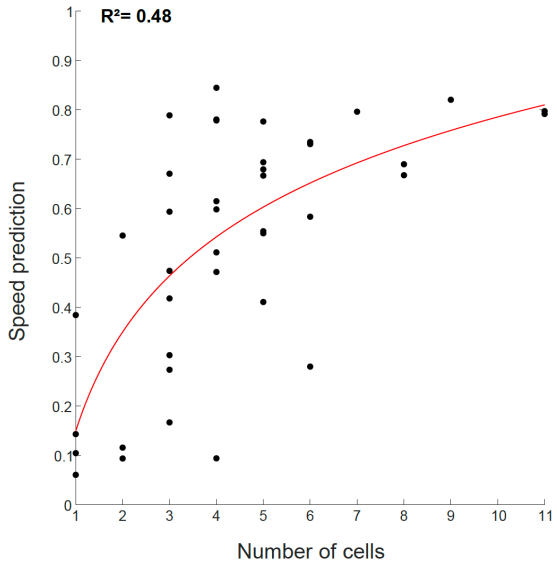
Supplemental Figure 5 Locomotion influence on all neurons (including sound-unresponsive) Same layout as Figure 2.3B and 3D. Left, P = 7.05 × 10−27; middle, P = 4.11 × 10−29; right, P = 0.0693, two-sided Wilcoxon signed-rank test.



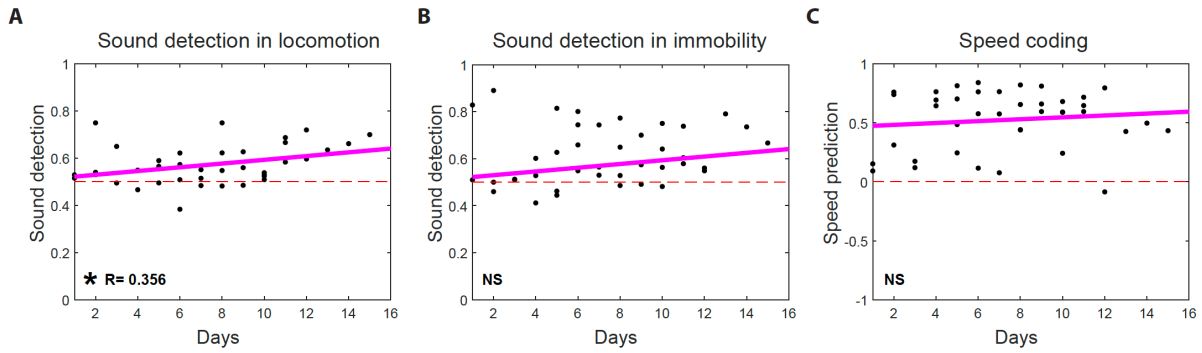
Supplemental Figure 6 Locomotion modulation of putative fast-spiking interneurons (A) Detection of putative FS interneurons (red) based on spike width and firing rate. **(B)** Waveforms of putative FS (red) and RS (black) neurons. **(C)** Influence of locomotion on sound-evoked responses of putative FS interneurons (as in Figure 2.2E). **(D)** Distribution of spiking-speed correlation for putative FS interneurons (orange) over the distribution for putative RS neurons (as in Fig 2.4D). The distribution of FS neurons was significantly higher than that of RS neurons (P = 0.0055, rank-sum test). There was no significant correlation between firing rate and spiking-speed correlation within putative excitatory neurons (R = −0.037, P = 0.557) or within putative FS interneurons (R = −0.075, P = 0.62). **(E)** Speed prediction performance of putative RS and FS single neurons. Prediction of FS neurons showed a higher trend, though this did not reach significance (P = 0.1036, rank-sum test).



Supplemental Figure 7 Auto correlation of speed (purple) and cross-correlation of speed and spiking (green) across the electrophysiology dataset, calculated across all data where the speed-spiking correlation was > = 0.3 Inset shows enlarged view of the highlighted area.



Supplemental Figure 8 Relationship between the number of cells in an ensemble and speed prediction performance in the electrophysiological data..



Supplemental Figure 9 Sound detection performance of electrophysiological data Sound detection in locomotion **(A)** sound detection in immobility **(B)** and speed coding **(C)** across training days for the electrophysiology data.

2.11 References

1. Audette NJ, Zhou W, La Chioma A, Schneider DM. Precise movement-based predictions in the mouse auditory cortex. Curr Biol. 2022; 32(22):4925–40 e6. Epub 20221024. https://doi.org/10.1016/j.cub.2022.09.064 PMID: 36283411; PubMed Central PMCID: PMC9691550.
2. Ayaz A, Saleem AB, Scholvinck ML, Carandini M. Locomotion controls spatial integration in mouse visual cortex. Curr Biol. 2013; 23(10):890–4. Epub 2013/05/15. https://doi.org/10.1016/j.cub.2013.04. 012 PMID: 23664971; PubMed Central PMCID: PMC3661981.
3. Ayaz A, Stauble A, Hamada M, Wulf MA, Saleem AB, Helmchen F. Layer-specific integration of locomotion and sensory information in mouse barrel cortex. Nat Commun. 2019; 10(1):2585. Epub 2019/ 06/15. https://doi.org/10.1038/s41467-019-10564-8 PMID: 31197148; PubMed Central PMCID:PMC6565743.
4. Bathellier B, Ushakova L, Rumpel S. Discrete neocortical dynamics predict behavioral categorizationof sounds. Neuron. 2012; 76(2):435–49. Epub 20121017. https://doi.org/10.1016/j.neuron.2012.07. 008 PMID: 23083744.
5. Bandyopadhyay S, Shamma SA, Kanold PO. Dichotomy of functional organization in the mouse auditory cortex. Nat Neurosci. 2010; 13(3):361–8. Epub 20100131. https://doi.org/10.1038/nn.2490 PMID:20118924; PubMed Central PMCID: PMC2866453
6. Bigelow J, Morrill RJ, Dekloe J, Hasenstaub AR. Movement and VIP Interneuron Activation Differentially Modulate Encoding in Mouse Auditory Cortex. eNeuro. 2019; 6(5). Epub 20190918. https://doi. org/10.1523/ENEURO.0164-19.2019 PMID: 31481397; PubMed Central PMCID: PMC6751373.
7. Campbell MG, Giocomo LM. Self-motion processing in visual and entorhinal cortices: inputs, integration, and implications for position coding. J Neurophysiol. 2018; 120(4):2091–106. Epub 2018/08/09.https://doi.org/10.1152/jn.00686.2017 PMID: 30089025; PubMed Central PMCID: PMC6230811.\
8. Cuppone AV, Cappagli G, Gori M. Audio Feedback Associated With Body Movement Enhances Audio and Somatosensory Spatial Representation. Front Integr Neurosci. 2018; 12(37). https://doi.org/10.3389/fnint.2018.00037 PMID: 30233334

9. Carr KW, Tierney A, White-Schwoch T, Kraus N. Intertrial auditory neural stability supports beat synchronization in preschoolers. Dev Cogn Neuros-Neth. 2016; 17:76–82. https://doi.org/10.1016/j.dcn. 2015.12.003 WOS:000368760600010. PMID: 26760457
10. Chen JL, Penhune VB, Zatorrel RJ. Moving on time: Brain network for auditory-motor synchronization is modulated by rhythm complexity and musical training. J Cognitive Neurosci. 2008; 20(2):226–239. https://doi.org/10.1162/jocn.2008.20018 WOS:000252532600004. PMID: 18275331
11. Clayton KK, Williamson RS, Hancock KE, Tasaka GI, Mizrahi A, Hackett TA, et al. Auditory Corticothalamic Neurons Are Recruited by Motor Preparatory Inputs. Curr Biol. 2021; 31(2):310–21 e5. Epub 20201105. https://doi.org/10.1016/j.cub.2020.10.027 PMID: 33157020; PubMed Central PMCID: PMC7855066.
12. Cohen L, Rothschild G, Mizrahi A. Multisensory integration of natural odors and sounds in the auditorycortex. Neuron. 2011; 72(2):357–369. https://doi.org/10.1016/j.neuron.2011.08.019 PMID: 22017993.
13. Cornwell T, Woodward J, Wu MM, Jackson B, Souza P, Siegel J, et al. Walking With Ears: Altered Auditory Feedback Impacts Gait Step Length in Older Adults. Frontiers in Sports and Active. Living. 2020; 2(38). https://doi.org/10.3389/fspor.2020.00038 PMID: 33345030
14. Dannenberg H, Lazaro H, Nambiar P, Hoyland A, Hasselmo ME. Effects of visual inputs on neural dynamics for coding of location and running speed in medial entorhinal cortex. Elife. 2020; 9. Epub 2020/12/11. https://doi.org/10.7554/eLife.62500 PMID: 33300873; PubMed Central PMCID:PMC7773338.
15. Dadarlat MC, Stryker MP. Locomotion Enhances Neural Encoding of Visual Stimuli in Mouse V1. JNeurosci. 2017; 37(14):3764–3775. https://doi.org/10.1523/JNEUROSCI.2728-16.2017 PMID: 28264980; PubMed Central PMCID: PMC5394894.
16. David SV, Fritz JB, Shamma SA. Task reward structure shapes rapid receptive field plasticity in auditory cortex. Proc Natl Acad Sci U S A. 2012; 109(6):2144–9. Epub 20120123. https://doi.org/10.1073/ pnas.1117717109 PMID: 22308415; PubMed Central PMCID: PMC3277538.

17. Falk B, Jakobsen L, Surlykke A, Moss CF. Bats coordinate sonar and flight behavior as they forage in open and cluttered environments. J Exp Biol. 2014; 217(Pt 24):4356–4364. https://doi.org/10.1242/ jeb.114132 PMID: 25394632; PubMed Central PMCID: PMC4375838.
18. Farrell JS, Lovett-Barron M, Klein PM, Sparks FT, Gschwind T, Ortiz AL, et al. Supramammillary regulation of locomotion and hippocampal activity. Science. 2021; 374(6574):1492–6. Epub 2021/12/17. https://doi.org/10.1126/science.abh4272 PMID: 34914519; PubMed Central PMCID: PMC9154354.
19. Fiser A, Mahringer D, Oyibo HK, Petersen AV, Leinweber M, Keller GB. Experience-dependent spatial expectations in mouse visual cortex. Nat Neurosci. 2016; 19(12):1658–1664. https://doi.org/10.1038/nn.4385 PMID: 27618309.
20. Fox MW. The whistling hunters: field studies of the Asiatic wild dog (Cuon alpinus). Albany: State University of New York Press; 1984. viii, p. 150.
21. Francis NA, Winkowski DE, Sheikhattar A, Armengol K, Babadi B, Kanold PO. Small Networks Encode Decision-Making in Primary Auditory Cortex. Neuron. 2018; 97(4):885–97 e6. Epub20180201. https://doi.org/10.1016/j.neuron.2018.01.019 PMID: 29398362; PubMed Central PMCID: PMC6289180.
22. Fritz JB, David SV, Radtke-Schuller S, Yin P, Shamma SA. Adaptive, behaviorally gated, persistent encoding of task-relevant auditory information in ferret frontal cortex. Nat Neurosci. 2010; 13(8):1011–9. Epub 20100711. https://doi.org/10.1038/nn.2598 PMID: 20622871; PubMed Central PMCID:PMC2921886.
23. Gao S, Webb J, Mridha Z, Banta A, Kemere C, McGinley M. Novel Virtual Reality System for Auditory Tasks in Head-fixed Mice. Annu Int Conf IEEE Eng Med Biol Soc. 2020; 2020:2925–2928. https://doi.org/10.1109/EMBC44109.2020.9176536 PMID: 33018619
24. Ghose K, Horiuchi TK, Krishnaprasad PS, Moss CF. Echolocating bats use a nearly time-optimal strategy to intercept prey. PLoS Biol. 2006; 4(5):865–73. ARTN e108 https://doi.org/10.1371/journal.pbio. 0040108 WOS:000237966900023. PMID: 16605303
25. Geisler C, Robbe D, Zugaro M, Sirota A, Buzsaki G. Hippocampal place cell assemblies are speed controlled oscillators. Proc Natl Acad Sci U S A. 2007; 104(19):8149–54. Epub

2007/05/02. https://doi. org/10.1073/pnas.0610121104 PMID: 17470808; PubMed Central PMCID: PMC1876586.

26. Gimenez TL, Lorenc M, Jaramillo S. Adaptive categorization of sound frequency does not require the auditory cortex in rats. J Neurophysiol. 2015; 114(2):1137–45. Epub 20150708. https://doi.org/10. 1152/jn.00124.2015 PMID: 26156379; PubMed Central PMCID: PMC4541140.
27. Gois Z, Tort ABL. Characterizing Speed Cells in the Rat Hippocampus. Cell Rep. 2018; 25(7):1872–84 e4. Epub 2018/11/15. https://doi.org/10.1016/j.celrep.2018.10.054 PMID: 30428354.
28. Goldberg JM, Neff WD. Frequency Discrimination after Bilateral Ablation of Cortical Auditory Areas. J Neurophysiol. 1961; 24(2):119. https://doi.org/10.1152/jn.1961.24.2.119 WOS:A19616099B00002. PMID: 13706457
29. Haggerty DC, Ji D. Activities of visual cortical and hippocampal neurons co-fluctuate in freely moving rats during spatial behavior. Elife. 2015; 4. Epub 2015/09/09. https://doi.org/10.7554/eLife.08902 PMID: 26349031; PubMed Central PMCID: PMC4595967.
30. Harrington IA, Heffner RS, Heffner HE. An investigation of sensory deficits underlying the aphasia-like behavior of macaques with auditory cortex lesions. Neuroreport. 2001; 12(6):1217–1221. https://doi. org/10.1097/00001756-200105080-00032 PMID: 11338194
31. Harrington IA, Stecker GC, Macpherson EA, Middlebrooks JC. Spatial sensitivity of neurons in the anterior, posterior, and primary fields of cat auditory cortex. Hear Res. 2008; 240(1–2):22–41. Epub20080219. https://doi.org/10.1016/j.heares.2008.02.004 PMID: 18359176; PubMed Central PMCID:PMC2515616.
32. Hinman JR, Brandon MP, Climer JR, Chapman GW, Hasselmo ME. Multiple Running Speed Signals in Medial Entorhinal Cortex. Neuron. 2016; 91(3):666–79. Epub 2016/07/19. https://doi.org/10.1016/j. neuron.2016.06.027 PMID: 27427460; PubMed Central PMCID: PMC4976037.
33. Jadhav SP, Rothschild G, Roumis DK, Frank LM. Coordinated Excitation and Inhibition of Prefrontal Ensembles during Awake Hippocampal Sharp-Wave Ripple Events. Neuron. 2016; 90(1):113–27.Epub 20160310.

https://doi.org/10.1016/j.neuron.2016.02.010 PMID: 26971950; PubMed CentralPMCID: PMC4824654.

34. Jaramillo S, Zador AM. The auditory cortex mediates the perceptual effects of acoustic temporal expectation. Nat Neurosci. 2011; 14(2):246–U340. https://doi.org/10.1038/nn.2688 WOS:000286595400023. PMID: 21170056
35. Kato HK, Gillet SN, Isaacson JS. Flexible Sensory Representations in Auditory Cortex Driven byBehavioral Relevance. Neuron. 2015; 88(5):1027–39. Epub 20151112. https://doi.org/10.1016/j. neuron.2015.10.024 PMID: 26586181; PubMed Central PMCID: PMC4670799.
36. Kavanagh GL, Kelly JB. Contribution of auditory cortex to sound localization by the ferret (Mustelaputorius). J Neurophysiol. 1987; 57(6):1746–1766. https://doi.org/10.1152/jn.1987 .57.6.1746 PMID: 3598629.
37. Kavanagh GL, Kelly JB. Hearing in the ferret (Mustela putorius): effects of primary auditory corticallesions on thresholds for pure tone detection. J Neurophysiol. 1988; 60(3):879–888. https://doi.org/10.1152/jn.1988.60.3.879 PMID: 3171665.
38. Karpati FJ, Giacosa C, Foster NEV, Penhune VB, Hyde KL. Dance and the brain: a review. Ann NyAcad Sci. 2015; 1337:140–146. https://doi.org/10.1111/nyas.12632 WOS:000351580900020. PMID:25773628
39. Keller GB, Bonhoeffer T, Hubener M. Sensorimotor mismatch signals in primary visual cortex of the behaving mouse. Neuron. 2012; 74(5):809–815. https://doi.org/10.1016/j.neuron.2012.03.040 PMID: 22681686.
40. Kelly JB, Glazier SJ. Auditory-Cortex Lesions and Discrimination of Spatial Location by Rat. Brain Res. 1978; 145(2):315–321. https://doi.org/10.1016/0006-8993(78)90865-X WOS:A1978EX16500008. PMID: 638790
41. King AJ, Bajo VM, Bizley JK, Campbell RA, Nodal FR, Schulz AL, et al. Physiological and behavioral studies of spatial coding in the auditory cortex. Hear Res. 2007; 229(1–2):106–15. Epub 20070117. https://doi.org/10.1016/j.heares.2007.01.001 PMID: 17314017; PubMed Central PMCID: PMC7116512.
42. Khoury CF, Fala NG, Runyan CA. Arousal and Locomotion Differently Modulate Activity of Somatostatin Neurons across Cortex. eNeuro. 2023; 10(5). Epub 20230525.

https://doi.org/10.1523/ ENEURO.0136-23.2023 PMID: 37169583; PubMed Central PMCID: PMC10216262

43. Kuchibhotla KV, Gill JV, Lindsay GW, Papadoyannis ES, Field RE, Sten TAH, et al. Parallel processing by cortical inhibition enables context-dependent behavior. Nat Neurosci. 2017; 20(1):62–71. https://doi.org/10.1038/nn.4436 WOS:000391085500013. PMID: 27798631
44. Lee J, Rothschild G. Encoding of acquired sound-sequence salience by auditory cortical offset responses. Cell Rep. 2021; 37(5):109927. https://doi.org/10.1016/j.celrep.2021.109927 PMID:34731615.
45. Lin PA, Asinof SK, Edwards NJ, Isaacson JS. Arousal regulates frequency tuning in primary auditorycortex. Proc Natl Acad Sci U S A. 2019; 116(50):25304–10. Epub 20191122. https://doi.org/10.1073/pnas.1911383116 PMID: 31757852; PubMed Central PMCID: PMC6911239.
46. Liu J, Kanold PO. Diversity of Receptive Fields and Sideband Inhibition with Complex Thalamocortical and Intracortical Origin in L2/3 of Mouse Primary Auditory Cortex. J Neurosci. 2021; 41(14):3142–62. Epub 20210216. https://doi.org/10.1523/JNEUROSCI.1732-20.2021 PMID: 33593857; PubMed Central PMCID: PMC8026349
47. Liu J, Whiteway MR, Sheikhattar A, Butts DA, Babadi B, Kanold PO. Parallel Processing of SoundDynamics across Mouse Auditory Cortex via Spatially Patterned Thalamic Inputs and Distinct Areal Intracortical Circuits. Cell Rep. 2019; 27(3):872–85 e7. https://doi.org/10.1016/j.celrep.2019.03.069 PMID: 30995483; PubMed Central PMCID: PMC7238664.
48. Maor I, Shalev A, Mizrahi A. Distinct Spatiotemporal Response Properties of Excitatory Versus Inhibitory Neurons in the Mouse Auditory Cortex. Cereb Cortex. 2016; 26(11):4242–4252. https://doi.org/ 10.1093/cercor/bhw266 PMID: 27600839; PubMed Central PMCID: PMC5066836.
49. Middlebrooks JC, Pettigrew JD. Functional classes of neurons in primary auditory cortex of the cat distinguished by sensitivity to sound location. J Neurosci. 1981; 1(1):107–120. https://doi.org/10.1523/NEUROSCI.01-01-00107.1981 PMID: 7346555; PubMed Central PMCID: PMC6564160.

50. McGinley MJ, David SV, McCormick DA. Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection. Neuron. 2015; 87(1):179–92. Epub 20150611. https://doi.org/10.1016/j. neuron.2015.05.038 PMID: 26074005; PubMed Central PMCID: PMC4631312.
51. Mineault PJ, Tring E, Trachtenberg JT, Ringach DL. Enhanced Spatial Resolution During Locomotion and Heightened Attention in Mouse Primary Visual Cortex. J Neurosci. 2016; 36(24):6382–6392. https://doi.org/10.1523/JNEUROSCI.0430-16.2016 PMID: 27307228; PubMed Central PMCID:PMC4909779.
52. Moss CF, Surlykke A. Auditory scene analysis by echolocation in bats. J Acoust Soc Am. 2001; 110 (4):2207–2226. https://doi.org/10.1121/1.1398051 WOS:000171526400054. PMID: 11681397
53. Niell CM, Stryker MP. Modulation of visual responses by behavioral state in mouse visual cortex. Neuron. 2010; 65(4):472–479. https://doi.org/10.1016/j.neuron.2010.01.033 PMID: 20188652; PubMed Central PMCID: PMC3184003.
54. Nelken I. Stimulus-specific adaptation and deviance detection in the auditory system: experiments and models. Biol Cybern. 2014; 108(5):655–63. Epub 20140130. https://doi.org/10.1007/s00422-014-0585-7 PMID: 24477619.
55. Nodal FR, Bajo VM, King AJ. Plasticity of spatial hearing: behavioural effects of cortical inactivation. JPhysiol-London. 2012; 590(16):3965–3986. https://doi.org/10.1113/jphysiol.2011.222828 WOS:000308128100027. PMID: 22547635
56. Ohl FW, Wetzel W, Wagner T, Rech A, Scheich H. Bilateral ablation of auditory cortex in Mongolian gerbil affects discrimination of frequency modulated tones but not of pure tones. Learn Mem. 1999; 6 (4):347–62. WOS:000082668400001. PMID: 10509706
57. O'Sullivan C, Weible AP, Wehr M. Auditory Cortex Contributes to Discrimination of Pure Tones. eNeuro. 2019; 6(5). Epub 20191016. https://doi.org/10.1523/ENEURO.0340-19.2019 PMID:31591138; PubMed Central PMCID: PMC6795560.
58. Otazu GH, Tai LH, Yang Y, Zador AM. Engaging in an auditory task suppresses responses in auditory cortex. Nat Neurosci. 2009; 12(5):646–54. Epub 20090412.

https://doi.org/10.1038/nn.2306 PMID: 19363491; PubMed Central PMCID: PMC4084972

59. Pachitariu M, Stringer C, Dipoppa M, Schro¨der S, Rossi LF, Dalgleish H, et al. Suite2p: beyond 10,000 neurons with standard two-photon microscopy. bioRxiv. 2017:061507.https://doi.org/10.1101/061507
60. Parker PRL, Brown MA, Smear MC, Niell CM. Movement-Related Signals in Sensory Areas: Roles in Natural Behavior. Trends Neurosci. 2020; 43(8):581–595. https://doi.org/10.1016/j.tins.2020.05.005 PMID: 32580899.
61. Porter BA, Rosenthal TR, Ranasinghe KG, Kilgard MP. Discrimination of brief speech sounds isimpaired in rats with auditory cortex lesions. Behav Brain Res. 2011; 219(1):68–74. https://doi.org/10.1016/j.bbr.2010.12.015 WOS:000289664100010. PMID: 21167211
62. Ravignani A, Cooke PF. The evolutionary biology of dance without frills. Curr Biol. 2016; 26(19):R878–R879. https://doi.org/10.1016/j.cub.2016.07.076 WOS:000385690800005. PMID: 27728787
63. Recanzone GH, Guard DC, Phan ML, Su TK. Correlation between the activity of single auditory cortical neurons and sound-localization behavior in the macaque monkey. J Neurophysiol. 2000; 83 (5):2723–2739. https://doi.org/10.1152/jn.2000.83.5.2723 PMID: 10805672.
64. Redd CB, Bamberg SJM. A Wireless Sensory Feedback Device for Real-Time Gait Feedback and Training. Ieee-Asme T Mech. 2012; 17(3):425–433. https://doi.org/10.1109/Tmech.2012.2189014WOS:000303502000004.
65. Reimer J, McGinley MJ, Liu Y, Rodenkirch C, Wang Q, McCormick DA, et al. Pupil fluctuations track rapid changes in adrenergic and cholinergic activity in cortex. Nat Commun. 2016; 7:13289. Epub 20161108. https://doi.org/10.1038/ncomms13289 PMID: 27824036; PubMed Central PMCID:PMC5105162
66. Reznik D, Guttman N, Buaron B, Zion-Golumbic E, Mukamel R. Action-locked Neural Responses in Auditory Cortex to Self-generated Sounds. Cereb Cortex. 2021; 31(12):5560–5569. https://doi.org/10. 1093/cercor/bhab179 PMID: 34185837.

67. Reznik D, Henkin Y, Schadel N, Mukamel R. Lateralized enhancement of auditory cortex activity and increased sensitivity to self-generated sounds. Nat Commun. 2014; 5:4059. Epub 20140605. https:// doi.org/10.1038/ncomms5059 PMID: 24898564.
68. Reznik D, Ossmy O, Mukamel R. Enhanced auditory evoked activity to self-generated sounds is mediatedby primary and supplementary motor cortices. J Neurosci. 2015; 35(5):2173–2180. https://doi.org/10.1523/JNEUROSCI.3723-14.2015 PMID: 25653372; PubMed Central PMCID: PMC6705360.
69. Reznik D, Mukamel R. Motor output, neural states and auditory perception. Neurosci Biobehav Rev.2019; 96:116–26. Epub 20181102. https://doi.org/10.1016/j.neubiorev.2018.10.021 PMID: 30391407.
70. Rodgers CC, DeWeese MR. Neural Correlates of Task Switching in Prefrontal Cortex and Primary Auditory Cortex in a Novel Stimulus Selection Task for Rodents. Neuron. 2014; 82(5):1157–1170. https://doi.org/10.1016/j.neuron.2014.04.031 WOS:000337359800020. PMID: 24908492
71. Rodger MWM, Young WR, Craig CM. Synthesis of Walking Sounds for Alleviating Gait Disturbances in Parkinson's Disease. IEEE T Neur Sys Reh. 2014; 22(3):543–548. https://doi.org/10.1109/TNSRE 2013.2285410 WOS:000342079300012. PMID: 24235275
72. Rothschild G, Nelken I, Mizrahi A. Functional organization and population dynamics in the mouse primary auditory cortex. Nat Neurosci. 2010; 13(3):353–60. Epub 20100131. https://doi.org/10.1038/nn.2484 PMID: 20118927.
73. Rothschild G, Mizrahi A. Global order and local disorder in brain maps. Annu Rev Neurosci. 2015; 38:247–68. Epub 20150409. https://doi.org/10.1146/annurev-neuro-071013-014038 PMID: 25897872.
74. Rothschild G, Eban E, Frank LM. A cortical-hippocampal-cortical loop of information processing during memory consolidation. Nat Neurosci. 2017; 20(2):251–9. Epub 20161212. https://doi.org/10.1038/nn. 4457 PMID: 27941790; PubMed Central PMCID: PMC5783826
75. Rummell BP, Klee JL, Sigurdsson T. Attenuation of Responses to Self-Generated Sounds in Auditory Cortical Neurons. J Neurosci. 2016; 36(47):12010–12026.

https://doi.org/10.1523/JNEUROSCI.1564-16.2016 PMID: 27881785; PubMed Central PMCID: PMC6604922.

76. Saderi D, Schwartz ZP, Heller CR, Pennington JR, David SV. Dissociation of task engagement and arousal effects in auditory cortex and midbrain. Elife. 2021; 10. Epub 20210211. https://doi.org/10. 7554/eLife.60153 PMID: 33570493; PubMed Central PMCID: PMC7909948.
77. Saleem AB, Ayaz A, Jeffery KJ, Harris KD, Carandini M. Integration of visual motion and locomotion in mouse visual cortex. Nat Neurosci. 2013; 16(12):1864–9. Epub 2013/11/05. https://doi.org/10.1038/ nn.3567 PMID: 24185423; PubMed Central PMCID: PMC3926520.
78. Saleem AB, Diamanti EM, Fournier J, Harris KD, Carandini M. Coherent encoding of subjective spatial position in visual cortex and hippocampus. Nature. 2018; 562(7725):124–7. Epub 2018/09/12. https:// doi.org/10.1038/s41586-018-0516-1 PMID: 30202092; PubMed Central PMCID: PMC6309439.
79. Schwartz ZP, Buran BN, David SV. Pupil-associated states modulate excitability but not stimulus selectivity in primary auditory cortex. J Neurophysiol. 2020; 123(1):191–208. Epub 20191113. https://doi.org/10.1152/jn.00595.2019 PMID: 31721652; PubMed Central PMCID: PMC6985860.
80. Sigurdsson T. Filtering of self-generated sounds by auditory cortical neurons. Acta Physiol. 2019; 227.WOS:000487978200047.
81. Schauer M, Mauritz K-H. Musical motor feedback (MMF) in walking hemiparetic stroke patients: randomized trials of gait improvement. Clin Rehabil. 2003; 17(7):713–722. https://doi.org/10.1191/0269215503cr668oa PMID: 14606736.
82. Scharloc DP, Neff WD, Stroming Nl. Discrimination of Tone Duration after Bilateral Ablation of Cortical Auditory Areas. J Neurophysiol. 1965; 28(4):673. https://doi.org/10.1152/jn.1965.28.4.673 WOS:A19656640700004. PMID: 14347627
83. Schneider DM, Nelson A, Mooney R. A synaptic and circuit basis for corollary discharge in the auditory cortex. Nature. 2014; 513(7517):189–94. Epub 20140827. https://doi.org/10.1038/nature13724 PMID: 25162524; PubMed Central PMCID: PMC4248668.

84. Schneider DM, Sundararajan J, Mooney R. A cortical filter that learns to suppress the acoustic consequences of movement. Nature. 2018; 561(7723):391–5. Epub 20180912. https://doi.org/10.1038/ s41586-018-0520-5 PMID: 30209396; PubMed Central PMCID: PMC6203933
85. Suri H, Rothschild G. Enhanced stability of complex sound representations relative to simple sounds in the auditory cortex. eNeuro. 2022; 9(4). Epub 20220721. https://doi.org/10.1523/ENEURO.0031- 22.2022 PMID: 35868858; PubMed Central PMCID: PMC9347310.
86. Tajadura-Jime´nez A, Basia M, Deroy O, Fairhurst M, Marquardt N, Bianchi-Berthouze N. As Light as your Footsteps: Altering Walking Sounds to Change Perceived Body Weight, Emotional State and Gait. CHI '15 Proceedings of the 33rd Annual ACM Conference on Human Factors in Computing Systems. 2015:2943–52. https://doi.org/10.1145/2702123.2702374
87. Tierney A, Kraus N. The Ability to Move to a Beat Is Linked to the Consistency of Neural Responses to Sound. J Neurosci. 2013; 33(38):14981–14988. https://doi.org/10.1523/JNEUROSCI.0612-13.2013 WOS:000324586500003. PMID: 24048827
88. Tierney A, Kraus N. Getting back on the beat: links between auditory-motor integration and precise auditory processing at fast time scales. Eur J Neurosci. 2016; 43(6):782–791. https://doi.org/10.1111/ ejn.13171 WOS:000373062700006. PMID: 26750313
89. Town SM, Brimijoin WO, Bizley JK. Egocentric and allocentric representations in auditory cortex.PLoS Biol. 2017; 15(6):e2001878. Epub 20170615. https://doi.org/10.1371/journal.pbio.2001878 PMID: 28617796; PubMed Central PMCID: PMC5472254.
90. Triblehorn JD, Yager DD. Timing of praying mantis evasive responses during simulated bat attack sequences. J Exp Biol. 2005; 208(Pt 10):1867–1876. https://doi.org/10.1242/jeb.01565 PMID:15879067
91. Turchet L, Camponogara I, Cesari P. Interactive footstep sounds modulate the perceptual-motor aftereffect of treadmill walking. Exp Brain Res. 2015; 233(1):205–214. https://doi.org/10.1007/s00221-014-4104-9 WOS:000348048100020. PMID: 25234404

92. Turchet L, Camponogara I, Nardello F, Zamparo P, Cesari P. Interactive footsteps sounds modulate the sense of effort without affecting the kinematics and metabolic parameters during treadmill-walking. Appl Acoust. 2018; 129:379–385. https://doi.org/10.1016/j.apacoust.2017.08.021 WOS:000413060300038.
93. Turchet L, Serafin S, Cesari P. Walking Pace Affected by Interactive Sounds Simulating Stepping on Different Terrains. Acm T Appl Percept. 2013; 10(4). Artn 23 https://doi.org/10.1145/2536764. 2536770 WOS:000329136700006.
94. Ulanovsky N, Las L, Nelken I. Processing of low-probability sounds by cortical neurons. Nat Neurosci. 2003; 6(4):391–398. https://doi.org/10.1038/nn1032 WOS:000181833100017. PMID: 12652303
95. Vinck M, Batista-Brito R, Knoblich U, Cardin JA. Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. Neuron. 2015; 86(3):740–754. https://doi.org/10.1016/j. neuron.2015.03.028 PMID: 25892300; PubMed Central PMCID: PMC4425590.
96. Whitton JP, Hancock KE, Polley DB. Immersive audiomotor game play enhances neural and perceptual salience of weak signals in noise. Proc Natl Acad Sci U S A. 2014; 111(25):E2606–E2615. https://doi.org/10.1073/pnas.1322184111 PMID: 24927596; PubMed Central PMCID: PMC4078866.
97. Wood KC, Town SM, Bizley JK. Neurons in primary auditory cortex represent sound source location in a cue-invariant manner. Nat Commun. 2019; 10(1):3019. Epub 20190709. https://doi.org/10.1038/ s41467-019-10868-9 PMID: 31289272; PubMed Central PMCID: PMC6616358.
98. Xiong QJ, Znamenskiy P, Zador AM. Selective corticostriatal plasticity during acquisition of an auditory discrimination task. Nature. 2015; 521(7552):348. https://doi.org/10.1038/nature14225 WOS:000354816500055. PMID: 25731173
99. Yavorska I, Wehr M. Effects of Locomotion in Auditory Cortex Are Not Mediated by the VIP Network. Front Neural Circuits. 2021; 15:618881. Epub 20210407. https://doi.org/10.3389/fncir.2021.618881 PMID: 33897378; PubMed Central PMCID: PMC8058405.

100. Znamenskiy P, Zador AM. Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. Nature. 2013; 497(7450):482. https://doi.org/10.1038/nature12077WOS:000319254000046. PMID: 23636333

101. Zhu F, Elnozahy S, Lawlor J, Kuchibhotla KV. The cholinergic basal forebrain provides a parallel channel for state-dependent sensory signaling to auditory cortex. Nat Neurosci. 2023; 26(5):810–9. Epub 20230327. https://doi.org/10.1038/s41593-023-01289-5 PMID: 36973512.

102. Zhou M, Liang F, Xiong XR, Li L, Li H, Xiao Z, et al. Scaling down of balanced excitation and inhibition by active behavioral states in auditory cortex. Nat Neurosci. 2014; 17(6):841–50. Epub 20140420. https://doi.org/10.1038/nn.3701 PMID: 24747575; PubMed Central PMCID: PMC4108079

Chapter 3 Sound Source Tracking: Continuous Movement of External Sounds Guides Behavior and is Encoded by Auditory Cortical Neuronal Ensembles

The ability to process, interpret, and act upon moving objects in the environment is critical for survival and adaptive behavior. It is this ability that allows humans and animals to be able to respond to and track a predator, prey, or, mate. While some work has been done to understand aspects of a moving object, how an object moves throughout the environment and if this influences behavior and neural activity remains to be fully addressed. Specifically in the auditory modality, discrete changes in location and sound intensity have been used to study components of the motion of an object. However, objects continuously move through the environment producing gradual changes in sensory information that distinguish its movement. Furthermore, whether the continuous movement of an object in the environment, or object tracking, is used to drive behaviors and if this is encoded by sensory neural populations has not been extensively addressed. Here, I test the hypothesis that movement information of a continuously moving sound-producing object is encoded by neural populations and used to learn adaptive behaviors. To test this hypothesis, I designed a continuously moving sound-producing object, The Doppler. Using The Doppler, I found that animals can be trained to track and predict the consequences of a continuously moving object across multiple speeds. Using The Doppler in combination with two-photon calcium imaging of local excitatory layer 2/3 auditory cortical neural populations in awake mice, I found that the continuous movement of a sound object had diverse influences on the activity of different neurons, and this was dependent on the location and speed of the object. I found that across slow and fast-moving conditions baseline-subtracted sound-evoked neural responses of auditory cortical neurons were larger in non-movement conditions compared to moving conditions. Interestingly, I found that compared to slow-moving objects, fast moving objects caused an increase in sound-evoked activity at discrete stationary locations, while causing a larger suppression of neural activity during moving states. Underlying this change in sound-evoked activity was a change in the responsiveness of auditory cortical neurons, with greater speed causing a larger proportion of auditory neurons to become

responsive. Together this data suggests that the continuous movement of an object can inform the animal of an upcoming consequence and that auditory neural populations can encode aspects of the object's behavior such as location, movement, and speed.

3.1 Introduction

Throughout the day, animals and humans are continuously processing and integrating sensory information to guide adaptive behavior and survival. In the environment sensory information can come from a static source or a moving source and both are equally important for survival. For example, if someone standing far away were to yell "duck," the listener would respond by getting closer to the ground to avoid being hit by a golf ball. However, if no one yelled, but there was a constant whirring sound getting louder and louder, this too would indicate an approaching object and cause a behavioral response to look around and crouch for cover. Thus, sounds can carry different information about the sound source and the nature of its movements. Animals and humans use sound sources and their movement information to produce sound source tracking behavior, or the ability to continuously monitor changes in location and intensity across time of sound-producing objects for adaptive purposes. A great example to illustrate this comes from the bat's ability to use echolocation, or to use the changes in sound vibrations to be able to identify and locate a moving target. In these studies, the auditory cortical activity of bats is enhanced to be able to identify and track objects as they move (Ghose et al.,2006; Moss et al., 2001; Falk et al., 2014; Triblehorn & Yager, 2005). Understanding how the brain can perceive moving sound sources and track their movement is critical to understanding how animals and humans navigate the real world. Despite this, very little to no work has been done to understand how a continuously moving sound source is processed and used to guide behaviors.

Across the sensory modalities, research has consistently used stationary objects to understand principles of object motion, with most of the work being done in the visual modality. By placing subjects in front of a stationary screen and using changing visual stimuli, scientists recreate external movement using a virtual reality system (Marques et al., 2018; Douglas et al., 2006; Niell & Stryker 2008; Metin et al., 1988; Stirman et al., 2016). By using this method, researchers demonstrated that V1 neurons can encode for the directionality of a moving object by training animals to lick to visual stimuli moving along a nasal or temporal plane. In this study

two-photon calcium imaging of V1 neurons showed that there are distinct subpopulations of neurons that preferentially respond to sounds moving in either the nasal or temporal direction (Marques et al., 2018). By using a closed-loop system tied to the animal's locomotion to make an external virtual reality track move, researchers showed that V1 neurons can encode the speed of the external object (Ayaz et al., 2013; Flossman & Rochefort, 2021). Beyond simply encoding external motion research has shown that visual cortical activity uses motion perception to guide adaptive behaviors, such as tracking the location of prey. When animals were placed in an open field with an appetitive prey positioned in the corner, animals quickly moved towards and captured the prey. However, when visual information was eliminated by placing animals in a dark box, animals took significantly longer to find their prey. Thus, visual information is necessary for animals to guide behavior in a tracking task (Hoy et al., 2016). In the visual modality, the primary visual cortex serves as a center for integrating visual stimuli and their movement information to guide behavioral performance (Hoy et al., 2016; Marques et al., 2018; Douglas et al., 2006; Ayaz et al, 2013; Flossman & Rochefort, 2021; Metin et al., 1988; Stirman et al., 2016). However, in these studies, objects were not moved, and ethologically speaking, there is a difference between approaching a stationary object and an object approaching you, or watching an object move on a stationary screen.

In the auditory modality, less work has been done to understand how the movement of an object is processed and its ability to guide adaptive behaviors. Studies in the auditory modality have focused on understanding sound source localization, or the ability to track changes in an object's spatial position. Work in this field has shown that auditory cortical neurons respond to and encode the sound of an external object as well as the discrete changes in spatial position along the azimuth (Town et al, 2017; King et al., 2007). Furthermore, animals can use this information to predict the movement direction of the object by correctly licking a reward port if a sound moves from left to right, or right to left (Wood et al., 2019). In the context of sound source localization, interaural time differences and interaural level differences heavily shape the ability to locate a sound source respective to the listener's head (Blauert & Lindleman,1986; Bizley et al.,2009; Town et al., 2017; King et al., 2007). When a sound-producing object moves in space, it will generate sound waves that will reach the ears for processing. Because humans and animals have binaural hearing, sounds produced by objects on either side of the observer, will take longer to reach one of the ears producing a slight difference in time and intensity. Using interaural time

and level differences, the movement, or change in spatial location, of an object can be inferred by changes in these cues (Bizley et al.,2009; Town et al., 2017; King et al., 2007). In one of the few studies to continuously move a sound source along the azimuth researchers found that individual neurons responded to the location of a sound source and were modulated by its movement (Ahissar et al., 1992). This suggests that in the AC movement of a sound source may modulate neural activity. However, in this study objects were moved along a horizontal azimuth. By moving along this plane, whether neural activity reflected an encoding of pure movement or a change in interaural level and time differences, cannot be disentangled.

When objects move along the frontal plane, sound intensities become louder or weaker depending on whether a sound is approaching or receding, respectively. In a pioneering study, researchers focused on the changes in sound properties, as opposed to changes in location to understand how the movement of sound objects was perceived. By using looming sounds, a sound quickly increases or decreases intensity to mimic the approaching or receding nature of an external sound source, researchers were able to elicit defensive behaviors to approaching looming sounds. In this study, inactivating the AC impaired the animal's ability to produce defensive behaviors to the looming sound, suggesting that the AC is necessary to understand a sound is moving toward the observer (Li et al., 2021). One limitation of this study is that researchers utilize a stationary speaker at a discrete location to mimic sound source movement. Thus, while reproducing the acoustic effects of movement, no movement of the external object occurred. In the real world, sound source tracking involves the integration of these components, or the continuous monitoring of a sound source as it moves locations, which produces gradual changes in sound intensity, creating a looming or Doppler effect (Li et al., Vessely et al., 2018).

To understand how a continuously moving object is processed I created a novel behavioral setup, The Doppler, that allows an object to continuously move while generating sensory stimuli. Because this study aims to understand if the motion of an object can guide behavior, the Doppler moves along the frontal axis to control for and eliminate the use of interaural and time differences by the auditory cortex. However spectral cues, or changes in sound perception due to changes in sound elevation or front-back distance remained. While spectral cues can be used to determine changes in position along the azimuth, they are intrinsically necessary to detect changes in position along the frontal plane (Ito et al., 2020). By allowing the object to move across space and time without any binaural cues, this study is a first

of its kind, aiming to understand if and how animals use the motion of an external object to guide behavior as it moves and changes distance along the frontal plane.

In this study, I use a novel sound source tracking task to test the hypothesis that mice use the movement of an external sound source to guide behavior. To do this I train mice to understand that an approaching sound source signals an incoming reward. Furthermore, by combining The Doppler, with two-photon calcium imaging I test whether auditory cortical neuronal ensembles actively encode information about the object's movement. Specifically, I test whether auditory cortical neuronal ensembles can encode the location, movement state, direction, and speed of an externally moving sound source. All together this study tests whether the external motion of an object is used to guide behavior and the neural properties that are encoded alongside this phenomenon.

3.2 Continuously moving sound sources guide learning and adaptive behavior

Previous studies have shown that changes in the spatial location of a sound-producing object can be used to guide behavior, however, have not addressed whether a continuously moving sound source can do the same (Wood et al., 2019). To determine if a continuously moving sound source can inform an animal about the consequences of movement, I developed a novel behavioral recording setup to measure sound-guided reward predictive licking during conditions in which a sound source was approaching (Figure3.1A). Male and female mice (N=11) were first implanted with titanium headbars and allowed to recover. Mice were then placed on water restriction and trained on an appetitive trace conditioning task. Head-fixed mice were placed on top of a rotatable plate, in front of a visual block, reward delivery system, and a continuously moving speaker on a linear actuator(Figure3.1A). Using an Arduino system to control the movement of the linear actuator, training trials were initiated by the continuous movement of the speaker from the out-most position, "Far", to in front of the visual block, "Close". During training trials, constant 8 kHz tones (125ms duration) were emitted by the moving speaker for the entirety of its movement path from "far" to "close". Once the speaker reached the "close" position a delay period was initiated (1s duration), immediately followed by a water reward ("delay" Fig 3.1A). During daily recording sessions, each animal was exposed to 3 blocks of (n=20) training trials. Each training block was varied in how fast the moving speaking approached and thus the total time taken in traveling the same distance, slow (12s), mid

(8s), and fast (6s) (Fig 3.1A). For each training block a Predictive Lick Index (PLI) was determined by comparing the lick rate during the predictive lick window, 1s before reaching "close" until the end of the delay, to the baseline licking of each animal, which consisted of 2s following movement initiation ("predictive lick" "baseline lick" Fig3.1A, B). Mice were trained until they learned to predict a reward to an approaching sound source. Learning was quantified as a significant increase in PLI, or amount of licking during the predictive window compared to the baseline window, across speed conditions. Animals were considered trained after two consecutive days of PLI scores reflecting a significant difference between, predictive and baseline licking (Fig 3.1B). As the total time traveled varied, but not the total distance traveled, our results show that across learning of an approaching sound source, the lick rate of mice significantly increases (Fig 3.1C). Using the PLI as an index of learning, animals show a significantly higher score in trained conditions compared to initial baseline conditions (Fig 3.1D). These animals also demonstrate a global increase in licking. Overall, these results demonstrate that mice can be trained to predict the expectancy of an upcoming reward based on the movement of an object approaching them. In this study design, binaural cues were controlled for by moving along the frontal plane, however, other forms of learning may not have been controlled for. To test the hypothesis that the movement of an object is what is driving behavioral learning, I designed a control behavioral paradigm to for location, timing, and sound intensity cues.

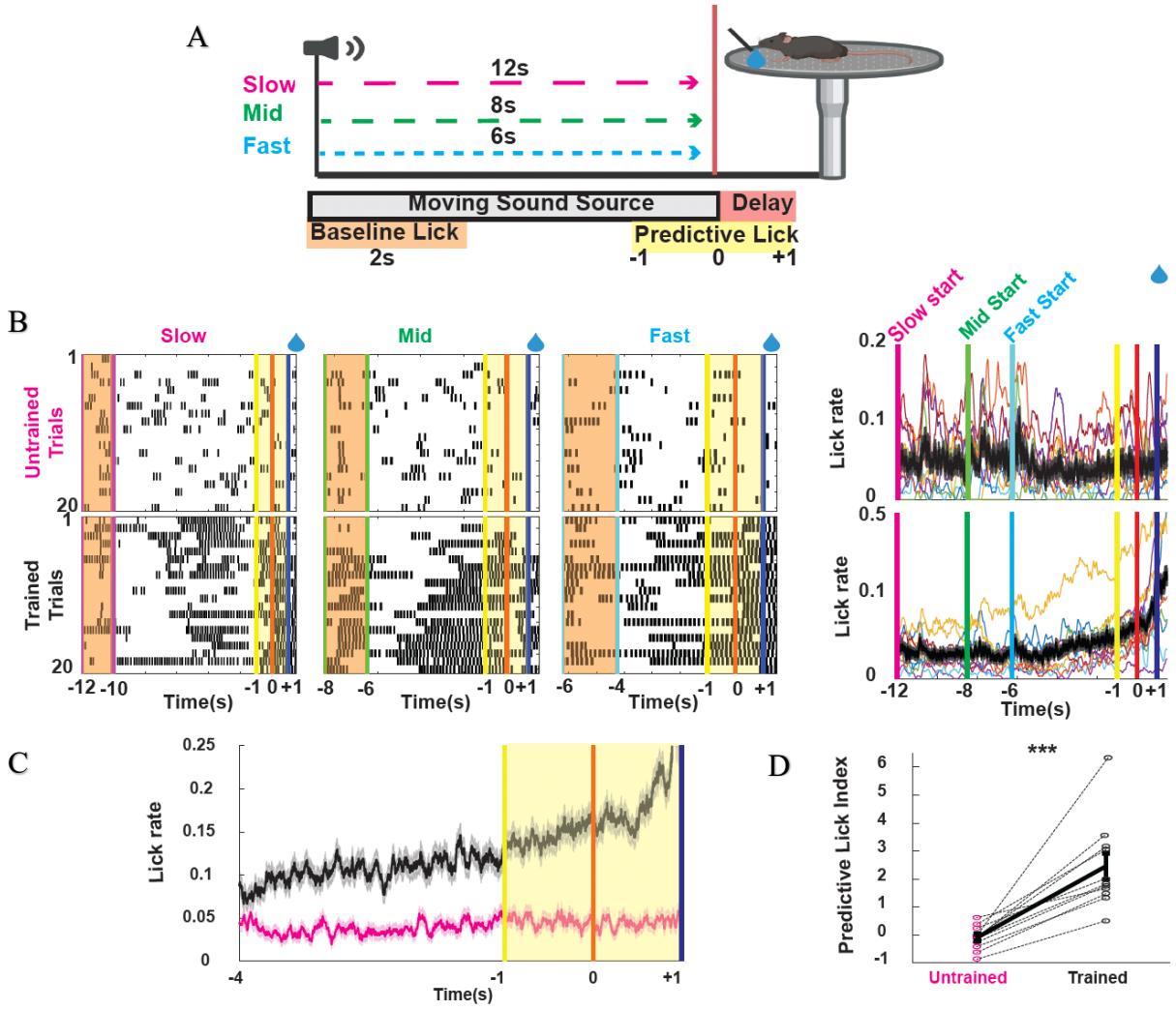


Figure 3.1 Continuously moving sound sources guide learning and behavior. (A) Illustration of the behavioral set up for predictive licking to a moving sound source. (B) Left: Peri-sound lick rasters of an example behavioral session from baseline and trained animals. Licking in the yellow shaded area 1s before and 1s after movement and sound cessation and before reward delivery represents prediction of an upcoming reward. Licking in the orange shaded area 2s after movement and sound initiation represents baseline licking. Right: Peri sound lick histogram across animals and conditions performing a behavioral task. (C) Peri sound lick histogram across animals and conditions performing a behavioral task focused on the last 4s of movement to highlight an increase in predictive licking as a sound source approached. (D) There was a significant increase in predictive lick index following training (P=0.0001, signed rank test) Error bars represent meant +/- SEM across animals. Lines connecting pink and black dots represent data from the same animal across learning.

3.3 Sound source tracking is not due to changes in location, intensity, or timing

Studies have shown that animals can use changes in location, intensity, and timing to learn a behavioral task (Li et al., 2021; Town et al, 2017; Wood et al., 2019; King et al., 2007;

Cook et al, 2022). Because the ability to track a continuously moving sound source requires processing and integration of all three components whether learning is due to one of these components was not clear. To address these confounds I tested whether the movement of an approaching sound source signaled an upcoming reward and not one of these confounding variables. To do this, following training, animals underwent a testing phase. In this testing phase, mice were exposed to three conditions, Approaching, Stationary-Far, and Stationary-Close (Fig 3.2A). In the Approaching condition, which consisted of 60% of all daily trials, the speaker approached the animal at a fixed speed of 8s per travel time followed by a 1s delay and subsequent water reward. PLI scores were calculated by comparing predictive licking to baseline licking, as noted above (Fig 3.2A top panel). In the Stationary-Far condition, the speaker remained stationary in the farthest position away from the animal, while continuously emitting the same number of sounds for the same duration as in the Approaching condition. This design tested for a timing mechanism of learning. Consisting of 20% of all daily trials, Stationary-Far PLI scores were calculated by comparing baseline lick as the first 2s of each trial, or what would've been movement initiation, to the last 2 s of each trial before what would've been a water reward (Fig 3.2A middle panel). Lastly, in the Stationary-Close condition, 20% of trials began with the speaker moving to the closest position directly in front of the animal, without any sound being produced. Once at the inmost position, the speaker remained stationary and emitted the same number of sounds for the same duration as the Approaching condition. This design tested for location and sound intensity as mechanisms of learning. Stationary-Close PLI scores were calculated by comparing baseline lick as the first 2s of sound initiation, or what would've been movement initiation, to the last 2 s of each trial before what would've been a water reward (Fig 3.2A bottom panel). Our results show that mice selectively increase predictive licking only in the Approaching conditions as compared to the Stationary-Far and Stationary-Close conditions (Fig 3.2 B). By comparing PLI scores across conditions, our analysis show that animals have significantly higher predictive licking in the Approaching condition as compared to the Stationary-Far and Stationary-Close conditions. There was no significant difference in PLI between the Stationary-Far and Stationary-Close conditions (Fig 3.2C). Overall, these results suggest that mice tracked the movement of an object to predict an upcoming reward. Tracking of the sound object was independent of timing or changes in intensity of spatial location. To test whether this information is also encoded by auditory neural populations, I conducted two-photon

calcium imaging of animals while exposing them to a continuously moving sound source. To better understand how external sound movement is encoded, I decided to record neural activity under two different speeds (Fig 3.3A). Given that fast increases in sound intensity or looming sounds, produce defensive behaviors mediated by auditory cortical activity, whether gradually increasing sounds are encoded in the same way remains to be addressed. I hypothesize that sound objects moving at faster speeds, being associated with more defensive and dynamic acoustic changes, will be differentially encoded than objects moving at slower speeds by layer 2/3 AC neurons.

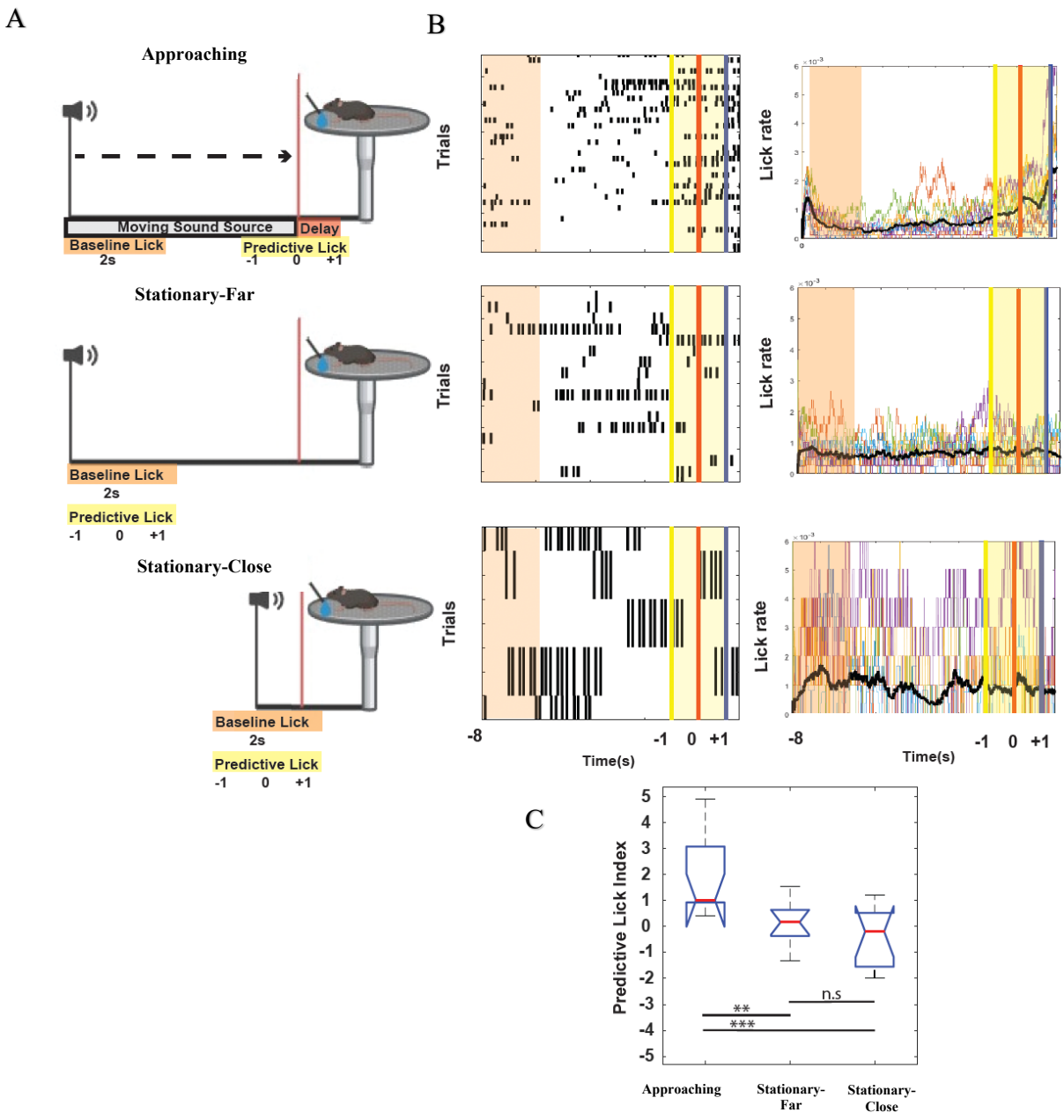


Figure 3.2 Sound source tracking is not due to changes in location, intensity, or timing. (A) Illustration of testing phase conditions. Top: Approaching sound source. Middle: Stationary-Far condition. Bottom: Stationary-Close condition. (B) Left: Peri-sound lick raster of an example behavioral testing session from a trained animal across testing phase conditions. Licking in the yellow shaded area represents 1s before and after movement cessation and before reward. In the stationary conditions, the yellow shaded area represents the same time points as moving conditions. The Orange shaded area represents the first 2s of movement or non-movement trials and sound initiation. Right: Peri-sound lick histogram across animals by testing phase conditions when performing a behavioral task (C) There was a significant difference between approaching sound source and stationary trials $F(2,30)=10.94$, $P=0.0003$. Multiple comparison analysis revealed a significant difference of Approaching and Stationary-Far ($P=0.012$) and Stationary-Close ($P=0.000$) but not between the Stationary-Far and Stationary-Close ($P=0.293$). Box Plot show the mean Predictive lick index as the red line and the lower and upper limits for 95% confidence intervals.

3.4 Acoustic properties of a continuously moving sound source during two-photon calcium imaging

Having established that a continuously moving sound source can be used to train animals to understand the movement of an object can have consequences, I aimed to test whether auditory cortical neuronal ensembles themselves encoded information about a continuously moving object. To do this, I modified The Doppler to interact with a two-photon calcium imaging microscope (Fig 3.3 A). Before examining the auditory cortical neural encoding of a moving sound source, I tested the acoustic intensity and frequency components of the setup to determine how a continuously moving sound source would influence auditory neural activity based on changes in acoustic properties. This was done by placing a microphone directly under the animal (Fig 3.3 A).

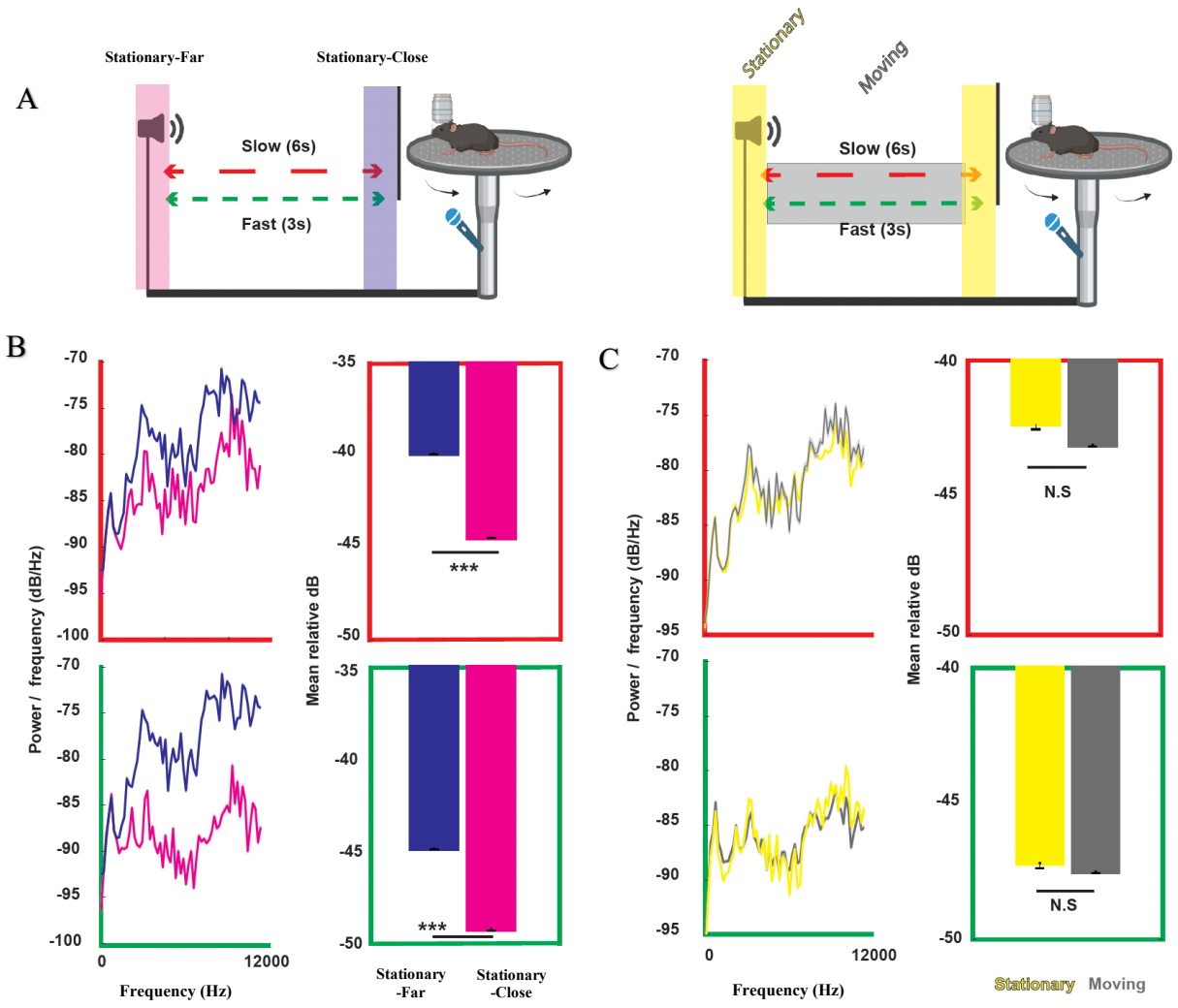


Figure 3.3 Acoustic properties of a continuously moving sound source during two-photon calcium imaging (A) Illustration of the behavioral setup under a two photon (B) Left: Power spectrums of a moving sound source across location (Stationary-Far-magenta, Stationary-Close-purple), and speed conditions (Red-slow, Green-fast). Right: There was a significant difference in sound intensity (dB) across location and speed conditions (Slow P=0.000, Fast P=0.000, signed rank test). Error bars represent mean +/- SEM across all trials. (C) Left: Power spectrums of a moving sound source across moving trials (Stationary-yellow, Moving-grey), and speed conditions (Red-slow, Green-fast). Left: There was no significant difference in sound intensity (dB) across moving state and speed condition (Slow P=0.304, Fast P=0.899, signed rank test). Error bars represent mean +/- SEM across all trials.

It is widely understood that auditory cortical neurons can encode changes in sound intensity that can be increased or decreased by location (Soeta & Nakagawa, 2012; Takahashi & Kaga, 2004; Schreiner & Malone, 2015). In this design, the Doppler moves from Stationary-Far to the Stationary-Close position while continuously emitting a 125ms broadband noise pulse and therefore I hypothesize a difference in the sound intensities across locations, but not frequency components as the sound remains the same. To address this, I tested if there were changes in frequency components between locations (Fig 3.3A left). Our results showed that there was no

discernable difference in the frequency components of the broadband noise between Stationary-Far and Stationary-Close locations (Fig 3.3B Left top, bottom). As expected, there was a significant decibel change between locations reflecting the gradual increase and decrease in sound intensity caused by an object moving closer and farther away (M=4.5dB difference Slow, M=4.3dB difference Fast) (Fig 3.3B Right top bottom). Overall, sound frequency components did not differ between speed conditions, sound intensity gradually changed across locations and was overall larger in the Stationary-Close conditions.

Next, I tested whether the moving sound source produced a discernable difference in frequency components and sound intensities as a function of being in motion compared to periods of being in a discrete stationary location (Fig 3.3A Right). Our results showed that when the object was moving there was no difference in the frequency components of the sound source relative to when the sound source was at discrete stationary positions (Fig 3.3C Left top, bottom). Furthermore, there was no significant difference in the sound intensities between stationary and moving conditions (M=0.75dB difference Slow, M=0.31dB difference Fast) (Fig 3.3C Right top, bottom).

Overall, these results demonstrate continuously moving sound source will produce gradual changes in sound intensity across locations that replicate how an object's movement would influence sound intensity changes. Furthermore, the average sound intensities of a sound produced during movement were not different from sounds in stationary positions. Next, using two-photon calcium imaging I examined how the movement of a sound source was encoded by auditory neural populations.

3.5 Auditory cortical neuronal ensembles encode for the location of a continuously moving sound source

In the previous section, I demonstrated that when an external object continuously moves from the Stationary-Far position to the Stationary-Close position, it causes a gradual change in sound intensity. Because auditory cortical neurons are known to encode sound intensity information, I aimed to replicate this finding in a moving sound source (Soeta & Nakagawa, 2012; Takahashi & Kaga, 2004; Schreiner & Malone, 2015). To do this I carried out two-photon calcium imaging of head-fixed Thy-1GCamp6f mice (N=5) that were free to stand or run on a rotatable plate, while a moving sound source continuously moved from the Far to Close position,

across slow and fast movement speeds (Fig 3.4 A). I first examined how location modulated the responses of neurons to broadband noise (BBN) bursts in (n=962) primary auditory cortical neurons. In keeping with most previous studies, I examined the baseline-subtracted responses, which are defined as the difference between the activity evoked by the sound and the activity immediately preceding the sound. Specifically, to test for discrete location encoding I examined sound-evoked activity only at locations when The Doppler was temporarily immobile at Stationary-Far (blue) and Stationary-Close (magenta) positions, before beginning to move again (Fig 3.4 A). Additionally, because animals were free to move and previous research shows self-locomotion influences neural activity, all trials in which the animal was moving were not used in this analysis (Vivaldo et al., 2023). Replicating previous findings in the literature, auditory cortical neurons had a diverse range of sound-evoked responses to different sound intensities caused by changes in location, including enhancement, invariance, and suppression (3.4B). Across all neurons that exhibited significant BBN-evoked responses (n=327) at discrete locations during slow-moving conditions to changes in Stationary-Far and Stationary-Close locations, the population-average responses were significantly greater in the Stationary-Far condition, inconsistent with our previous acoustic analysis and studies demonstrating more neural activity is recorded during louder conditions (Fig3.4C top). Additionally, I examined all neurons that exhibited significant BBN-evoked responses (n=296) at discrete locations in fast-moving conditions to changes in Stationary-Far and Stationary-Close locations. These results showed that the population-average responses of neurons in fast moving conditions were greater in the Stationary-Far conditions as well (Fig 3.4C middle). To examine how the speed of an object before reaching a stationary position influenced auditory processing, I examined the average difference of significant BBN-evoked responses across speed conditions at the Stationary-Far and Stationary-Close positions. My results show that there was a significant difference in the relative change in neural activity from Stationary-Far to Stationary-Close locations based on speed. These results showed that slower-moving conditions have a greater effect on the difference in neural activity between discrete locations and that this difference is smaller when objects are moving faster (Fig 3.4C bottom). Interestingly, how an object moved prior to reaching a stationary position influenced the overall population average responses of neurons to different locations. This difference was most pronounced for neural activity in the Stationary-Close position, such that objects moving at a faster speed before reaching a specific location

enhanced population-average neural activity to sounds in the Stationary-Close position but had little effect on population averages to sound-evoked activity in the Stationary-Far position (Fig 3.4C top middle). To further explore the nature of this finding I examined the distribution of sound-evoked responses to Stationary-Far and Stationary-Close conditions when objects gradually approached these distinct locations. In the slow-moving conditions, there was a heterogenous spread of neurons preferring a specific location (327/962 total, 34% of which sound-evoked magnitudes of 42/327, 46/327, and 239/327 were individually significantly Stationary-Close preferring, Stationary-Far preferring, and not showing a significant difference, respectively) (Fig3.4D top). Similarly in the fast-moving conditions, there was a heterogenous spread of neurons preferring a specific location (296/962, 28% of which sound-evoked magnitudes of 21/296, 40/296, and 208/296 were individually significantly Stationary-Close preferring, Stationary-Far preferring, and not showing a significant difference, respectively) (Fig 3.4D bottom). Overall, more neurons were significantly BBN-evoked responsive in the slower condition compared to faster condition (n=327 slow, n=296 fast). Interestingly, of these neurons, the proportion of neurons that preferred the Stationary-Far location remained relatively the same across speeds, but there was an increase in the neurons that preferred the Stationary-Close condition in the faster moving condition, in agreement with an increase in population average neural activity to this condition at this speed.

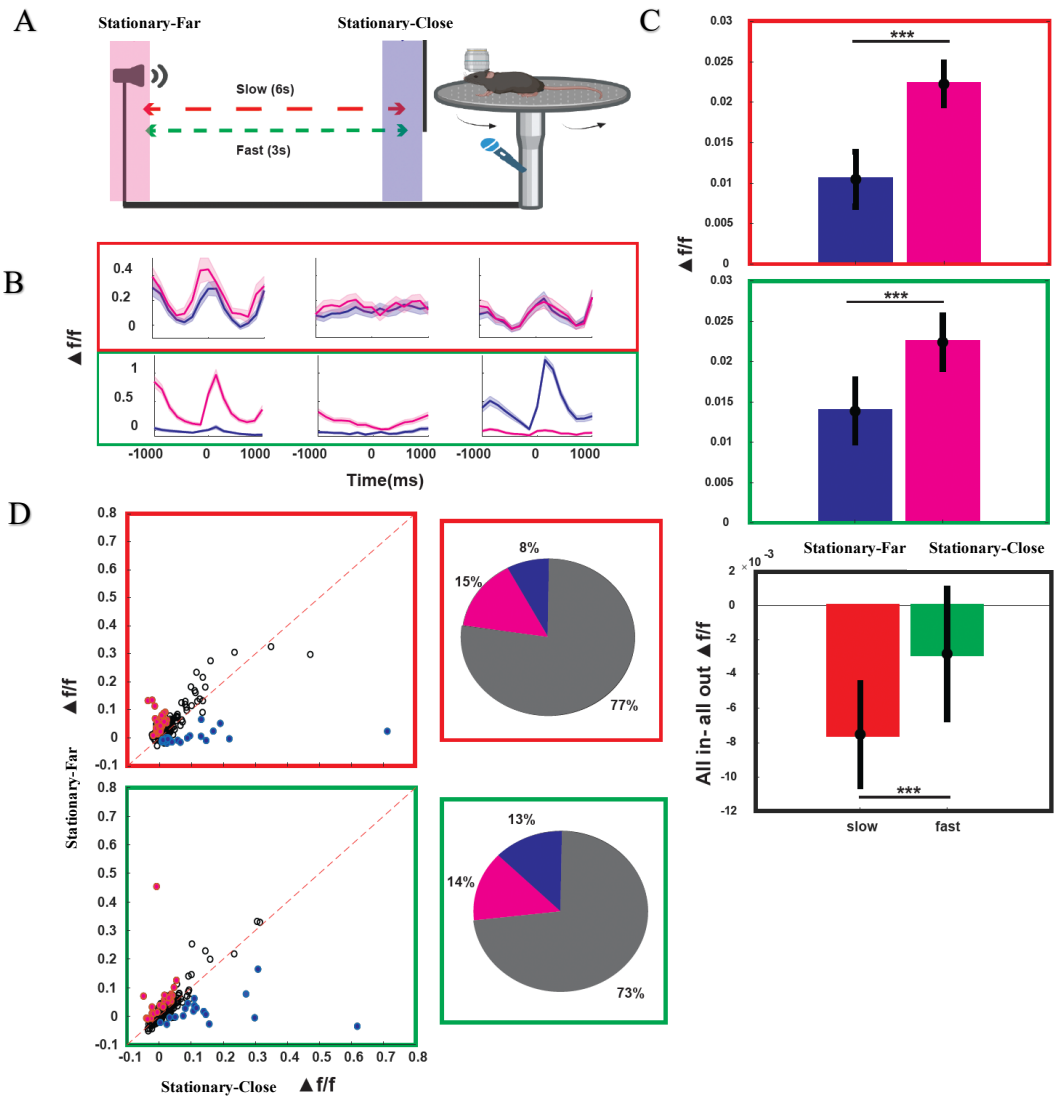


Figure 3.4 Auditory cortical neuronal ensembles encode for the location of a continuously moving sound source (A) Illustration of the behavioral setup for recording a continuously moving sound source under two-photon calcium imaging. (B) Sound-triggered PSTHs from 6 example neurons. Sound presentations under slow conditions, red box. Sound presentations in the fast condition, green box. Sound presentation in the Stationary-Far condition, magenta. Sound presentation in the Stationary-Close, purple. Moving sound sources showed a heterogeneous spread of sound-evoked responses at different locations including invariance (Red box neuron 2&3 Purple box neuron2) Stationary-Far preferring (Red box neuron 1 Purple box neuron 1) and Stationary-Close preferring (Blue box neuron3). (C) Top: There was significantly more sound-evoked activity to Stationary-Far positions compared to Stationary-Close, in slow moving conditions (P=0.002, signed rank test). Error bars represent the median +/- SEM across all trials. Middle: There was significantly more sound-evoked activity to Stationary-Far-positions compared to Stationary-Close, in fast-moving conditions (P=0.000, signed rank test). Error bars represent the median +/- SEM across all trials. Bottom: The difference in sound-evoked activity across locations was significantly larger in the slow-moving conditions than fast moving conditions (P=0.000, signed rank test). Error bars represent the median +/- SEM across all trials. (D) Top left: Sound evoked responses in Stationary-Far and Stationary-Close locations across all BBN responsive neurons during slow moving sessions. Magenta and purple dots represent neurons that individually exhibited a significantly stronger response to Stationary-Far and Stationary-Close respectively. Black dots did not exhibit a significant difference. Top right: Proportion of BBN-responsive neurons showing a significantly stronger response to Stationary-Far and Stationary-Close respectively during slow moving conditions. Bottom left: Sound evoked responses in Stationary-Far and Stationary-Close locations across all BBN responsive neurons during fast-moving sessions. Magenta and purple dots represent neurons that individually exhibited a significantly stronger response to Stationary-Far and Stationary-Close respectively. Black dots did not exhibit a significant

difference. Bottom right: Proportion of BBN-responsive neurons showing a significantly stronger response to Stationary-Far and Stationary-Close respectively during fast-moving conditions.

Overall, these results suggest that auditory cortical neuronal populations can encode for the location of a continuously moving object and this does not match onto the intensity of the object. Interestingly, slower-moving objects produce a large difference in activity due to differences in location. Faster moving objects overall enhance population level activity while simultaneously increasing the overall number of location-selective neurons and more so to the all-in condition, despite an overall weaker sound intensity. Because this analysis examined how the movement of an object influenced neural activity at discrete stationary locations, I next examined how neural activity during continuous movement modulated auditory cortical activity.

3.6 Auditory cortical neuronal ensembles encode the movement state and speed of an external sound source

Previous research has attempted to replicate aspects of a moving sound source while recording neural activity, but no study to my knowledge has recorded neural activity under conditions in which an object is continuously moving across space and time in the frontal plane (Ahissar et al., 1992). To test the hypothesis that auditory cortical neurons encode the movement state and speed of the object, neuronal activity was recorded in sound-source stationary (yellow) and moving conditions (grey) (Figure 3.5 A). Auditory cortical neurons exhibited a diverse range of sound-evoked responses to BBN sounds during stationary and moving conditions, including enhancement, invariance, and suppression (3.5 B). Across all neurons that exhibited significant BBN-evoked responses (n=327) in slow speed conditions to changes in stationary or moving conditions, the population-average responses were significantly greater in the stationary positions compared to moving states (Fig3.5C top). Across all neurons that exhibited significant BBN-evoked responses (n=296) in fast speed conditions to changes stationary or moving conditions, the population-average responses were significantly greater in the stationary positions compared to moving states (Fig 3.5 C middle). To examine how the speed of the moving sound source influenced movement encoding, I examined the average difference of significant BBN-evoked responses across speed conditions to the stationary and moving conditions. Results showed that there was a significant difference in the relative change in neural activity caused by movement speed. These results showed that faster moving conditions have a

greater effect on the difference in neural activity between movement and stationary conditions, causing a significantly larger reduction in sound-evoked activity. (Fig 3.5C bottom). To further explore the nature of this change I examined how sound-evoked responsiveness changed across movement-state by the speed of moving object. In the slow-moving conditions, there was a heterogeneous spread of neurons preferring a specific movement state (327/962 total, 34% of which sound-evoked magnitudes of 56/327, 12/327, and 259/327 were individually significantly stationary preferring, movement preferring, and not showing a significant difference, respectively) (Fig3.5D top). Similarly in the fast-moving conditions, there was a heterogenous spread of neurons preferring a specific movement state (296/962, 28% of which sound-evoked magnitudes of 127/296, 62/296, and 80/296 were individually significantly stationary preferring, movement preferring, and not showing a significant difference, respectively) (Fig 3.5D bottom). Interestingly, more neurons were significantly BBN-evoked responsive to a movement state in the faster condition compared to the slower condition. Thus, despite overall movement-induced suppression, and an increase in speed enhancing this reduction in population-average BBN-evoked neural activity, a much larger proportion of neurons encoded for the movement state of the object as speed increased. This proportion however was still smaller than stationary preferring neurons, which also saw an increase in responsiveness proportion across speed conditions. Conversely, neurons that were invariant to a movement state but sound responsive saw a drastic reduction in their proportion, switching to encoding for a movement state (Fig 3.5D).

Overall, these results suggest that auditory cortical neuronal ensembles can encode the movement state of an external object. Furthermore, as objects move faster in the environment, population-level sound-evoked activity in the AC is suppressed. Underlying this overall external-movement-induced suppression is a significant increase in the number of neurons that encode for the movement state of the object, with fewer neurons becoming purely sound-responsive. Thus, auditory cortical populations integrate the movement state of the external object with their neural activity. Our results have shown that auditory cortical neurons encode for the location and movement-state of an object during locomotion, and thus I next test whether neurons encode for the integration of these two pieces of information or the direction of movement.

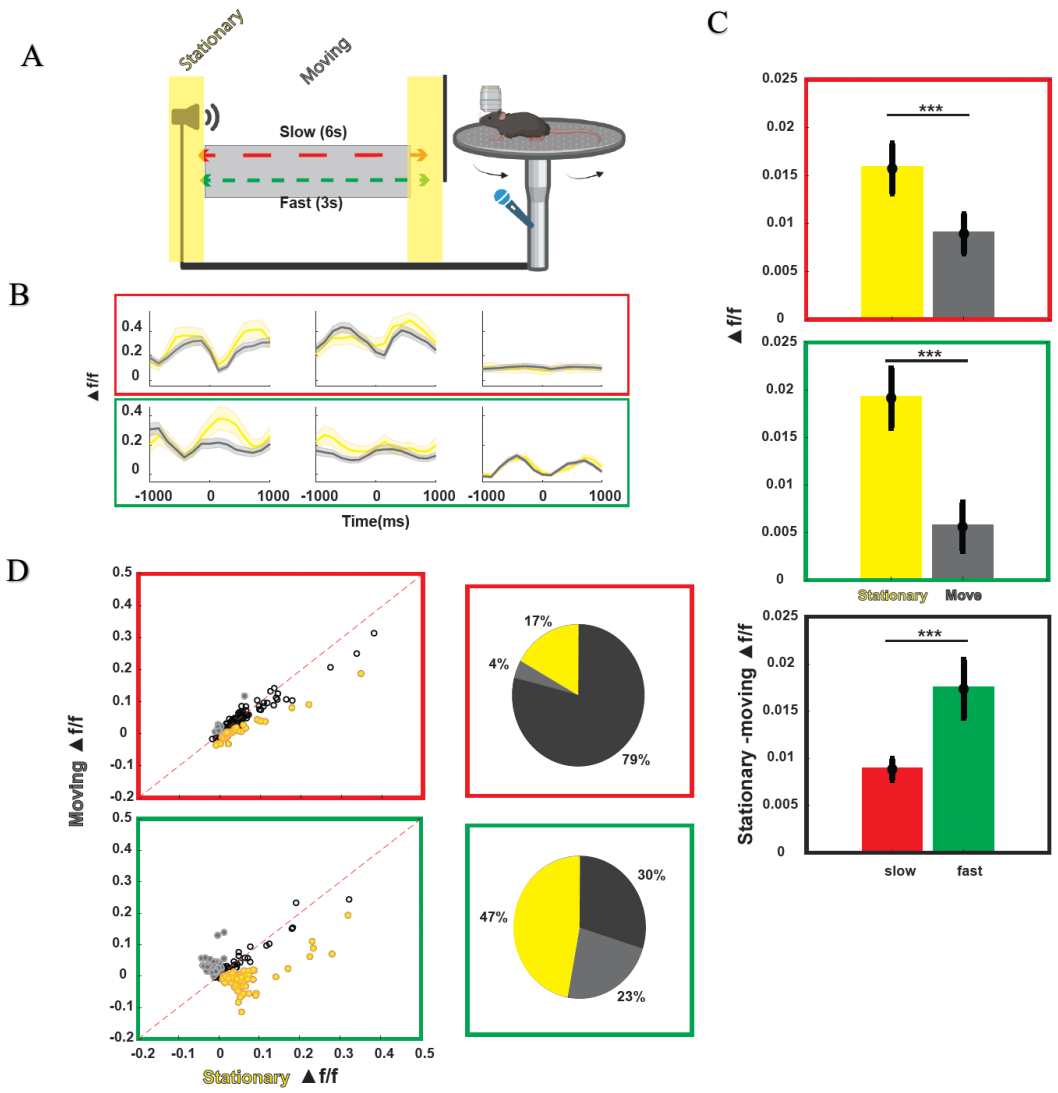


Figure 3.5 Auditory cortical neuronal ensembles encode for the movement state and speed of an external sound source (A) Illustration of the behavioral setup for recording a continuously moving sound source under two-photon calcium imaging. (B) Sound-triggered PSTHs from 6 example neurons. Sound presentations under slow conditions are in the red box, and sound presentations under fast condition are in the green box. Sound presentation in the stationary condition is colored in yellow, and sound presentation in the moving condition is colored in grey. Moving sound sources showed a heterogeneous spread of sound-evoked responses at different movement states including invariance (Yellow box neuron 3 Green box neuron 2) stationary preferring (Yellow box neuron 1& 2 Green box neuron 1) and moving preferring (Green box neuron 3). (C) Top: There was significantly more sound-evoked activity to stationary compared movement, in slow-moving conditions (P=0.000, signed rank test). Error bars represent the median +/- SEM across all trials. Middle: There was significantly more sound-evoked activity to stationary positions compared to movement, in fast moving conditions (P=0.000, signed rank test). Error bars represent the median +/- SEM across all trials. Bottom: The difference in sound-evoked activity across movement states was significantly larger in the fast moving conditions than the slow moving conditions (P=0.000, signed rank test). Error bars represent the median +/- SEM across all trials. (D) Top left: Sound evoked responses in stationary and movement-state across all BBN responsive neurons during slow-moving sessions. Yellow and grey dots represent neurons that individually exhibited a significantly stronger response to stationary and movement respectively. Black dots did not exhibit a significant difference. Top right: Proportion of BBN-responsive neurons showing a significantly stronger response to stationary and moving state respectively during slow-moving conditions. Bottom left: Sound evoked responses to stationary and movement-state across all BBN responsive neurons during fast moving sessions. yellow and grey dots represent neurons that individually exhibited a significantly stronger response to stationary and movement conditions, respectively. Black dots did not exhibit a significant difference. Bottom right: Proportion of BBN-responsive neurons showing a significantly stronger response to stationary and movement respectively during fast-moving conditions.

3.7 Auditory cortical neuronal ensembles encode the direction of a moving object

Previous work has shown that visual cortical neurons can encode for and are selective to the direction of an object and that AC neurons might be doing the same along the horizontal azimuth (Marques, et a.,2018; Ahissar et al., 1992). In my previous results, I demonstrated that AC neurons can encode for the location and movement state of an object and that the speed of an object influences these effects. Here I test whether these same neurons encode for and are selective to the direction of a continuously moving object, which is an integration of changes and in location and movement-state information. To do this auditory cortical neural activity of BBN-evoked sound responses were recorded in moving-in (blue) and moving-out (conditions) (Fig 3.6 A). Auditory cortical neurons exhibited a diverse range of sound-evoked responses to BBN sounds during stationary and moving conditions, including enhancement, invariance, and suppression (Fig3.6 B). Across all neurons that exhibited significant BBN-evoked responses in slow speed conditions to moving-in and moving-out conditions, the population-average responses were significantly greater when objects were moving in (Fig3.6C top). As expected, faster-moving objects caused a suppression of auditory cortical activity, but had no overall effect on population-level direction encoding (Fig3.6C middle). To further test this effect, I calculated the average difference of significant BBN-evoked responses across speed conditions to moving in and moving conditions and found that there was a significant difference in the relative change in neural activity caused by movement speed on direction encoding. These results showed that slower moving conditions have a greater effect on the difference in neural activity between moving-in and moving out conditions, causing a significantly larger reduction in sound-evoked from moving-in and moving-out activity (Fig3.6C bottom). To test if this population-level encoding of directionality was due to distinct auditory cortical populations, I examined how BBN sound-responsive neurons selectively preferred a moving-in or moving-out condition. This analysis showed that there was a heterogenous spread of neurons preferring a specific direction, with only a significant but small population of neurons encoding for moving-in direction (327/962 total, 34% of which sound-evoked magnitudes of 20/327, 8/327, and 299/327 were individually significantly moving-in preferring, moving-out preferring, and not showing a significant difference, respectively) (Fig3.6D top). Unexpectedly, in conditions where the object moved faster, significant populations of direction encoding neurons emerged (269/962, 28% of which sound-evoked magnitudes of 25/269, 26/269, and 218/269 were individually significantly

moving-in preferring, moving-out preferring, and not showing a significant difference, respectively) (Fig3.6D bottom). Thus, despite an overall reduction of sound-evoked activity and elimination of direction encoding at the population level by speed, more individual neurons significantly encoded for a specific direction.

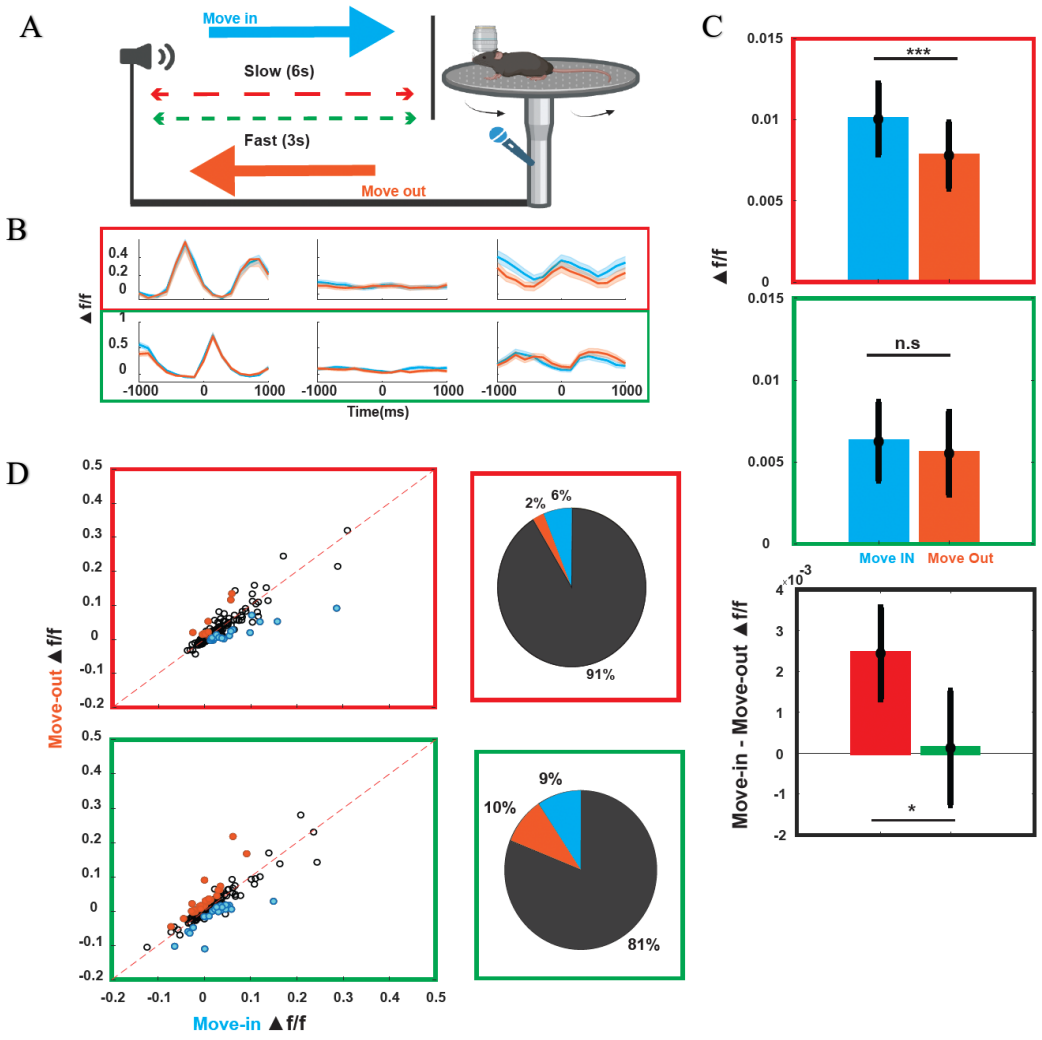


Figure 3.6 Auditory cortical neuronal ensembles encode the direction of a moving object (A) Illustration of the behavioral setup for recording a continuously moving sound source under two-photon calcium imaging. (B) Sound-triggered PSTHs from 6 example neurons. Sound presentation under slow conditions are in the red box, and sound presentations in the fast condition are in the green box. Sound presentation in the Moving-in condition are colored in cyan, and sound presentation in the moving-out condition are in orange. Moving sound sources showed a heterogeneous spread of sound-evoked responses at different movement-states including invariance (Cyan box neuron1& 2, Orange box neuron& 2) move-in preferring (Cyan box neuron 3) and move-out (Orange box neuron 3). (C) Top: There was significantly more sound-evoked activity to move in compared move out, in slow moving conditions (P=0.001, signed rank test). Error bars represent the median +/- SEM across all trials. Middle: There was no significant different in sound-evoked activity to move-in and move-out, in fast moving conditions (P=0.75, signed rank test). Error bars represent the median +/- SEM across all trials. Bottom: The difference in sound-evoked activity across direction was significantly larger in the slow moving conditions than the fast moving conditions (P=0.027, signed rank test). Error bars represent the median +/- SEM across all trial. (D) Top left: Sound evoked responses to move-in and move-out directions across all BBN responsive neurons during slow moving sessions. Cyan and orange dots represent neurons that individually exhibited a significantly stronger response to move-in and move out respectively. Black dots did not exhibit a

significant difference. Top right: Proportion of BBN-responsive neurons showing a significantly stronger response to move-in and move out direction respectively during slow moving conditions. Bottom left: Sound evoked responses to move-in and move-out directions across all BBN responsive neurons during fast moving sessions. Cyan and orange dots represent neurons that individually exhibited a significantly stronger response to move-in and move-out conditions, respectively. Black dots did not exhibit a significant difference. Bottom right: Proportion of BBN-responsive neurons showing a significantly stronger response to move-in and move-out directions respectively during fast moving conditions.

Overall, these results demonstrate that auditory cortical neuronal ensembles can encode the direction of a moving object and that the speed of the object modulates if this is encoded at the individual or population-level analysis of neural activity. In slower conditions, directionality is encoded for population-level sound evoked activity, with a small yet significant proportion of neurons encoding for objects moving in, or closer, but not for moving out. In faster conditions, directionality is not encoded at the population level sound evoked activity as moving in and moving out conditions produce similar levels of neural activity. However, underlying this net null effect is a significant increase and emergence of distinct subpopulations of neurons that encode for the directionality of a moving object, greater for both moving-in and moving-out compared to slow-moving conditions.

3.8 Discussion

In this study, I tested the hypothesis that the external motion of a sound-producing object guides and informs adaptive behavior and information about the object is encoded by neural populations. To do this I created a novel behavioral recording system, The Doppler, that allows an object to continuously move, while allowing for behavioral training or neural recordings. Using, the Doppler, this study demonstrated that animals could learn that a continuously approaching sound source signals an upcoming reward. Furthermore, using a series of behavioral controls this study demonstrated that mice used the continuous movement of a sound source to predict an upcoming reward and confounding cues such as an increase in sound intensity, change in location, or time that an object emits sounds. Using two-photon calcium imaging of L2/3 auditory cortical neurons, this study showed that neural populations encoded aspects of movement, such as location and sound intensity changes, as well as the continuous movement state of an object and that this is modulated by the speed of the object. Additionally, this study showed that auditory cortical neurons can integrate aspects of motion to be able to encode for directionality of a moving sound source. Furthermore, this study demonstrated that during bouts of movement, sound-evoked responses to moving sounds were significantly reduced as

compared to stationary conditions. Finally, the faster the object moved the larger the suppression of sound-evoked neural activity. Interestingly, as objects move faster, more individual neurons become selectively responsive for location, movement, and directionality.

Previous work has found that auditory cortical activity is necessary to drive adaptive behaviors that signal the movement of an external object (Li et al., 2021). Namely, by increasing sound intensity at a stationary location to replicate an object approaching, mice produce evasive behaviors suggesting an understanding that a potential threat is approaching and that the AC is necessary for this. Because, large, unexpected sounds can produce an acoustic startle reflex, whether mice understand that an object is approaching, or whether they are responding to this loud burst of noise cannot be separated (Yeomans & Frankland, 1995). Because mice in our study selectively increased predictive behavior to conditions in which the sound-source continuously approached and not to control conditions in which the sound intensity was increased by location we provide evidence that mice understand that a continuously approaching movement object is perceptually different than a large deflection in sound intensity. Interestingly, mice in our study showed some startle-like behavior as licking slightly increased at sound and movement onset, but this quickly was suppressed and emerged to a significantly higher degree the closer the object approached (Fig 3.2). Previous work has established that the auditory cortex is necessary for behavioral, context, and self-locomotive state dependent sound processing to guide learning and production of adaptive behavior (Cohen et al., 2011; David et al., 2012; Fritz et al., 2010; Jaramillo & Zador, 2011; Kuchibhotla et al., 2017; McGinley et al., 2015b; Nelken, 2014; Rodgers & DeWeese, 2014; Saderi et al., 2021; Ulanovsky et al., 2003; Xiong et al., 2015; Znamenskiy & Zador, 2013). Thus, I hypothesize that external-movement state dependent sound processing is also auditory cortex mediated, especially given its necessary role in the production of adaptive behaviors to the perception of a replication of a moving sound. However, because this study did not inactivate or impair the AC this remains to be confirmed. The AC, however, was necessary for mice to produce evasive maneuvers to a looming sound (Li et al., 2021).

In a recent study, auditory cortex was shown to be necessary for sound perception during self-locomotion, despite a net-global suppression of sound-evoked activity by locomotive state (Vivaldo et al., 2023). To allow for sound perception during self-locomotion, this study found that underlying neural suppression, auditory cortical neuronal ensembles, encode for and

integrate locomotive state and speed with sound information into a single neural code. Here, we show that external movement of a sound source is also encoded for and integrated by auditory cortical neuronal ensembles. Furthermore, much like self-locomotion, external-movement produces a net-global suppression of sound-evoked activity in the AC. Moreover, much like self-locomotion, underlying this net-suppression is an enhancement of coding for the locomotion or movement information itself. In self-locomotion a net suppression of sound-evoked activity was mediated by an enhancement of ongoing activity that encoded for the movement state, and speed of the animal. In this study we demonstrate that underlying a net suppression of sound evoked activity by external movement state, subpopulations of individual neurons are selectively enhanced to be able to encode for specific aspects of the external object's movement. Overall, these studies suggest that self and external movement suppress auditory cortical sound-evoked activity to enhance the encoding and integration of non-sensory information such as movement-state and speed into a single neural code. This is supported by work suggesting that with increased locomotion there is shift towards spatial coding (Bigelow, et al. 2019). As objects move their spatial location changes and this would be supported by this enhancement of spatial coding. Because of the influence of self-locomotion on auditory processing, in this study we selectively examined external locomotion only when the animal was remained stationary. Some evidence of neural encoding of self-locomotion and external-movement comes from studies in the visual modality where visual cortical neurons integrated self-locomotion speed and the speed of an external stimulus on a virtual reality screen (Ayaz et al.2013). If and how self-locomotion and external locomotion are integrated during sound perception remains to be addressed in the auditory cortex. Furthermore, whether internal and external movement is encoded by influencing distinct neural properties, such that internal locomotion is encoded by modulating ongoing activity and external locomotion is encoded in the formation of distinct sound-evoked subpopulations, remains to be addressed.

In this study, our results show that not only is the movement state of an external object encoded in auditory cortical neural activity but that the nature, or speed, of this object can modulate activity at stationary positions. Before examining the effect of neural activity of auditory cortical neurons, I examined the acoustic properties of the Doppler, and found that as expected sound intensity increased as objects approached. It is widely, understood that as sounds approach their intensities increase (Soeta & Nakagawa, 2012; Takahashi & Kaga, 2004;

Schreiner & Malone, 2015), so I expected the larger neural response in the Stationary-Close conditions. However, this was not the case as there was more activity in the Stationary-Far condition. This may have been confounded by the introduction of a visual block in front of the animal, which caused an unintentional sound block as the object moved closer, allowing for fewer sound waves to reach the mice but not the microphone.

An interesting result was that as the Doppler increased in speed and moved between these two locations at a faster rate, Stationary-Far activity remained relatively unchanged, but activity in the Stationary-Close condition was increased, but still overall less. Since the Doppler overall had a weaker sound intensity in the faster moving conditions, I expected overall activity to be suppressed at both locations, however, it was selective for the Stationary-Close position and remained stable at the Stationary-Far position. Studies have shown that auditory cortical neurons can maintain sustained activity at specific locations, perhaps explaining the relatively unchanged activity to Stationary-Far conditions (Wang et al., 2005). This however cannot explain the selective enhancement to the Stationary-Close position. An alternative explanation reflects a stimulus adaptation paradigm (Ulanovsky et al., 2003; Nelken et al 2014; Taaseh et all., 201; Klein et al., 2014; Kim et al.,2020). Over time, with repeated exposure, sounds will undergo stimulus adaptation, or produce a smaller neural response to the same sound because the novelty of the object decreases. Because the change in decibel from all to all in is relatively small, each sound-evoked response in the slower conditions will be only slightly different than the previous, so there is less overall change in the stimulus. In a classic odd-ball paradigm to test stimulus adaptation, after presenting repeated sounds, a novel or different sound will elicit a larger neural response (Zhang et al., 2003; Malmierca et al., 2014; Anderson & Malmierca, 2013). When objects move faster, there is a greater distance traveled between sounds and thus two consecutive sounds will have larger differences in their stimulus properties and sound evoked responses may reflect this. However, this does not fully explain why sounds in the Stationary-Close position were enhanced, as this should also apply to Stationary-Far sounds. A last hypothesis could reflect an evolutionary need to pay more attention to sounds that are approaching at faster speeds. In the real world, if something approaches, we pay attention to it as it could mean a predator, prey, or mate. If something is moving slowly, we have time to determine what it is and how to appropriately respond. However, objects that are moving much faster towards us give us less time to process what they are and in turn how to respond to them. In both scenarios, objects

that are moving away maintain the same relevance because they are becoming less meaningful to the immediate environment. Perhaps this effect of the moving speed of the external object reflects this phenomenon, to have more responsiveness to faster approaching sounds as it more relevant. Research has that auditory responsiveness in the cortex can be modulated by the behavioral relevance of an object (Kato et al., 2015; Kuchibhotla & Bathellier, 2018; Liu & Schreiner, 2007).

Lastly, in the visual modality, studies have shown that there are direction selective neurons (Marques et al., 2018), and in the visual modality neurons encode direction based on changes in interaural and time differences (Ahissar et al., 1992). Here we show that in the AC similarly, we have direction selective neurons that do not rely on binaural cues. Interestingly, when we looked at how AC neurons respond to the directionality of the moving sound source, we see that in slow speed there is a significant population of neurons that selectively respond to an object moving in, but not to moving out. Adding support for the hypothesis that the behavioral relevance of the object influences neural activity (Kato et al., 2015; Kuchibhotla & Bathellier, 2018; Liu & Schreiner, 2007). As speed increases this population increases, and a distinct population of neurons encoding all out emerges. Despite this, population-level activity shows a reverse effect, having distinct differences in neural activity between moving in and moving out when objects move slower, and this goes away with an increase in external sound source speed. Because increased external speed has a greater suppression of sound-evoked activity, it makes sense that faster movement would suppress sound-evoked activity and decrease the overall difference of directionality at the population level. This Possibly reflects a floor effect or sound-evoked activity is suppressed enough that the differences between directionality slow-moving states are negated. Similarly, as we have noted underlying this net-suppression of external movement is an enhancement of individual neuronal subpopulations that encode for aspects of movement, in this case, directionality. This excitation-inhibition balance is common across the sensory cortices, including the AC and reflects a neural computational property allowing the encoding of more information (Zhou et al., 2014; Poole, 2023; Briggs et al., 2013). Specifically, by inhibiting global sound-evoked activity, while exciting subpopulations of neurons, neural ensembles increase the signal-to-noise ratio of sensory encoding. Overall, specific neurons become more important when overall activity is suppressed allowing for the distinct encoding of directionality and movement-state of an object.

3.9 Methods

All procedures followed laboratory animal care guidelines approved by the University of Michigan Institutional Animal Care and Use Committee and conformed to National Institutes of Health guidelines.

3.9.1 Animals

A total of 5 male and female Thy1-GCaMP6f mice (C57BL/6J-Tg(Thy1-GCaMP6f)GP5.17Dkim/J, JAX stock No: 025393) between the ages of 12-23 weeks were used in this study. Mice were kept on a reverse light cycle and all imaging and behavioral sessions were performed in the dark cycle.

3.9.2 Mouse surgery

Mice were anesthetized with Ketamine-Xylazine or isoflurane and implanted with a custom lightweight (<1 gr.) titanium head bar. For the cortical inactivation experiments, small bilateral craniotomies were drilled above the AC and either 2 mm or 3 mm length custom cannulas (Plastics One, MA) were lowered into the AC. Mice received postop antibiotic ointment and Carprofen and were allowed to recover for at least 5 days before any imaging or behavioral sessions.

3.9.3 Sound Source Tracking conditioning

Mice were placed on water restriction 48 hours prior to behavioral training and received ad libitum access to food. During training and testing, mice were placed in a custom-built behavioral training box, in which they were head fixed on top of a rotatable plate with an accessible water reward port. A custom Arduino-based linear actuator system that received input from an external power source allowed presenting moving sounds from a speaker fastened to the end of the actuator. Upon receiving voltage, the linear actuator would move the speaker forward and back while emitting continuous 1s 8 kHz pulses, from ~ 42 inches away from to ~6 inches in front of the animal in either immobility or locomotion.

Sound source tracking learning phase: Animals were trained to associate a 1 s 8 kHz tone with subsequent water reward delivered after a delay of 1s following sound termination. Sounds

(followed by water rewards) were presented following a period of continuous approaching of the speaker that randomly varied across trials between 5-10 s. Animals advanced to the testing phase only after they displayed consistent post-sound reward-predictive licking in locomotion for 2 consecutive days.

Sound source tracking control phase: Animals were trained to associate a 1s 8 kHz tone with subsequent water reward delivered after a delay of 1s following sound termination, which was called a movement trial (40% of trials). To test that animal understood the movement of an object meant reward and not the timing of the trial an All-out condition occurred (20% of the time). In this condition the doppler remained stationary while playing sounds at the same intensity and duration from a far. To test that animal understood the movement of an object meant reward and not the location or intensity of the trial an All-in condition occurred (20% of the time). In this condition the doppler remained stationary while playing sounds at the same intensity and duration however began close to the animal.

To quantify the association between sound and subsequent water reward, we quantified the degree of increased licking in the 1 s window before the sound reached it target and 1s window following movement of the sound source termination and before reward delivery ((-1000) – (+1000)) ms from movement onset relative to the pre-sound baseline lick rate ((0) – (2000) ms from sound onset. To this end we defined a "predictive lick index" as the across-trials average difference between the number of licks in the predictive window and that of the baseline window.

3.9.4 Two-photon imaging

During imaging sessions, mice were placed on a rotating plate while being head fixed under the microscope objective. Imaging was carried out while the head of the animal was straight, with the objective tilted using an orbital nosepiece to allow optical access to the AC. Mice were allowed to initiate movement at their leisure. Imaging was performed using an Ultima IV two-photon microscope (Bruker), a pulsed tunable laser (MaiTai eHP DeepSee by Spectra Physics) providing excitation light at 940nm and 16X or 40X water-immersion objectives (Nikon). Images (256X256 pixels) were acquired using galvanometric mirrors at ~3 Hz to optimize signal quality and cell separation. The microscope was placed in an enclosed chamber

in a dark, quiet room. Neurons were imaged at depths of 150-350 μM, corresponding to cortical L2/3.

During imaging sessions, the mouse's behavior was video recorded using an infrared camera, which was synchronized offline with the imaging data acquisition. Locomotion and immobility were determined offline using semi-automatic movement-detection MATLAB code with manual thresholding and supervision. In addition, in all imaging sessions a rotary encoder was positioned at the base of the rotating plate allowing to acquire continuous locomotion speed, which was used to filter out all trials where the animal was moving. In each daily imaging session, responses of the same neurons were imaged to multiple speed protocols as the Doppler, a moving sound source continuously approached and receded. For the slow conditions the doppler took 6 s to go from all out to all in, and in the fast conditions that was sped up to 3 s. While the doppler was moving sounds were continuously played. During each session, directly under the animal, a microphone was placed to be able to record and measure the intensities of the sounds as they approached and receded. Lastly a visual block was placed 3 inches in front of the animal, to block visual input from the moving speaker.

Auditory stimuli were delivered via an open-field magnetic speaker (MF1, Tucker Davis Technologies) at 75 dB. The broadband noise bursts protocol consisted continuous repeats of 125 ms white noise bursts padded with 825ms of silence for a total of 1s per sound presentation.

3.9.5 Imaging data preprocessing and analysis

Daily imaging data of the same ensemble across multiple sound protocols was concatenated and then preprocessed using the open source Suite2P software (Pachitariu et al., 2017) for movement correction and neuronal ROI detection within the ensemble. Neural data, sound stimuli and locomotion speed signals were aligned.

Data analysis was performed using custom software written in Matlab (MathWorks). Relative change in fluorescence (ΔF/F) across time (t) was calculated for each detected cell as (F(t)-median(F))/(median(F)), where F(t) is the mean brightness of the cell's pixels at time t. For determination of BBN-responsiveness of individual neurons and quantification of activity in the pre-stimulus time window (Ongoing) and stimulus time window (Evoked), the mean ΔF/F was taken across 1-2 samples preceding stimulus onset (corresponding to ~-0.5 – 0 s), and 1-3 samples following stimulus onset (corresponding to ~0 – 1 s), respectively. A cell was

determined as BBN-responsive if ΔF/F during the stimulus time window was significantly higher than during the pre-stimulus time window using a one-tailed paired t-test at $P<0.05$ across all immobile trials.

Using a closed-loop system, all trials began with the actuator in the all-out position and continuously moved positions for the duration of 600s. Actuator start times and movement direction were calculated as alternating deflections in voltage recordings signaling movement onset and change in direction. Slow moving conditions required an external voltage of 5.2mV, and fast conditions were recorded with an external voltage of 9.3mV to power the actuator. To calculate acoustic properties, voltage data from the microphone was collected and was converted to decibels using MATLAB.

To determine analysis conditions BBN-evoked responses and acoustic recordings were only from certain locations, moving-states, and directions. Stationary-Far neural activity was taken when the actuator was stationary and in the starting and outmost position and given a percent value of 100, signifying 100 percent of the actuator was extended. All in neural activity was taken when the actuator was stationary and at the inmost, closest to the animal position and given a percent value of 0, signifying that 0 percent of the actuator was extended. In slow conditions the Doppler remained stationary at each location for ~3 seconds, while in the fast conditions the time stationary at each location was ~1s. All-stationary neural activity was the sum of all-in neural activity and all-out neural activity, or only when the actuator and sound-evoked activity occurred at 100 and 0 percent of actuator extension. All-moving in were calculated as all sound evoked activity when the actuator was moving, or all values between 99 and 1 percent actuator extension, signaling it was moving. Moving in conditions were calculated as all sound evoked activity when the actuator was moving from the outmost position to the inmost position or as values of percent extension went from 99 to 1, selectively. Moving out conditions were calculated as the sound evoked activity when the actuator was moving from the inmost position to the outmost position or as percent values of extension went from 1 to 99, selectively. Calculations for conditions remained consistent across speeds.

To calculate effects of speed for each condition BBN-evoked activity for each cell was subtracted across condition, to calculate an average change in effect. Differences were calculated by subtracting the fluorescent activity of each cell across conditions, such that for each cell condition 1 activity was subtracted from condition 2. Condition 1 variables were Stationary-

close, stationary, and moving-in, while condition 2 variables were, all out, moving, and moving out. Population averages of the differences were used to generate figures.

To calculate decibels of sounds, microphone voltage data was only taken at Statioary-Far, Stationary-Close, and Moving conditions. All per condition effects and speed effects were tested for significance using a nonparametric Wilcoxon rank-sum test.

3.10 References

1. Anderson, L. A., & Malmierca, M. S. (2013). The effect of auditory cortex deactivation on stimulus-specific adaptation in the inferior colliculus of the rat. European Journal of Neuroscience, 37(1), 52-62.
2. Ayaz A, Saleem AB, Scholvinck ML, Carandini M. Locomotion controls spatial integration in mouse visual cortex. Curr Biol. 2013; 23(10):890–4. Epub 2013/05/15. https://doi.org/10.1016/j.cub.2013.04. 012 PMID: 23664971; PubMed Central PMCID: PMC3661981.
3. Ahissar, M., Ahissar, E. H. U. D., Bergman, H., & Vaadia, E. (1992). Encoding of sound-source location and movement: activity of single neurons and interactions between adjacent neurons in the monkey auditory cortex. Journal of neurophysiology, 67(1), 203-215.
4. Bigelow J, Morrill RJ, Dekloe J, Hasenstaub AR. Movement and VIP Interneuron Activation Differentially Modulate Encoding in Mouse Auditory Cortex. eNeuro. 2019; 6(5). Epub 20190918. https://doi. org/10.1523/ENEURO.0164-19.2019 PMID: 31481397; PubMed Central PMCID: PMC6751373.
5. 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. Journal of neuroscience, 29(7), 2064-2075.
6. Blauert, J., & Lindemann, W. (1986). Spatial mapping of intracranial auditory events for various degrees of interaural coherence. The Journal of the Acoustical Society of America, 79(3), 806–813. https://doi.org/10.1121/1.393471
7. Briggs, F., Mangun, G. R., & Usrey, W. M. (2013). Attention enhances synaptic efficacy and the signal-to-noise ratio in neural circuits. Nature, 499(7459), 476-480.
8. Cook, J. R., Li, H., Nguyen, B., Huang, H.-H., Mahdavian, P., Kirchgessner, M. A., Strassmann, P., Engelhardt, M., Callaway, E. M., & Jin, X. (2022). Secondary auditory cortex mediates a sensorimotor mechanism for action timing. Nature Neuroscience, 25(3), 330–344. https://doi.org/10.1038/s41593-022-01025-5
9. Cohen L, Rothschild G, Mizrahi A. Multisensory integration of natural odors and sounds in the auditorycortex. Neuron. 2011; 72(2):357–369. https://doi.org/10.1016/j.neuron.2011.08.019 PMID: 22017993.

10. David SV, Fritz JB, Shamma SA. Task reward structure shapes rapid receptive field plasticity in auditory cortex. Proc Natl Acad Sci U S A. 2012; 109(6):2144–9. Epub 20120123. https://doi.org/10.1073/ pnas.1117717109 PMID: 22308415; PubMed Central PMCID: PMC3277538.
11. Douglas, R. M., Neve, A., Quittenbaum, J. P., Alam, N. M., & Prusky, G. T. (2006). Perception of visual motion coherence by rats and mice. Vision Research, 46(18), 2842–2847. https://doi.org/10.1016/j.visres.2006.02.025
12. Falk B, Jakobsen L, Surlykke A, Moss CF. Bats coordinate sonar and flight behavior as they forage in open and cluttered environments. J Exp Biol. 2014; 217(Pt 24):4356–4364. https://doi.org/10.1242/ jeb.114132 PMID: 25394632; PubMed Central PMCID: PMC4375838.
13. Fritz JB, David SV, Radtke-Schuller S, Yin P, Shamma SA. Adaptive, behaviorally gated, persistent encoding of task-relevant auditory information in ferret frontal cortex. Nat Neurosci. 2010; 13(8):1011–9. Epub 20100711. https://doi.org/10.1038/nn.2598 PMID: 20622871; PubMed Central PMCID:PMC2921886.
14. Ghose K, Horiuchi TK, Krishnaprasad PS, Moss CF. Echolocating bats use a nearly time-optimal strategy to intercept prey. PLoS Biol. 2006; 4(5):865–73. ARTN e108 https://doi.org/10.1371/journal.pbio. 0040108 WOS:000237966900023. PMID: 16605303
15. Hoy, J. L., Yavorska, I., Wehr, M., & Niell, C. M. (2016). Vision drives accurate approach behavior during prey capture in laboratory mice. Current Biology, 26(22), 3046–3052. https://doi.org/10.1016/j.cub.2016.09.009
16. Ito, S., Si, Y., Feldheim, D.A. et al. Spectral cues are necessary to encode azimuthal auditory space in the mouse superior colliculus. Nat Commun 11, 1087 (2020). https://doi.org/10.1038/s41467-020-14897-7
17. Jaramillo S, Zador AM. The auditory cortex mediates the perceptual effects of acoustic temporal expectation. Nat Neurosci. 2011; 14(2):246–U340. https://doi.org/10.1038/nn.2688 WOS:000286595400023. PMID: 21170056
18. 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.
19. King AJ, Bajo VM, Bizley JK, Campbell RA, Nodal FR, Schulz AL, et al. Physiological and behavioral studies of spatial coding in the auditory cortex. Hear Res. 2007; 229(1–

2):106–15. Epub 20070117. https://doi.org/10.1016/j.heares.2007.01.001 PMID: 17314017; PubMed Central PMCID: PMC7116512.

20. Kim, S. G., Poeppel, D., & Overath, T. (2020). Modulation change detection in human auditory cortex: Evidence for asymmetric, non-linear edge detection. European Journal of Neuroscience, 52(2), 2889-2904.
21. Klein, C., von der Behrens, W., & Gaese, B. H. (2014). Stimulus-specific adaptation in field potentials and neuronal responses to frequency-modulated tones in the primary auditory cortex. Brain topography, 27, 599-610.
22. Kuchibhotla KV, Gill JV, Lindsay GW, Papadoyannis ES, Field RE, Sten TAH, et al. Parallel processing by cortical inhibition enables context-dependent behavior. Nat Neurosci. 2017; 20(1):62–71. https://doi.org/10.1038/nn.4436 WOS:000391085500013. PMID: 27798631
23. Kuchibhotla, K., & Bathellier, B. (2018). Neural encoding of sensory and behavioral complexity in the auditory cortex. Current opinion in neurobiology, 52, 65-71.
24. Li, Z., Wei, J.-X., Zhang, G.-W., Huang, J. J., Zingg, B., Wang, X., Tao, H. W., & Zhang, L. I. (2021). Corticostriatal control of defense behavior in mice induced by auditory looming cues. Nature Communications, 12(1). https://doi.org/10.1038/s41467-021-21248-7
25. Malmierca, M. S., Sanchez-Vives, M. V., Escera, C., & Bendixen, A. (2014). Neuronal adaptation, novelty detection and regularity encoding in audition. Frontiers in systems neuroscience, 8, 111.
26. Liu, R. C., & Schreiner, C. E. (2007). Auditory cortical detection and discrimination correlates with communicative significance. PLoS biology, 5(7), e173.
27. Marques, T., Summers, M. T., Fioreze, G., Fridman, M., Dias, R. F., Feller, M. B., & Petreanu, L. (2018). A role for mouse primary visual cortex in motion perception. Current Biology, 28(11). https://doi.org/10.1016/j.cub.2018.04.012
28. McGinley, M. J., Vinck, M., Reimer, J., Batista-Brito, R., Zagha, E., Cadwell, C. R., Tolias, A. S., Cardin, J. A., & McCormick, D. A. (2015). Waking state: Rapid variations modulate neural and behavioral responses. Neuron, 87(6), 1143–1161. https://doi.org/10.1016/j.neuron.2015.09.012

29. Metin, C., Godement, P., & Imbert, M. (1988). The primary visual cortex in the mouse: Receptive field properties and functional organization. Experimental Brain Research, 69(3). https://doi.org/10.1007/bf00247312
30. Moss CF, Surlykke A. Auditory scene analysis by echolocation in bats. J Acoust Soc Am. 2001; 110 (4):2207–2226. https://doi.org/10.1121/1.1398051 WOS:000171526400054. PMID: 1168139
31. Nelken I. Stimulus-specific adaptation and deviance detection in the auditory system: experiments and models. Biol Cybern. 2014; 108(5):655–63. Epub 20140130. https://doi.org/10.1007/s00422-014-0585-7 PMID: 24477619.
32. Niell, C. M., & Stryker, M. P. (2008). Highly selective receptive fields in mouse visual cortex. The Journal of Neuroscience, 28(30), 7520–7536. https://doi.org/10.1523/jneurosci.0623-08.2008
33. Poole, K. C. (2023). How does the brain extract acoustic patterns? A behavioural and neural study (Doctoral dissertation, UCL (University College London)).
34. Rodgers CC, DeWeese MR. Neural Correlates of Task Switching in Prefrontal Cortex and Primary Auditory Cortex in a Novel Stimulus Selection Task for Rodents. Neuron. 2014; 82(5):1157–1170. https://doi.org/10.1016/j.neuron.2014.04.031 WOS:000337359800020. PMID: 24908492
35. Saderi D, Schwartz ZP, Heller CR, Pennington JR, David SV. Dissociation of task engagement and arousal effects in auditory cortex and midbrain. Elife. 2021; 10. Epub 20210211. https://doi.org/10. 7554/eLife.60153 PMID: 33570493; PubMed Central PMCID: PMC7909948
36. Soeta, Y., & Nakagawa, S. (2012). Auditory evoked responses in human auditory cortex to the variation of sound intensity in an ongoing tone. Hearing Research, 287(1-2), 67-75.
37. Schreiner, C. E., & Malone, B. J. (2015). Representation of loudness in the auditory cortex. Handbook of clinical neurology, 129, 73-84.
38. Stirman, J. N., Townsend, L. B., & Smith, S. L. (2016). A touchscreen based global motion perception task for Mice. Vision Research, 127, 74–83. https://doi.org/10.1016/j.visres.2016.07.006
39. Taaseh, N., Yaron, A., & Nelken, I. (2011). Stimulus-specific adaptation and deviance detection in the rat auditory cortex. PloS one, 6(8), e23369.

40. Takahashi, H., Nakao, M., & Kaga, K. (2004). Distributed representation of sound intensity in the rat auditory cortex. Neuroreport, 15(13), 2061-2065.
41. Town SM, Brimijoin WO, Bizley JK. Egocentric and allocentric representations in auditory cortex.PLoS Biol. 2017; 15(6):e2001878. Epub 20170615.
42. Triblehorn JD, Yager DD. Timing of praying mantis evasive responses during simulated bat attack sequences. J Exp Biol. 2005; 208(Pt 10):1867–1876. https://doi.org/10.1242/jeb.01565 PMID:15879067.
43. Ulanovsky N, Las L, Nelken I. Processing of low-probability sounds by cortical neurons. Nat Neurosci. 2003; 6(4):391–398. https://doi.org/10.1038/nn1032 WOS:000181833100017. PMID: 12652303
44. Vesely, S. L., Dolci, C. A., Dolci, S. R. (2018). The doppler effect in the perception of sound. AIP Conference Proceedings. https://doi.org/10.1063/1.5047781
45. Vivaldo, C. A., Lee, J., Shorkey, M., Keerthy, A., & Rothschild, G. (2023). Auditory cortex ensembles jointly encode sound and locomotion speed to support sound perception during movement. PLOS Biology, 21(8). https://doi.org/10.1371/journal.pbio.3002277
46. Wang, X., Lu, T., Snider, R. K., & Liang, L. (2005). Sustained firing in auditory cortex evoked by preferred stimuli. Nature, 435(7040), 341-346.
47. Wood KC, Town SM, Bizley JK. Neurons in primary auditory cortex represent sound source location in a cue-invariant manner. Nat Commun. 2019; 10(1):3019. Epub 20190709. https://doi.org/10.1038/ s41467-019-10868-9 PMID: 31289272; PubMed Central PMCID: PMC6616358.
48. Xiong QJ, Znamenskiy P, Zador AM. Selective corticostriatal plasticity during acquisition of an auditory discrimination task. Nature. 2015; 521(7552):348. https://doi.org/10.1038/nature14225 WOS:000354816500055. PMID: 25731173
49. Yeomans, J. S., & Frankland, P. W. (1995). The acoustic startle reflex: neurons and connections. *Brain research reviews*, *21*(3), 301-314.
50. Znamenskiy P, Zador AM. Corticostriatal neurons in auditory cortex drive decisions during auditory discrimination. Nature. 2013; 497(7450):482. https://doi.org/10.1038/nature12077 WOS:000319254000046. PMID: 23636333

51. Zhang, L. I., Tan, A. Y., Schreiner, C. E., & Merzenich, M. M. (2003). Topography and synaptic shaping of direction selectivity in primary auditory cortex. Nature, 424(6945), 201-205.
52. Zhou, M., Liang, F., Xiong, X. R., Li, L., Li, H., Xiao, Z., Tao HW & Zhang, L. I. (2014). Laminar-specific Scaling Down of Balanced Excitation and Inhibition in Auditory Cortex by Active Behavioral States. *Nature neuroscience*, *17*(6), 841.

Chapter 4 Discussion

4.1 Summary

In this dissertation, I aimed to address how continuous processing and integration of sensory and non-sensory information is crucial for adaptive behavior. Specifically, I set forth to show that both self-locomotion and external-object motion can be used to guide behavior and that auditory sensory neural populations can process and integrate these two pieces of non-sensory information. In my first set of experiments, I tested the hypothesis that a net-suppressive effect of self-locomotion on auditory sound evoked responses does not impair active auditory behavior and learning but reflects an alternate neural computation. Secondly, I tested the hypothesis that external sound source object movement can be used to guide adaptive behaviors and external sound source object movement is processed and encoded by auditory cortical neuronal ensembles.

To address my first hypothesis, I designed a novel behavioral system that trained animals to understand that their own locomotive state was indicative of an upcoming reward. Using AC inactivation in these trained and behaving mice, I found that AC activity is required for sound-guided behavior during locomotion. Furthermore, by using two-photon calcium imaging in L2/3 of AC of head-fixed mice, I found that locomotion had a diverse but overall inhibitory influence on sound-evoked responses of individual neurons, which resulted in a mild but significant reduction in ensemble-level stimulus detection. Across ensembles, stimulus detection in immobility and locomotion were positively correlated, suggesting that sound processing across these states is supported by shared local neural populations in L2/3 of AC. Furthermore, I found that the net reduction in sound-evoked responses during locomotion are at least partly a result of increased ongoing neural activity, and importantly, that this ongoing activity robustly encoded the animal's running speed. Thus, lower sound-evoked responses during locomotion reflected a tradeoff with the emergence of locomotion speed coding. Decoding analyses revealed that local neuronal ensembles of a few dozen neurons could jointly code locomotion speed and sound with

high fidelity. Finally, I report consistent patterns of co-encoding of sound and locomotion speed in electrophysiologically recorded freely-moving rats.

To address my second hypothesis, I designed a second novel behavioral recording system, The Doppler, that allows an object to continuously move, while allowing for behavioral training or neural recordings. Using, the Doppler, this study demonstrated that animals could learn that a continuously approaching sound source signals an upcoming reward. Furthermore, using a series of behavioral controls, this study demonstrated that mice understand that it is the continuous movement of a sound source that signals and upcoming reward and not aspects of motion such as an increase in sound intensity, change in location, or time that an object emits sounds. Using two-photon calcium imaging of L2/3 auditory cortical neurons, this study showed that neural populations encoded aspects of movement, such as location and sound intensity changes, as well as the continuous movement-state of an object and that this is modulated by the speed of the object. Additionally, this study showed that auditory cortical neurons can integrate aspects of motion to be able to encode for directionality of a moving sound source. Furthermore, this study demonstrated that during bouts of movement, sound-evoked responses to moving sounds were significantly reduced as compared to stationary conditions. Finally, the faster the object moved the larger the suppression of sound-evoked neural activity. Interestingly, as objects move faster, more individual neurons become selectively responsive for location, movement, and directionality.

Overall, this dissertation provides evidence that both self-locomotion and external locomotion can guide auditory learning and behavior. Additionally, here, I provided evidence that self-locomotion and external object locomotion information is processed and encoded by auditory cortical neuronal ensembles.

4.2 Self-locomotion is a fundamental property of sensory cortices

In my first study, I addressed how self-locomotion guided behavior and the neural computations needed to produce this behavior. The results of this study filled in a missing gap to a larger phenomenon, encoding of self-locomotion is a fundamental property of sensory cortices. Previous work in the visual somatosensory, barrel, and to some extent auditory cortices has shown that self-locomotion is processed by its respective ensembles and modulated neural activity (Ayaz et al., 2013, Saleem et al., 2013; Vinck et al., 2015; Schneider et al., 2014;

McGinley et al., 2015a; McGinley et al., 2015b; Niell & Stryker 2010; Polack et al., 2013; Zhou et al., 2014; Schneider, 2020) . In the other sensory modalities work has also established a functional role of self-locomotion to guide in behavior, however this remained to be addressed in the auditory cortex. By filling in this gap, this dissertation shows that the locomotive state of the human or animal, immobile or moving, is not only encoded but that animals can respond to sensory stimuli in an adaptive way using this information. Because animals and humans are continuously changing locomotive states as they behave through the world, switching from periods of rest or sitting, to moving, walking, or running, being able to process and react to sensory information is necessary across all these states. This study replicates work done in the visual and somatosensory cortices, demonstrating that auditory neural populations encode for locomotive state and speed of movement. Furthermore, by establishing the functional role of this integration as necessary for behavior, this study further provides evidence to this larger phenomenon. Sensory cortices integrate locomotion state and speed into an integrated neural code, to be able to learn and make quick informed behaviors.

It is interesting to note that the encoding of self-locomotion, is not limited to sensory modalities. In hippocampal regions, self-locomotion is encoded by neural activity in a speed dependent manner (Saleem et al., 2018; Gois et al.,2018; Farrell et al., 2021; Geisler et al., 2007; Haggerty et al., 2015) Additionally, increases in locomotion can coordinate the activity of hippocampal and sensory cortices (Ghosh et al, 2022)., suggesting that perhaps self-locomotion coordinates the transfer of information from sensory cortices to memory and spatial regions, or vice versa. A cortico-hippocampal-cortical circuit, known to be important in the formation of sensory learning and memories, might be responsible for more than a simple transfer of sensory information (Rothschild et al., 2017). To be able to properly learn a sensory cue, the behavioral context, or locomotive state that the learning occurred is perhaps also transferred in this loop. If one were to think about our dreams, we often dream in multiple states, think about running from that scary clown. Having sensory information consolidated along with the behavioral state it occurred would allow learning to be more effective (Ghosh et al. 2022; Rothschild et al., 201; Maluck et al 2019; Windt, 2018; Rosen, 2019). Given self-locomotion modulated activity along this pathway this is something worth addressing in future work.

4.3 External motion is not the same perceptually as mimicking motion in stationary conditions.

In the second series of experiments I conducted, I fundamentally wanted to test if the perception of external motion by replicating aspects of motion was indeed the same as a moving object in space. Much, if not all, of the work done on the understanding how the motion of external objects is encoded has been done by replicating components of motion on stationary objects. In the visual modality, this is done by using a virtual reality system where objects can be moved in different directions, locations, and along different speeds (Marques et al., 2018; Douglas et al., 2006; Ayaz et al., 2013; Hoy et al., 2016; Stirman et al., 2016). In the auditory modality this has been done by modulating sound intensity, and by playing sounds along a series of discrete speakers (Li et al., 2021; Wood et al., 2019, Ahissar et al., 1992). While these methods produce aspects of external motion, true movement of objects in the real world is a gradual and complex integration of all these properties simultaneously. Thus, by developing, a first of its kind, behavioral system, I was able to move an external object producing sensory information in a naturalistic and holistic manner, not a stationary one or aspects of movement. In this manner, I demonstrated that mice understand movement in the environment as independent from replications of aspects of motion. A mouse understands that an incoming object is not the same as an increase in sound intensity, and this is a key finding of this dissertation. Much of our knowledge of understanding external motion in the context of behavioral relevance must distinguish if what is being recorded and encoded for is actual motion, or simply changes in sensory information. Because this study was able to show that auditory cortical neurons encode for aspects of motion such as location, directionality, movement-state, and speed, we can argue that it is the simultaneous encoding and processing of changes in along these parameters that define true external motion. Furthermore, in this study I demonstrated that even at stationary locations, how the external object was behaving before reaching a still, influences how neurons encode for that stimulus, movement components can be processed in stationary locations, but they are modulated by the behavioral relevance (Kato et al., 2015; Kuchibhotla & Bathellier, 2018; Liu & Schreiner, 2007).

In the real world, external objects are free to move independent of the self, and here we show that the dynamics of the objects, or speed of the objects, influences how sensory

information is processed and interpreted. If humans and animals are in environments where there is no external motion, or everything is still, then when something moves it requires attention. If humans and animals were in environments where there were many moving objects in space than that would require more fine-tuned processing (Verdeny-Vilalta et al., 2015; Hermundstad et al., 2014; Machens et al., 2005; Malmierca et al., 2014; Nelken et al., 2014). Furthermore, if there were multiple moving objects and suddenly one increased its speed that too would carry information. Thus, this dissertation highlights a new phenomenon of interest in the sensory neurosciences. How do external objects modulate sensory processing and how is that used for behavior.

4.4 Dynamic environments require complex neural mechanism of excitation and inhibition

In the studies conducted here a unique phenomenon was also discovered. Whether it is self or external locomotion, auditory cortical activity becomes suppressed. However, underlying this suppression is a neural mechanism that allows for the integration of locomotion and auditory information, in self-locomotion it's the encoding of speed by ongoing activity, and in external locomotion it is the enhancement of subpopulations to encode for movement and directionality. As information and environments become more complex neural ensembles, must alter their neural computations to allow for this increase in information without losing function. An excitation-inhibition paradigm to increase signal to noise ratio of neuronal activity had been proposed that fits into this idea (Zhou et al., 2014; Zagha et al., 2016; Schinkel et al., 2012; Dorrn et al., 2010). Neuronal ensembles will excite or enhance some neural properties to make up for the inhibition and suppression of others, maintaining an equilibrium of information and neural activity. In the auditory cortex, self-locomotion inhibits sound-evoked activity, buy enhances on going activity (Vivaldo et al., 2023). In the context of, external locomotion this also inhibits sound-evoked activity, but enhances neural specificity. On a larger scale, this same principle applies to multimodal integration and processing (Bigelow 2019). In the context of self-locomotion, visual and somatosensory sensory-evoked activity is excited, and auditory activity is suppressed (Ayaz et al., 2013; Schneider et al., 2014; Ayaz et al., 2019). Because this is the first study to examine a moving sensory object in three-dimensional space, without any cues beyond simple motion, whether the same principles generate across all forms of locomotion remains to be addressed.

4.5 Applications of the doppler for audio-visual integration

If an external object is moving in space, there are only a few sensory modalities that can realistically encode for this. For example, if a predator is coming towards you, you might be able to see the predator approaching, hear the approaching footsteps, but once you can smell or feel or even taste, then it has reached you and that would not advantageous. Thus, it stands to reason that auditory and visual modalities primarily are responsible for being able to detect external objects and infer about their movement (Bruns & Getzmann, 2008; Kwon et al, 2014; Schroger & Widmann, 1998). Additionally, if a predator is approaching from behind only auditory perceptions would be able to detect an incoming or approaching object. (Furukawa et al., 2000; Town & Bizley, 2022; Konishi, 2003) Thus, here I propose additional studies and use for Doppler to understand how external objects are processed.

In this dissertation, I specifically blocked out visual input to understand how a moving sound source guides behavior. By removing the visual block and disconnecting the speaker the doppler functions as a visually moving object in space. By using the behavioral protocols mentioned in chapter 2, one could test whether the motion of an approaching object guides learning and behavior. Additionally, by placing animals in a two-photon microscope with the visual Doppler, one could compare if external motion elicits similar neural responses as movement on a virtual reality screen. By using both a speaker, without a visual block, Audiovisual integration strategies can be assessed. Given the unique role both sensory modalities have in processing stimuli in similar manners, for location, direction, movement-state, and speed, whether there is multimodal integration of information has yet to be addressed in the context of an externally moving object. Lastly, because auditory modality is uniquely equipped to monitor changes in the environment beyond peripheral vision, if external locomotion of objects moving behind the perceiver enhanced or suppressed auditory activity has yet to be addressed. Furthermore, whether and how these influences visual activity remains to be addressed.

4.6 Ethologically relevant study designs are necessary to understand how the brain and behavior interact

In this dissertation, I took an ethologically grounded approach to studying sensory perception. Because most of our understanding of sensory processing has occurred in slice

recordings, anesthetizes states, or by replicating aspects of self or external locomotion, how this generalizes to the real world is still up for debate (Nelson et al., 2013; Schneider et al., 2014; Marques et al., 2018; Douglas et al., 2006; Ayaz et al., 2013; Hoy et al., 2016; Stirman et al., 2016; Li et al., 2021; Wood et al., 2019, Ahissar et al., 1992). With the advancement of technologies like two-photon calcium imaging and the increase in affordability of Arduino microprocessors and mechanical parts, this dissertation designed novel behavior recording systems that recorded activity much more ethologically grounded manner (Svoboda & Yasuda, 2006). In my first study to test the effects of locomotion, animals were free to initiate locomotion voluntarily, in awake and behaving state. Much like you or I would react to a car horn while on a walk or jog, my first experimental training paradigm mimicked a freely moving animal responding to a sound while in motion. It was using this method that I was able to causally show that despite self-locomotion's net inhibitory effect auditory cortical processing, it was still necessary to produce sound-guided behaviors. In my second set of studies, I designed the first ever sensory object that could freely move in space, while the animal was also free to move, and recorded from awake and behaving conditions. Just as you or I would respond to the sound of an incoming car no matter how fast, I designed my second behavioral training paradigm to mimic a freely moving object across multiple speeds to ensure that the concept of movement was understood. By doing this I was able to train animals to understand that no matter the speed an approaching object has consequences. Furthermore, I was able to understand that not only do neurons encode for external locomotion and direction, but that how the object is moving influences neural activity. I end this dissertation with a general call to produce and replicate more ethologically relevant behavioral training recording systems and training paradigms (Bekoff 2006; Dixon et al., 1989; Hernstein, 1977; Timberlake, 1997). If neuroscientist truly want to understand how the brain influences behavior and vice versa, then we must model behavior as it occurs naturally in a dynamic world.

4.7 References

1. Ahissar, M., Ahissar, E. H. U. D., Bergman, H., & Vaadia, E. (1992). Encoding of sound-source location and movement: activity of single neurons and interactions between adjacent neurons in the monkey auditory cortex. Journal of neurophysiology, 67(1), 203-215.
2. Ayaz A, Saleem AB, Scholvinck ML, Carandini M. Locomotion controls spatial integration in mouse visual cortex. Curr Biol. 2013; 23(10):890–4. Epub 2013/05/15. https://doi.org/10.1016/j.cub.2013.04. 012 PMID: 23664971; PubMed Central PMCID: PMC3661981.
3. Ayaz A, Saleem AB, Scholvinck ML, Carandini M. Locomotion controls spatial integration in mouse visual cortex. Curr Biol. 2013; 23(10):890–4. Epub 2013/05/15. https://doi.org/10.1016/j.cub.2013.04. 012 PMID: 23664971; PubMed Central PMCID: PMC3661981.
4. Ayaz A, Stauble A, Hamada M, Wulf MA, Saleem AB, Helmchen F. Layer-specific integration of locomotion and sensory information in mouse barrel cortex. Nat Commun. 2019; 10(1):2585. Epub 2019/ 06/15. https://doi.org/10.1038/s41467-019-10564-8 PMID: 31197148; PubMed Central PMCID:PMC6565743.
5. Bekoff, M. (2006). Animal passions and beastly virtues : Cognitive ethology as the unifying science for understanding the subjective, emotional, empathic, and moral lives of animals. *Zygon*, 41, 71-104. https://doi.org/10.1111/J.1467-9744.2006.00727.X.
6. Bigelow J, Morrill RJ, Dekloe J, Hasenstaub AR. Movement and VIP Interneuron Activation Differentially Modulate Encoding in Mouse Auditory Cortex. eNeuro. 2019; 6(5). Epub 20190918. https://doi. org/10.1523/ENEURO
7. Bruns, P., & Getzmann, S. (2008). Audiovisual influences on the perception of visual apparent motion: exploring the effect of a single sound.. *Acta psychologica*, 129 2, 273-83 . https://doi.org/10.1016/j.actpsy.2008.08.002.
8. Campbell MG, Giocomo LM. Self-motion processing in visual and entorhinal cortices: inputs, integration, and implications for position coding. J Neurophysiol. 2018; 120(4):2091–106. Epub 2018/08/09.https://doi.org/10.1152/jn.00686.2017 PMID: 30089025; PubMed Central PMCID: PMC6230811.

9. Dixon, A., Fisch, H., Huber, C., & Walser, A. (1989). Ethological studies in animals and man, their use in psychiatry.. *Pharmacopsychiatry*, 22 Suppl 1, 44-50 . https://doi.org/10.1055/S-2007-1014624.
10. Dorrn, A., Yuan, K., Barker, A., Schreiner, C., & Froemke, R. (2010). Developmental sensory experience balances cortical excitation and inhibition. *Nature*, 465, 932 - 936. https://doi.org/10.1038/nature09119.
11. Douglas, R. M., Neve, A., Quittenbaum, J. P., Alam, N. M., & Prusky, G. T. (2006). Perception of visual motion coherence by rats and mice. Vision Research, 46(18), 2842–2847. https://doi.org/10.1016/j.visres.2006.02.025
12. Farrell JS, Lovett-Barron M, Klein PM, Sparks FT, Gschwind T, Ortiz AL, et al. Supramammillary regulation of locomotion and hippocampal activity. Science. 2021; 374(6574):1492–6. Epub 2021/12/17. https://doi.org/10.1126/science.abh4272 PMID: 34914519; PubMed Central PMCID: PMC9154354.
13. Furukawa, S., Xu, L., & Middlebrooks, J. C. (2000). Coding of sound-source location by ensembles of cortical neurons. *Journal of Neuroscience*, *20*(3), 1216-1228.
14. Geisler C, Robbe D, Zugaro M, Sirota A, Buzsaki G. Hippocampal place cell assemblies are speed controlled oscillators. Proc Natl Acad Sci U S A. 2007; 104(19):8149–54. Epub 2007/05/02. https://doi. org/10.1073/pnas.0610121104 PMID: 17470808; PubMed Central PMCID: PMC1876586.
15. Ghosh, M., Yang, F.-C., Rice, S. P., Hetrick, V., Gonzalez, A. L., Siu, D., Brennan, E. K. W., John, T. T., Ahrens, A. M., & Ahmed, O. J. (2022). Running speed and REM sleep control two distinct modes of rapid interhemispheric communication. Cell Reports, 40(1), 111028. https://doi.org/10.1016/j.celrep.2022.111028
16. Gois Z, Tort ABL. Characterizing Speed Cells in the Rat Hippocampus. Cell Rep. 2018; 25(7):1872–84 e4. Epub 2018/11/15. https://doi.org/10.1016/j.celrep.2018.10.054 PMID: 30428354.
17. Haggerty DC, Ji D. Activities of visual cortical and hippocampal neurons co-fluctuate in freely moving rats during spatial behavior. Elife. 2015; 4. Epub 2015/09/09. https://doi.org/10.7554/eLife.08902 PMID: 26349031; PubMed Central PMCID: PMC4595967.
18. Heath, J. (Ed.). (2018). Multiphoton Microscopy (1st ed.). John Wiley & Sons.

19. Hermundstad, A., Briguglio, J., Conte, M., Victor, J., Balasubramanian, V., & Tkačik, G. (2014). Variance predicts salience in central sensory processing. eLife, 3. https://doi.org/10.7554/eLife.03722.
20. Herrnstein, R. (1977). The evolution of behaviorism.. *American Psychologist*, 32, 593-603. https://doi.org/10.1037/0003-066X.32.8.593.
21. Hoy, J. L., Yavorska, I., Wehr, M., & Niell, C. M. (2016). Vision drives accurate approach behavior during prey capture in laboratory mice. Current Biology, 26(22), 3046–3052. https://doi.org/10.1016/j.cub.2016.09.009
22. 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.
23. Konishi, M. (2003). Coding of Auditory Space. Annual Review of Neuroscience, 26(1), 31–55. https://doi.org/10.1146/annurev.neuro.26.041002.131123
24. Kuchibhotla, K., & Bathellier, B. (2018). Neural encoding of sensory and behavioral complexity in the auditory cortex. Current opinion in neurobiology, 52, 65-71.
25. Kwon, J., Ogawa, K., & Miyake, Y. (2014). The Effect of Visual Apparent Motion on Audiovisual Simultaneity. *PLoS ONE*, 9. https://doi.org/10.1371/journal.pone.0110224.
26. Liu, R. C., & Schreiner, C. E. (2007). Auditory cortical detection and discrimination correlates with communicative significance. PLoS biology, 5(7), e173.
27. Li, Z., Wei, J.-X., Zhang, G.-W., Huang, J. J., Zingg, B., Wang, X., Tao, H. W., & Zhang, L. I. (2021). Corticostriatal control of defense behavior in mice induced by auditory looming cues. Nature Communications, 12(1). https://doi.org/10.1038/s41467-021-21248-7
28. Machens, C., Gollisch, T., Kolesnikova, O., & Herz, A. (2005). Testing the Efficiency of Sensory Coding with Optimal Stimulus Ensembles. Neuron, 47, 447-456. https://doi.org/10.1016/j.neuron.2005.06.015.
29. Maluck, E., Busack, I., Besseling, J., Masurat, F., Turek, M., Busch, K., & Bringmann, H. (2019). A wake-active locomotion circuit depolarizes a sleep-active neuron to switch on sleep. PLoS Biology, 18. https://doi.org/10.1371/journal.pbio.3000361
30. Malmierca, M. S., Sanchez-Vives, M. V., Escera, C., & Bendixen, A. (2014). Neuronal adaptation, novelty detection and regularity encoding in audition. Frontiers in systems neuroscience, 8, 111.

31. Marques, T., Summers, M. T., Fioreze, G., Fridman, M., Dias, R. F., Feller, M. B., & Petreanu, L. (2018). A role for mouse primary visual cortex in motion perception. Current Biology, 28(11). https://doi.org/10.1016/j.cub.2018.04.012
32. McGinley, M. J., Vinck, M., Reimer, J., Batista-Brito, R., Zagha, E., Cadwell, C. R., Tolias, A. S., Cardin, J. A., & McCormick, D. A. (2015). Waking state: Rapid variations modulate neural and behavioral responses. Neuron, 87(6), 1143–1161. https://doi.org/10.1016/j.neuron.2015.09.012
33. McGinley MJ, David SV, McCormick DA. Cortical Membrane Potential Signature of Optimal States for Sensory Signal Detection. Neuron. 2015; 87(1):179–192. https://doi.org/10.1016/j.neuron.2015.05.038 WOS:000360977400017. PMID: 26074005
34. Niell CM, Stryker MP. Modulation of visual responses by behavioral state in mouse visual cortex. Neuron. 2010; 65(4):472–479. https://doi.org/10.1016/j.neuron.2010.01.033 PMID: 20188652; PubMed Central PMCID: PMC3184003.
35. Nelken I. Stimulus-specific adaptation and deviance detection in the auditory system: experiments and models. Biol Cybern. 2014; 108(5):655–63. Epub 20140130. https://doi.org/10.1007/s00422-014-0585-7 PMID: 24477619.
36. Polack, P.-O., Friedman, J., & Golshani, P. (2013). Cellular mechanisms of brain state–dependent gain modulation in visual cortex. Nature Neuroscience, 16(9), 1331–1339. https://doi.org/10.1038/nn.3464
37. Rothschild G, Eban E, Frank LM. A cortical-hippocampal-cortical loop of information processing during memory consolidation. Nat Neurosci. 2017; 20(2):251–9. Epub 20161212. https://doi.org/10.1038/nn. 4457 PMID: 27941790; PubMed Central PMCID: PMC5783826.
38. Rosen, M. (2019). Dreaming of a stable world: vision and action in sleep. Synthese, 1-36. https://doi.org/10.1007/s11229-019-02149-1.
39. Saleem AB, Ayaz A, Jeffery KJ, Harris KD, Carandini M. Integration of visual motion and locomotion in mouse visual cortex. Nat Neurosci. 2013; 16(12):1864–9. Epub 2013/11/05. https://doi.org/10.1038/ nn.3567 PMID: 24185423; PubMed Central PMCID: PMC3926520.
40. Saleem AB, Diamanti EM, Fournier J, Harris KD, Carandini M. Coherent encoding of subjective spatial position in visual cortex and hippocampus. Nature. 2018; 562(7725):124–7. Epub 2018/09/12. https:// doi.org/10.1038/s41586-018-0516-1 PMID: 30202092; PubMed Central PMCID: PMC6309439.

41. Schneider DM, Nelson A, Mooney R. A synaptic and circuit basis for corollary discharge in the auditory cortex. Nature. 2014; 513(7517):189–94. Epub 20140827. https://doi.org/10.1038/nature13724 PMID: 25162524; PubMed Central PMCID: PMC4248668.
42. Schneider, D. M. (2020). Reflections of action in sensory cortex. Current Opinion in Neurobiology, 64, 53–59. https://doi.org/10.1016/j.conb.2020.02.004
43. Schinkel-Bielefeld, N., David, S., Shamma, S., & Butts, D. (2012). Inferring the role of inhibition in auditory processing of complex natural stimuli.. *Journal of neurophysiology*, 107 12, 3296-307 . https://doi.org/10.1152/jn.01173.2011.
44. Schröger, E., & Widmann, A. (1998). Speeded responses to audiovisual signal changes result from bimodal integration.. *Psychophysiology*, 35 6, 755-9 . https://doi.org/10.1111/1469-8986.3560755.
45. Stirman, J. N., Townsend, L. B., & Smith, S. L. (2016). A touchscreen based global motion perception task for Mice. Vision Research, 127, 74–83. https://doi.org/10.1016/j.visres.2016.07.006
46. Svoboda, K., & Yasuda, R. (2006). Principles of two-photon excitation microscopy and its applications to neuroscience. Neuron, 50(6), 823–839. https://doi.org/10.1016/j.neuron.2006.05.019
47. Timberlake, W. (1997). An animal-centered, causal-system approach to the understanding and control of behavior. *Applied Animal Behaviour Science*, 53, 107-129. https://doi.org/10.1016/S0168-1591(96)01154-9.
48. Town, S. M., & Bizley, J. K. (2022). Sound localization of world and head-centered space in ferrets. *Journal of Neuroscience*, *42*(22), 4580-4593.
49. Vinck M, Batista-Brito R, Knoblich U, Cardin JA. Arousal and locomotion make distinct contributions to cortical activity patterns and visual encoding. Neuron. 2015; 86(3):740–754. https://doi.org/10.1016/j. neuron.2015.03.028 PMID: 25892300; PubMed Central PMCID: PMC4425590.
50. Vivaldo, C. A., Lee, J., Shorkey, M., Keerthy, A., & Rothschild, G. (2023). Auditory cortex ensembles jointly encode sound and locomotion speed to support sound perception during movement. PLOS Biology, 21(8). https://doi.org/10.1371/journal.pbio.3002277

51. Verdeny-Vilalta, O., Aluja, M., & Casas, J. (2015). Relative roles of resource stimulus and vegetation architecture on the paths of flies foraging for fruit. Oikos, 124, 337-346. https://doi.org/10.1111/OIK.01557.
52. Windt, J. (2018). Predictive brains, dreaming selves, sleeping bodies: how the analysis of dream movement can inform a theory of self- and world-simulation in dreams. Synthese, 195, 2577-2625. https://doi.org/10.1007/s11229-017-1525-6.
53. Wood KC, Town SM, Bizley JK. Neurons in primary auditory cortex represent sound source location in a cue-invariant manner. Nat Commun. 2019; 10(1):3019. Epub 20190709. https://doi.org/10.1038/ s41467-019-10868-9 PMID: 31289272; PubMed Central PMCID: PMC6616358.
54. Zagha, E., Murray, J., & McCormick, D. (2016). Simulating Cortical Feedback Modulation as Changes in Excitation and Inhibition in a Cortical Circuit Model. eNeuro, 3. https://doi.org/10.1523/ENEURO.0208-16.2016.
55. Zhou M, Liang F, Xiong XR, Li L, Li H, Xiao Z, et al. Scaling down of balanced excitation and inhibition by active behavioral states in auditory cortex. Nat Neurosci. 2014; 17(6):841–50. Epub 20140420. https://doi.org/10.1038/nn.3701 PMID: 24747575; PubMed Central PMCID: PMC4108079.
56. Zhou, M., Liang, F., Xiong, X. R., Li, L., Li, H., Xiao, Z., Tao HW & Zhang, L. I. (2014). Laminar-specific Scaling Down of Balanced Excitation and Inhibition in Auditory Cortex by Active Behavioral States. *Nature neuroscience*, *17*(6), 841.