11-7-2019 2:00 PM

Multisensory responses in primary auditory cortex of the cat

Catherine Boucher, *The University of Western Ontario*

Supervisor: Lomber, Stephen G., *The University of Western Ontario*

A thesis submitted in partial fulfillment of the requirements for the Master of Science degree in Neuroscience

Recommended Citation
Boucher, Catherine, "Multisensory responses in primary auditory cortex of the cat" (2019). *Electronic Thesis and Dissertation Repository*. 6718.
https://ir.lib.uwo.ca/etd/6718

Abstract

Core auditory cortex of the cat is comprised of primary auditory cortex (A1) and the anterior auditory field (AAF). Neurons in both fields respond strongly to acoustic stimuli and are tonotopically organized. In hearing animals, a small number of cells in AAF respond to tactile stimulation. However, it is unclear if multisensory input influences responses in A1. In this study, multisensory stimuli were developed by pairing a pure tone stimulus with a flash stimulus at various stimulus onset asynchronies. A linear multielectrode array recorded multi-unit activity in A1 across cortical layers. We identified unisensory auditory, unisensory visual, bimodal, and subthreshold multisensory multi-unit activity. We also found neurons where auditory-visual interactions either suppressed or enhanced neuronal activity. Additionally, visual stimulation can modulate the neural response to auditory inputs depending on the stimulus onset asynchrony. Taken together, the majority of neural activity in A1 in the cat is influenced by visual inputs.

Keywords

Hearing, vision, multisensory, neuroplasticity, auditory cortex, electrophysiology, cat

Summary for Lay Audience

We know our brains process information from our eyes and ears, however little is known about how the brain processes and combines these two senses. Integration is known to occur, as seeing lip movements influences the speech sounds we hear. In this thesis, we investigated how the auditory part of the brain can be affected by vision. We will be examining the electrical activity of individual neurons in an animal model to determine if changes in activity affect perception. We hypothesize that neurons in the auditory part of the brain are mainly activated by sound, but vision can modulate this activation. By comparing the sensory differences of neurons, we will be able to examine how the integration of sound and vision occurs in the brain. Understanding this sensory integration will better our ability to understand auditory processing in the brain, and changes in the brain following hearing loss.

Co-Authorship Statement

Catherine Boucher, Xiaohan Bao, Yaser Merrikhi, Alex M. Meredith, Stephen G. Lomber

As primary author of this thesis, Catherine Boucher was responsible for experimental design, data collection, analysis, and writing of the manuscript. Xiaohan Bao and Yaser Merrikhi offered guidance and assisted in data collection. M. Alex Meredith offered support with experimental design and data analysis. Stephen G. Lomber was the principal investigator for this project, and oversaw the experimental design, surgical procedures, data collection, analysis, and writing of the manuscript.

Acknowledgments

There are many people who have supported me and ensured the completion of this project. First and foremost, I would like to thank my supervisor Dr. Steve Lomber for guiding and mentoring me. I owe my passion and dedication for science to Dr. Lomber, who gave me the opportunity to transition from psycholinguistics to neural recordings, and fully supported me as a graduate student. Thank you to Dr. M. Alex Meredith, for showing me how to be a better electrophysiologist and scientific writer. I would also like to thank the members of my advisory committee, Dr. Brian Corneil, Dr. Brian Allman, Dr. Blake Butler, and Dr. Ingrid Johnsrude for their insight and expertise.

Thank you to every member of the Cerebral Systems Lab for your support and collaboration. I would especially like to thank Xiaohan Bao and Dr. Yaser Merrikhi for helping me complete these grueling recordings. I could not have done it without both of you. I am also grateful to Paisley Barnes, for ensuring I had healthy, hearing participants for this project. This work was a group effort and would not have been possible otherwise.

This project would not have been possible without the support of my loved ones. My grandparents encouraged me to pursue a career in science and supported me as an undergraduate. My dearest friends Aly, Victoria, and Sapphire shared their love and laughter that kept me sane. My partner Charlie Frantowski pushed me to use Python, taught me functional programming, and was always there no matter what. I love you all, nobody should do science alone.

Table of Contents

List of Figures

List of Abbreviations and Symbols

A	Anterior
A1	Primary auditory cortex
A2	Second auditory cortex
AAF	Anterior auditory field
ABR	Auditory brainstem response
aes	Anterior ectosylvian sulcus
ALLS	Anterolateral lateral suprasylvian area
cm	Centimetre
D	Dorsal
dB	Decibel
DZ	Dorsal zone of auditory cortex
EEG	Electroencephalography
fMRI	Functional magnetic resonance imaging
hr	Hour
Hz	Hertz
IC	Inferior colliculus
i.m.	Intramuscular
i.v.	Intravenous

kg	Kilogram
kHz	Kilohertz
LFP	Local field potential
mg	Milligram
MGN	Medial geniculate nucleus
mL	Millilitre
ms	Millisecond
nHL	Normal hearing level
P	Posterior
PAF	Posterior auditory field
pes	Posterior ectosylvian sulcus
PSTH	Peristimulus time histogram
s	Second
s.c.	Subcutaneous
SOA	Stimulus onset asynchrony
SPL	Sound pressure level
ss	Suprasylvian sulcus
V	Ventral
vPAF	Ventral posterior auditory field
μm	Micrometre

%	Percent

Chapter 1

1 Introduction

Over a century ago, the neocortex was found to be partitioned into structurally related areas (Brodmann, 1909). The structure and organization of sensory cortical areas were examined, and neocortical maps and processing models were developed (see Kaas and Collins, 2001 for review). Areas of sensory cortex were parcellated into specific regions for information processing from a particular sense. Different visual cortical regions, for example, process features like motion (Dubner and Zeki, 1971), faces (Gross et al., 1972; Perrett et al., 1982), and objects (Tsao et al., 2003). These unisensory areas, which process information from one sense, project to higher-order cortical regions. These were thought to be responsible for the integration of processing originating from multiple unisensory regions. This multisensory integration has behavioural advantages such as improving stimulus detection (Stein et al., 1989; Spence et al., 1998; McDonald et al., 2000; Lovelace et al., 2003) and decreasing reaction times (Hershenson, 1962). Despite strong evidence supporting a model centered around unisensory processing, functional investigations have identified neurons at early stages of cortical activation capable of multisensory integration (see Ghazanfar and Schroeder, 2006 for review). This development suggests that cortical sensory processing models remain poorly understood.

The aim of the present study is to describe how multisensory input influence neuronal responses at the earliest stage of cortical activation in the auditory system, namely, the primary auditory cortex (A1). I will first review peripheral auditory processing and the ascending auditory pathway up to the auditory cortex. Second, I present knowledge of structural, resting state, and functional connectivity in A1. Third, the principles of multisensory integration will be explained and evidence of multisensory integration in A1 will be discussed. Finally, the rationale and hypothesis for the investigation conducted in this thesis will be specified.

1.1 The ascending auditory pathway

The human peripheral auditory system includes the outer ear, middle ear, and inner ear, and transforms sound waves into electrochemical signals for further processing in the central nervous system. The outer ear is composed of the pinna and the entrance of the ear canal. Sound waves enter the ear canal and are detected by the tympanic membrane, which converts and amplifies the sound waves into mechanical pulses for the ossicles. The ossicles transmit the sound through the oval window to the cochlea, which includes the Organ of Corti on the basilar membrane. The Organ of Corti is composed of specialized sensory hair cells arranged in a tonotopic, frequency to place, organization and it responds differently to the spectral information of sounds. High frequency sounds displace the base of the basilar membrane, while low frequency sounds displace the apex. Additionally, the displacement of the basilar membrane produces a Fourier transformation, as the auditory hair cells convert mechanical time-dependent signals into electrical signals corresponding to the frequency and amplitude of a sound. Each auditory hair cell connects to auditory nerve fibers forming the auditory nerve, where different frequencies activate specific auditory nerve fibers, while the intensity is represented by the firing rate of those fibers. The auditory nerve transmits electrical signals to the brainstem. The auditory brainstem is composed of the cochlear nucleus and the superior olivary complex. The cochlear nucleus is found in the medulla, and the nuclei from both sides converge, forming synaptic connections with the superior olivary complex. Auditory processing then occurs in the inferior colliculus within the midbrain via the lateral lemniscus. Finally, the inferior colliculus projects to the medial geniculate nucleus (MGN) of the thalamus, and it relays auditory signals to the primary auditory cortex. The MGN can alter incoming auditory signaling based on feedforward and feedback connections (Winer et al., 2005), and structural and functional differences exist between the ventral, dorsal and medial subregions (Banks and Smith, 2011) which affect the output to the auditory cortex.

1.2 Connectivity of the primary auditory cortex

1.2.1 Structural connectivity

The core auditory cortex receives its dominant projection from the ventral MGN of the thalamus, has the shortest latencies in auditory cortex, and is comprised of A1 and the anterior auditory field (AAF) in mice (Hunt et al., 2006), ferrets (Bizley et al., 2005), and cats (Reale and Imig, 1980). **Figure 1** illustrates the location of the core auditory cortex in mice, ferrets, and cats. In primates, the core auditory cortex is positioned on the lower bank of the lateral sulcus, and includes A1 caudally and area R rostrally (Merzenich and Brugge, 1973; Morel et al., 1993; Hackett et al., 1998; Baumann et al., 2013). Within A1, there are thalamocortical, corticocortical, corticothalamic, and commissural inputs. Identification of these types of connections is necessary when it comes to understanding information processing within A1.

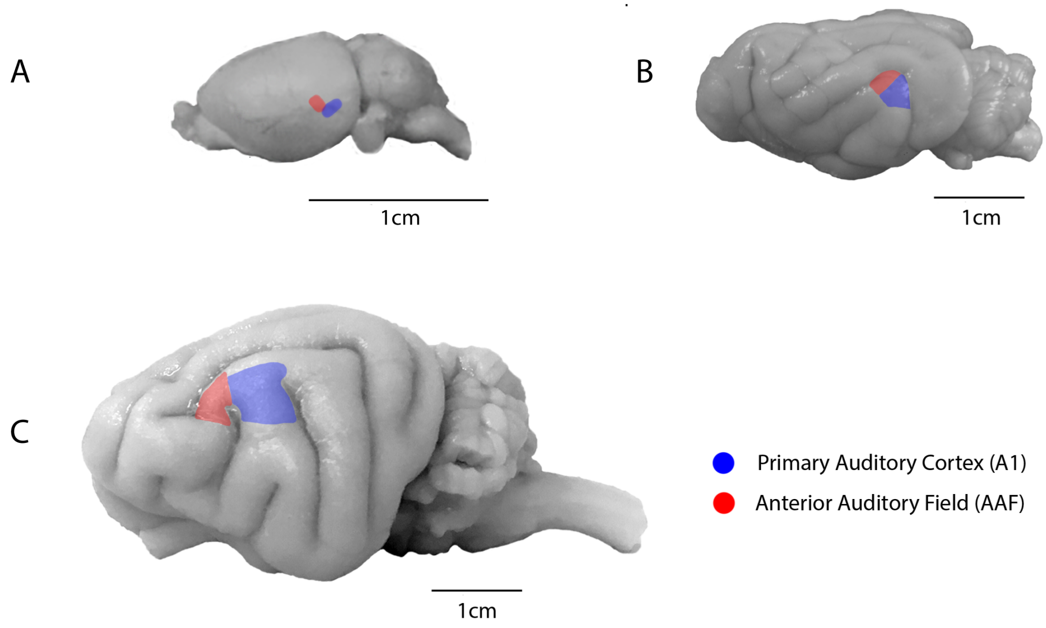


Figure 1. A lateral view of the left core auditory cortex in the mouse, ferret, and cat.

In each case, AAF is located rostral to A1. **A)** In mice, auditory cortex is found in the caudal half of the parietal cortex (Stiebler et al., 1997). **B)** In ferrets, core auditory cortex is located on the dorsal region of the middle ectosylvian gyrus (Kelly et al., 1986; Kowalski et al., 1995; Bizley et al., 2005). **C)** In cats, A1 is found across the middle ectosylvian gyrus and within a portion of the posterior ectosylvian sulcus, and AAF is found along the anterior ectosylvian gyrus (Merzenich et al., 1975; Knight, 1977; Reale and Imig, 1980; Carrasco and Lomber, 2009a; Hall and Lomber, 2015).

In the cat, thalamocortical projections from the MGN to A1 include projections from the ventral division of the MGN that are largest in number, projections from the medial division that are lesser in number, and projections from the dorsal division that are fewest in number (Huang and Winer, 2000). Corticocortical connections within A1 primarily project to dorsal regions of this auditory cortical area, and connections to the ventral portion are sparse (Chabot et al., 2015). Most of the corticocortical connectivity originating and terminating in A1 involves AAF, second auditory area (A2), posterior auditory field (PAF), and ventral posterior auditory field (vPAF) (Winer, 1992). Corticothalamic connections from A1 to the MGN have not been investigated in detail, but originate in the infragranular layers (Winer, 1992). Lastly, commissural connections between the two hemispheres include A1 (Code and Winer, 1985). Other commissural connections originating in A1 also project to AAF, A2, and PAF, and connections terminating in A1 also come from AAF (Winer, 1992). This structural connectivity network resembles the resting state connectivity within A1.

1.2.2 Resting state connectivity

Resting state connectivity derived from functional magnetic resonance imaging (fMRI) correlates with structural connectivity (Greicius et al., 2009) and facilitates the translation of animal models. The dorsoposterior auditory network (including primarily A1, PAF, and DZ) in hearing cats is positively correlated with the ventral auditory network, the anterior auditory network, the right lateral sulcus, and the left lateral sulcus (Stolzberg et al., 2018). The dorsoposterior auditory network is also negatively correlated with the superior colliculus, the posterior medial visual network, the anterior lateral visual network, and the somatosensory network (Stolzberg et al., 2018). This resting state connectivity is similar to the anatomical evidence discussed previously, and these may contribute to the functional organization of A1.

1.2.3 Functional connectivity

The core auditory cortex, made up of A1 and AAF, is functionally organized for processing low-level auditory stimuli. Across species, the core auditory cortex has a tonotopic organization maintained by thalamocortical, corticocortical, and commissural projections (Lee and Winer, 2005). Tonotopic regions of auditory cortex, including A1, maximally respond to pure tone stimuli compared to broadband noise (Hall and Lomber, 2015) and more complex auditory stimuli (Hall et al., 2016). In the mouse, frequency representations increase from the caudal to rostral ends of A1 and increase from the rostral to caudal ends of AAF (Stiebler et al., 1997). Thus, both core auditory regions share a high frequency border. The core auditory cortex is also tonotopically organized in the ferret, where high frequencies are represented dorsally and low frequencies ventrally in both subregions (Kelly et al., 1986; Phillips et al., 1988; Kowalski et al., 1995; Bizley et al., 2005). In the cat, the lowest frequencies are located rostroventrally in AAF and caudoventrally in A1, and the highest frequencies are situated at the border between AAF and A1 (Merzenich et al., 1975; Reale and Imig, 1980; Carrasco and Lomber, 2009a; Hall and Lomber, 2015). The orientation of isofrequency bands in core auditory cortex is reversed in primates. In macaques, A1 represents high to low frequencies caudorostrally and R has a reverse representation, such that both regions share a low frequency border (Merzenich and Brugge, 1973; Morel et al., 1993; Hackett et al., 1998; Kaas and Hackett, 2000; Baumann et al., 2013). Organization of bandwidth measurements (Schreiner and

Sutter, 1992) and stimulus intensity (Schreiner et al., 1992) are also present in A1. The most ventral and dorsal portions of A1 display large receptive fields, while central A1 has the sharpest receptive fields (Schreiner and Sutter, 1992). Additionally, clusters within A1 will alternate with preferences for low response thresholds (dB SPL) and for high response thresholds (Schreiner et al., 1992). The representational organizations of the auditory cortex influences activation in response to auditory stimuli.

Studies using reversible cooling deactivation have shown that tonotopic regions, including A1, AAF, and PAF, are highly interconnected. For example, deactivation of AAF results in decreased activation of unaffected frequencies within A1, sharpened response bandwidths, and increased response thresholds (Carrasco and Lomber, 2009a). Response latency and characteristic frequency tuning are not affected in A1 from cooling AAF. On the other hand, cooling deactivation of A1 does not change the responsiveness of AAF (Carrasco and Lomber, 2009a). Also, A1 deactivation significantly reduces the strength of PAF responses (Carrasco and Lomber, 2009b). These results suggest that AAF has a greater influence on A1 processing, than A1 does in AAF, and PAF processing is highly dependent on A1.

1.3 Multisensory Integration

Multisensory integration is the process where information from the senses is combined at the level of the individual neuron to develop and influence perception, cognition, and behaviour. A focus of previous investigations was on the convergence of inputs in multisensory regions. The anterior ectosylvian sulcus (Wallace et al., 1992) and the superior colliculus (Meredith and Stein, 1983) of the cat, for example, are regions where auditory, visual, and somatosensory inputs converge and form multisensory circuits. These circuits include bimodal, subthreshold and unimodal neurons, and multisensory integration can be observed from response enhancement or suppression. Bimodal neurons will respond to a stimulus "A" and a stimulus "B", and integrate the two stimuli when presented together. Subthreshold neurons will respond to a stimulus "A" but will not respond to a stimulus "B", and when two stimuli are presented together the response to stimulus "A" will be either enhanced or suppressed by the presence of stimulus "B". Lastly, unisensory neurons will only respond to either a stimulus "A" or a stimulus "B". Different forms of sensory processing arise from distinct anatomical architectures. Bimodal connections are formed with neurons that receive multiple inputs from two sensory modalities, subthreshold connections are formed with neurons that receive more inputs from a dominant modality and fewer inputs from another modality, and unisensory connections are formed with neurons that only receive inputs from one sensory modality (Clemo et al., 2012).

Traditionally, multisensory integration was believed to be a function of higher-order association areas of the cortex. Higher-order association cortex (Jones and Powell, 1970), the prefrontal cortex (Fuster et al., 2000), the anterior ectosylvian sulcus (Jiang et al., 2002), the lateral suprasylvian sulcus (Jiang et al., 2002), the ventral parietal area, the lateral intraparietal area, and the superior temporal sulcus (Bruce et al., 1981; Hikosaka et al., 1993) integrate information from presumed unisensory regions of auditory and visual cortex. Yet, while A1 does show a preference for the auditory sensory modality, it also exhibits multisensory integration (see Ghazanfar and Schroeder, 2006 for review). This integration is characterized by weak spatial precision (Fu et al., 2004) and high temporal acuity (Ghazanfar et al., 2005). A few neural pathways could contribute to multisensory integration in A1, such as direct feedforward projections from cortical or thalamic regions, proximity to multisensory areas, and feedback connections from higher-order multisensory areas (Driver and Noesselt, 2008).

1.3.1 Multisensory integration in the primary auditory cortex

Multisensory integration within the auditory system occurs early in A1 across species and is supported by anatomical connections from different sensory modalities. A1 of non-human primates is anatomically connected to visual cortex (Falchier et al., 2002) and somatosensory cortex (Luethke et al., 1989; Cappe and Barone, 2005), and in the cat, only very minor projections are present from areas 17 and 18 to A1 (Innocenti et al., 1988). The relative absence of projections from area 17 to A1 in cats is similar to the ferret, where few projections from early visual cortical areas 17, 18, 19, and 20 to core auditory cortex have been observed in normal hearing animals (Bizley et al., 2007, but see Allman et al., 2009). No substantial input to the core auditory cortex from somatosensory regions on the suprasylvian gyrus and the anterior ectosylvian gyrus, or from the somatosensory thalamus (ventrobasal complex) have been observed in normal hearing ferrets (Meredith & Allman, 2012). On the other hand, direct projections from the primary visual cortex to A1 have been reported in the rat (Paperna and Malach, 1991), vole (Campi et al., 2010), and gerbil (Budinger et al., 2006), which demonstrates that the core auditory cortex of these species is structurally different from that of higher-order animal models (see Meredith and Lomber, 2017 for review).

Multisensory integration is reported in A1 of some species. A visual stimulus can modulate the response to a pure tone stimulus in A1 of the gerbil, and metabolic activity in A1 was suppressed relative to activity in AAF or the posterior auditory cortex (Cahill et al., 1996).

In hearing mice, spiking activity in core auditory neurons is elicited predominantly by auditory stimulation, while a small number respond to somatosensory and audiovisual stimuli (Hunt et al., 2006). Similarly, only 6% of neurons responded to visual stimuli and 9% of neurons responded to somatosensory stimulation within A1 of the rat (Wallace et al., 2004). However, the number of multisensory neurons greatly increased at the borders between regions. Non-auditory activation of core auditory cortex, which includes A1 and AAF, occurs in hearing ferrets as well. Visual stimulation affects 15-17% of identified neurons in the core auditory cortex, which typically suppresses auditory inputs (Bizley, Nodal, Bajo, Nelken, & King, 2007; Meredith & Allman, 2015). Additionally, tactile stimulation affects 23% of identified core auditory neurons (Meredith & Allman, 2015). In macaques, somatosensory and visual multisensory integration also occurs in A1. Auditory-evoked activity is modulated by eye positions in A1 (Fu et al., 2004), and eye position also affects the spontaneous activity of single neurons in A1 (Werner-Reiss et al., 2003). Somatosensory inputs reset the phase, and multisensory interactions modulate the amplitude of neuronal oscillations in A1 (Lakatos et al., 2007). Additionally, auditory-visual interactions in A1 are present in local field potentials (LFPs) and single-unit recordings (Kayser et al., 2008). Similar to the findings from Lakatos et al. (2007), auditory LFPs are either enhanced or suppressed by visual stimulation, and enhancement resets the phase and modulates ongoing neuronal oscillations. An additive multisensory response is exhibited in 19% of LFPs and 4% of single-units, and multisensory effects are similar between A1 and the caudal belt (Kayser et al., 2008). These multisensory interactions depend on the efficacy of the visual stimulus and the relative audiovisual timing. Taken together, multisensory integration in A1 and the core auditory cortex is present in gerbils, mice, rats, ferrets, and macaques.

On the other hand, it is less clear if multisensory integration occurs in A1 of the cat. Visually evoked potentials can only be recorded in primary auditory cortex of completely deaf white cats, and of cats that are cochleoectomized during the first week postnatal (Rebillard et al., 1977). A subsequent study found no significant visual or somatosensory activation in A1 of hearing cats or congenitally deaf white cats (Kral et al., 2003). Poststimulus time histograms from one multi-unit exhibited changes in firing rate. The remaining 99% of multi-units did not respond to sensory stimulation in deaf cats, or only respond to auditory stimulation in hearing cats. Also, visual field potentials are present in 60% of recordings sites in hearing cats, however, local generators of these potentials are not found in A1 from current source density analysis (Kral et al., 2003). Since A1 processes non-auditory inputs in many other species, it is surprising to find that A1 is not subject to multisensory integration in the cat.

1.4 Thesis Overview

This thesis aims to determine if multisensory integration in the auditory cortex of the cat involves A1. Previous investigations in A1 of the cat employed visual stimuli only (Rebillard et al., 1977; Kral et al., 2003), and did not report visual integration with an auditory stimulus. Even though A1 dominantly responds to auditory inputs, evidence from other species suggests that multisensory integration may be present in this region. Most importantly, most of the non-auditory activity in A1 of ferrets and macaques is present in the form of subthreshold connections (Ghazanfar et al., 2005; Bizley et al., 2007; Kayser et al., 2008; Meredith and Allman, 2015). The first hypothesis proposes that the number of subthreshold multisensory recording sites will be greater than the number of unimodal visual or bimodal sites in A1 of the cat.

Frequency, sound level, and sound location are low-level auditory stimuli processed by A1 (Reale and Imig, 1980; Schreiner and Urbas, 1988; Malhotra et al., 2008; Carrasco and Lomber, 2009a; Hall and Lomber, 2015). In previous studies of multisensory integration, audiovisual processing was investigated with broadband noise as auditory stimuli (Werner-Reiss et al., 2003; Fu et al., 2004; Wallace et al., 2004; Bizley et al., 2007; Meredith and Allman, 2009, 2015), and fewer studies used pure tones as auditory stimuli (Hunt et al., 2006). Since A1 maximally responds to pure tones (Hall and Lomber, 2015; Hall et al., 2016), it seems more appropriate to use these as auditory stimuli when investigating multisensory integration in A1. The second hypothesis proposes that the presence of a visual stimulus may affect the neural response to a pure tone in A1 of the cat.

Chapter 2

2 Methods

2.1 Overview

Neuronal responses during auditory, visual, and bimodal (visual and auditory) stimulation were measured in the left A1 of 5 adult (> 6 months) cats. Animals were housed in a sensory enriched environment where toys and social interaction were accessible. Experimental procedures were approved by the University of Western Ontario Animal Use Subcommittee of the University Council on Animal Care and conducted in accord with the US National Research Council's *Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research* (2003), the Canadian Council on Animal Care's *Guide to the Care and Use of Experimental Animals* (Olfert ED, Cross BM, McWilliam AA, 1993).

2.2 Surgical procedures.

A hearing threshold of 20dB was confirmed in all animals by the presence of an auditory brainstem response (ABR), prior to electrophysiological recording. Subdermal electroencephalography (EEG) recording leads were inserted above the ears and on the vertex of the scalp, and a ground was placed on the lower back. Auditory stimuli (0.1ms squarewave clicks; range: 20-80 dB NHL) were delivered via speakers positioned in front of each ear. Electrophysological recording involved a two step procedure.

Step 1) First, under general anesthesia, a head-holder was attached to the frontal bone and a recording chamber was placed over primary auditory cortex and flanking cortices. The cat's anesthesia was induced with Dexdomitor (0.04 mg/kg i.m.) to facilitate the insertion of an indwelling catheter. The eyes were covered with an ophthalmic ointment to protect and moisten the cornea. Topical lidocaine was sprayed onto the laryngeal walls to inhibit the gag reflex, and the trachea was intubated with a cuffed endotracheal tube. Dexamethasone (0.5 mg/kg – i.v.) was delivered and Cefazolin (25 mg/kg, i.v.) was administered 30 mins prior to incision of the scalp and every 90 minutes during the procedure. The cat's head was depilated and placed in a stereotaxic frame (David Kopf Instruments, model 1430), lidocaine (20mg/mL, s.c.) was administered along the midline of the scalp (future incision site). Anesthetic depth was maintained with a continuous rate infusion of Alfaxan (7 mg/kg/hr, i.v.). Fentanyl was administered (0.005mg/kg, i.v.) and continuously infused (0.002 mg/kg/hr, i.v.). Core temperature was maintained at 37°C using a water-filled heating pad (Gaymar, model T/pump). Rectal temperature, blood pressure, expired CO_2 levels, heart rate and respiration rate were continuously monitored. A midline scalp incision was made and the left temporalis muscle was removed. A craniotomy exposed A1 in the left hemisphere (Horsley and Clarke, 1908 coordinates A0-A10, **Fig, 2A**), revealing the anterior ectosylvian, posterior ectosylvian, and suprasylvian sulci. A recording chamber was constructed around the craniotomy and the chamber was sealed. A stainless-steel head holder was attached to the frontal bone of the skull, the incision was repaired (3.0 silk sutures) and the cat was removed from the stereotaxic frame for recovery. Animals received buprenorphine (0.02 mg/kg, i.v.), and dexamethasone (0.5 mg/kg, i.v.) to reduce post-surgical inflammation. Routine postoperative care was provided and recovery was uneventful.

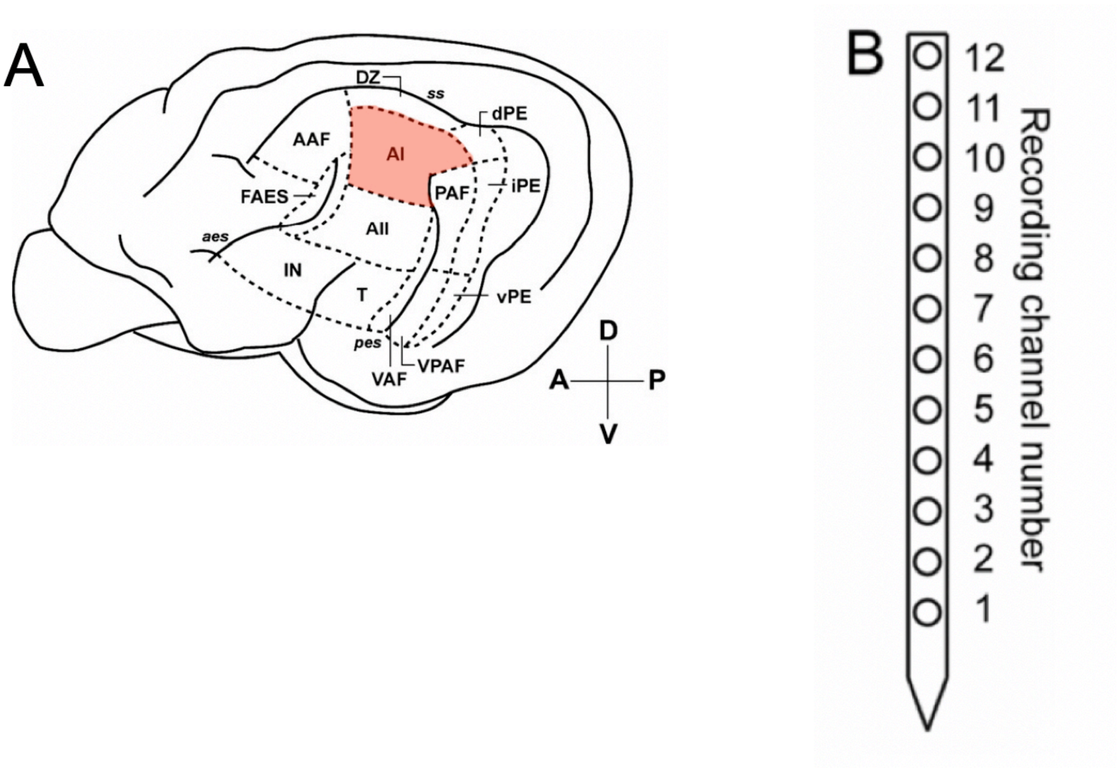


Figure 2. Illustrations of A1 recording procedures.

A) View of the left auditory cortex. A1 is coloured in red, located ventral to the suprasylvian sulcus (ss), and in between the anterior (aes) and poster ectosylvian sulci (pes). **B)** Model of the linear microelectrode array used for recordings. Contact sites are 25µm in diameter and spaced 200µm apart.

Step 2) Seven days later, recording sessions commenced by cannulating the right cephalic vein and the saphenous vein bilaterally with feline indwelling catheters. Anesthesia was induced with ketamine (35 mg/kg, i.m.) and acepromazine (0.4 mg/kg, i.m.). Topical lidocaine was with a cuffed endotracheal tube. Midfrin (pupillary dilator) and atropine drops were administered to each eye. A feline contact lens with optimal focal distance of 25cm was placed on the right/contralateral eye. A black contact lens was used to block visual signals from reaching the left eye (ipsilateral to recording hemisphere). Animals were secured to a stereotaxic frame (David Kopf Instruments, model 1530) via the head holder placed during the first procedure and continuous administration of supplemental anesthetic and fluids (9mg/kg/hr ketamine; 0.5mg/kg/hr acepromazine i.v.) were initiated. Animals were connected to a ventilator and expired CO_2 levels were monitored. Heart rate and blood pressure were monitored to establish baseline levels of anesthesia throughout recording sessions. Core temperature was maintained at 37°C using a water-filled heating pad (Gaymar, model T/pump). Ocular drift and limb movement were prevented by delivering a muscle relaxant (Nimbex, cistracurium besylate; 2mg/kg initial dose; 1.5mg/kg/hr supplement i.v.). The recording chamber was opened and the dura was resected. Tissue desiccation was prevented by applying a layer of Gelfoam. A digital image of the exposed cortical region was taken to maintain a record of cortical penetration tracks. Atropine (0.02 mg/kg, s.c.) and dexamethasone (0.5 mg/kg, s.c.) were administered every 24 hours.

Recording sessions lasted from 70-120 hours. At the end of the experiment, an anticoagulant (heparin, 10,000U; 1 mL) and a vasodilator (1% sodium nitrite, 1 mL) were administered to the animal, followed by Euthanol overdose (sodium pentobarbital, 50 mg/kg, i.v.). Animals were perfused intracardially through the ascending aorta with physiological saline (0.01 M PBS), followed by fixative (4% paraformaldehyde) and 10% sucrose. The brain was removed, photographed, stereotaxically blocked, and placed in 30% sucrose.

2.3 Recording procedures

Multi-unit responses were recorded with twelve-channel platinum-iridium linear array microelectrodes (Microprobes for Life Science, 241µm diameter, 0.3-0.5 MΩ impedances, **Fig, 2B**). Probes were orthogonally lowered from the cortical surface until neuronal responses to pure tones were measured in the deepest and most superficial recording channels of the array. Multi-unit activity was bandpass filtered (500Hz to 5,000Hz), amplified (x10,000) and digitized at 25,000Hz (Tucker Davis Technologies, model RZ2).

Stimulus generation and presentation. Recordings were conducted on a vibration-free table (Technical Manufacturing Corporation, model 63-500) within a double-walled sound chamber. Sensory stimulation was composed of unimodal or bimodal signals delivered across 3 different conditions (**Fig. 3**), described in detail below.

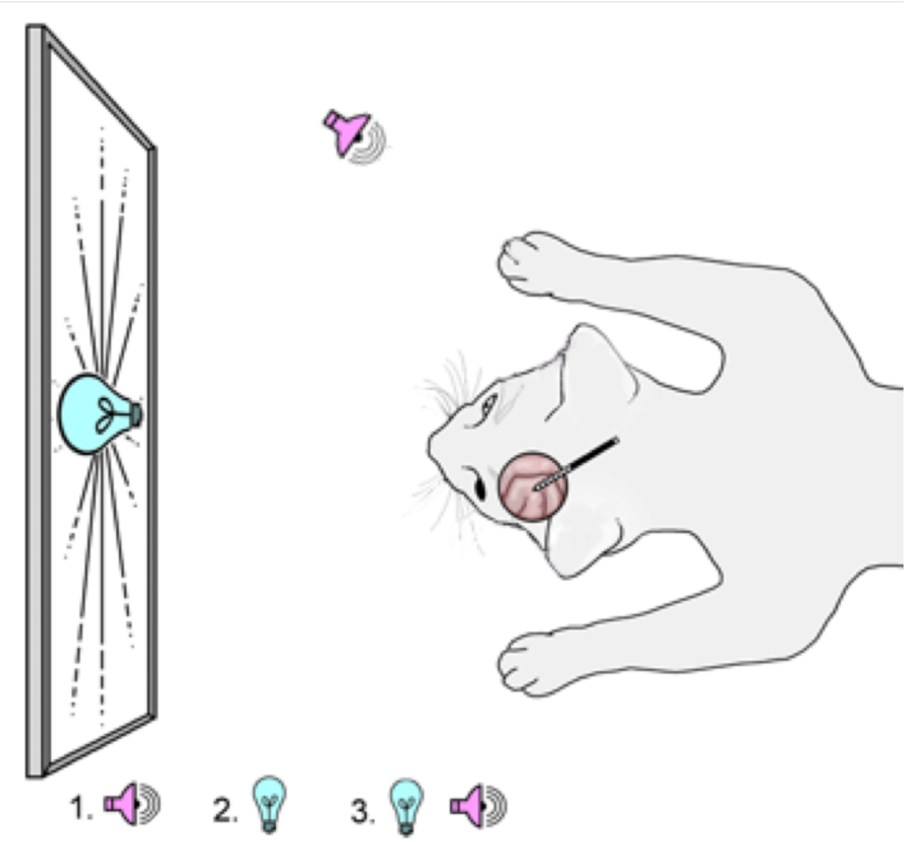


Figure 3. Stimulus presentation for recording.

Responses to auditory, visual, and bimodal stimuli were recorded in A1 of the left hemisphere. Auditory stimuli consisted of pure tones and were played on a speaker placed by the right ear. Visual stimuli consisted of light flashes on a monitor, which stimulated the entire visual field of the right eye. Bimodal stimuli paired the auditory and visual stimuli, where a monitor flash was delivered prior to a pure tone at various stimulus onset asynchronies (SOAs).

Auditory. Pure tones were presented binaurally via a speaker placed 15cm away from the cat's right ear (Tucker Davis Technologies, model MF1 magnetic speaker). Signals were digitally generated with a 24-bit digital-to-analog converter at 156 kHz (Tucker Davis Technologies, model RX6). Acoustic intensity was calibrated using a one-quarter-inch microphone (Brüel and Kjær, model 4939). Multi-unit activity to auditory search stimuli (pure tones) formed a frequency response area. In total, 275 pure tones (5ms cosine squared gated, 50ms in duration) were presented in pseudo-random order. Signals ranged from 500Hz to 32,000Hz in 1/4 octave steps, and 11 intensities extending from 15 to 65dB SPL in 5dB steps. Each frequency-intensity combination was presented for five repetitions. Once auditory responsive multi-units were found online, a responsive frequency with a sound level above the minimum threshold was determined manually from the frequency response areas across the twelve recording sites, was presented for 30 trials, and used for subsequent multisensory analysis.

Visual. Visual stimulation was generated using MATLAB (MathWorks) and the Psychophysics Toolbox (www.psychtoolbox.org), and was delivered via a liquid-crystal display monitor placed ~25cm away from the cat's eyes. A photodiode (Mouser, model 720-BPW21) near the bottom right corner of the display monitor measured the precise time of visual presentation. Flashes were composed of a 50ms long change in the monitor's background luminosity, from black (3 lux) to white (50 lux).

Bimodal. During multi-modal stimulation, visual flashes were delivered prior to auditory pure tone presentation to compensate for differences in modality response latency. Stimulus onset asynchronies (SOA) were 1ms, 40ms, 80ms, 120ms, 160ms, 200ms, and

240ms, and these increased the prospect of multimodal response identification by looking at the effects of visual stimulation across a time window rather than a single point in time.

2.4 Data analysis.

Multi-unit extracellular activity of A1 neurons during periods of acoustic, visual, and/or audiovisual stimulation was examined. Offline single-unit sorting was manually performed using Plexon Sorter. Custom made programs written in Python 3.7 were written to generate dot rasters and peristimulus time histograms (PSTHs) for each of the conditions tested at each recorded site. The spiking activity from the period of no stimulation was determined by averaging -1500ms to -500ms before the stimulus onset. Responses were normalized by subtracting the mean baseline response (from -1500ms to -500ms across trials) from each PSTH. Each PSTH was Gaussian smoothed with a rolling window of 10ms. The spiking activity after stimulus onset from unimodal auditory trials were averaged from 10ms to 75ms, and from unimodal visual trials were averaged from 250 to 500ms. Comparisons in multi-unit response activity across conditions were conducted with Wilcoxon signed-rank tests ($p < 0.05$). Neuronal responses to unisensory stimulation that significantly differed from periods of no stimulation were calculated for each modality tested (auditory, visual, and bimodal) including all audiovisual stimulus onset asynchronies, and were inspected manually. A second Wilcoxon signed-rank test ($p<0.05$) determined multisensory modulation (bimodal, subthreshold) for each detected unit by comparing the averaged response windows of a unit's largest unimodal response to each audiovisual response across seven SOAs (Kayser et al., 2008), and these were corrected for multiple comparisons using Holm-Bonferroni post-hoc tests. If this second Wilcoxon test yielded $p>0.05$ at a particular SOA, no multisensory modulation was observed for that temporal binding window. However, a positive z-score and an adjusted p-value >0.05 indicated multisensory enhancement, and a negative z-score and an adjusted p-value <0.05 indicated suppression. A unit was classified as subthreshold if either enhancement or suppression occurred for at least one SOA. The degree to which the response peak was enhanced or suppressed was quantified using the interactive index (Meredith and Stein, 1983):

$$\left[\frac{BI - Umax}{Umax}\right] \times\ 100 = Interaction\,\%$$

Where *BI* is the response to bimodal stimulation and *Umax* is the largest unimodal response. A score of zero indicates no multisensory modulation, a positive score indicates the percentage of multisensory enhancement, and a negative score indicates the percentage of multisensory suppression.

Chapter 3

3 Results

The goal of this experiment was to evaluate whether auditory responses in A1 can be modulated by visual stimuli and undergo multisensory integration. Responses were collected using a linear microelectrode array from 6 single units, and from 151 multiunit sites. To begin the experiment, every channel at each recording location was presented with auditory pure tone stimuli that formed frequency response areas ranging from 707Hz to 26908Hz and from 15dB SPL to 65dB SPL. A combination of a responsive frequency within a half-octave of the characteristic frequency and sound level above the minimum threshold were presented at each recording location. The frequencies and sound levels of the responsive pure tones presented are depicted in a scatter plot (**Fig. 4**). A variety of pure tone combinations, apart from high frequencies at low sound levels, were obtained from recording locations in A1. Responses to the pure tones were gathered and used to investigate multisensory integration in A1.

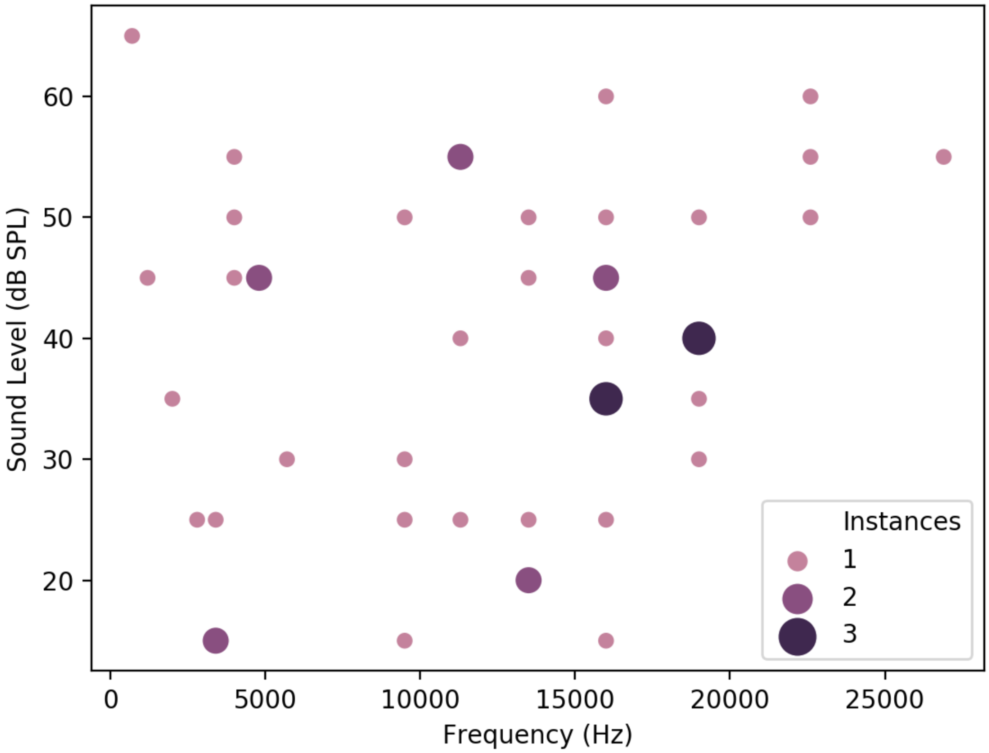
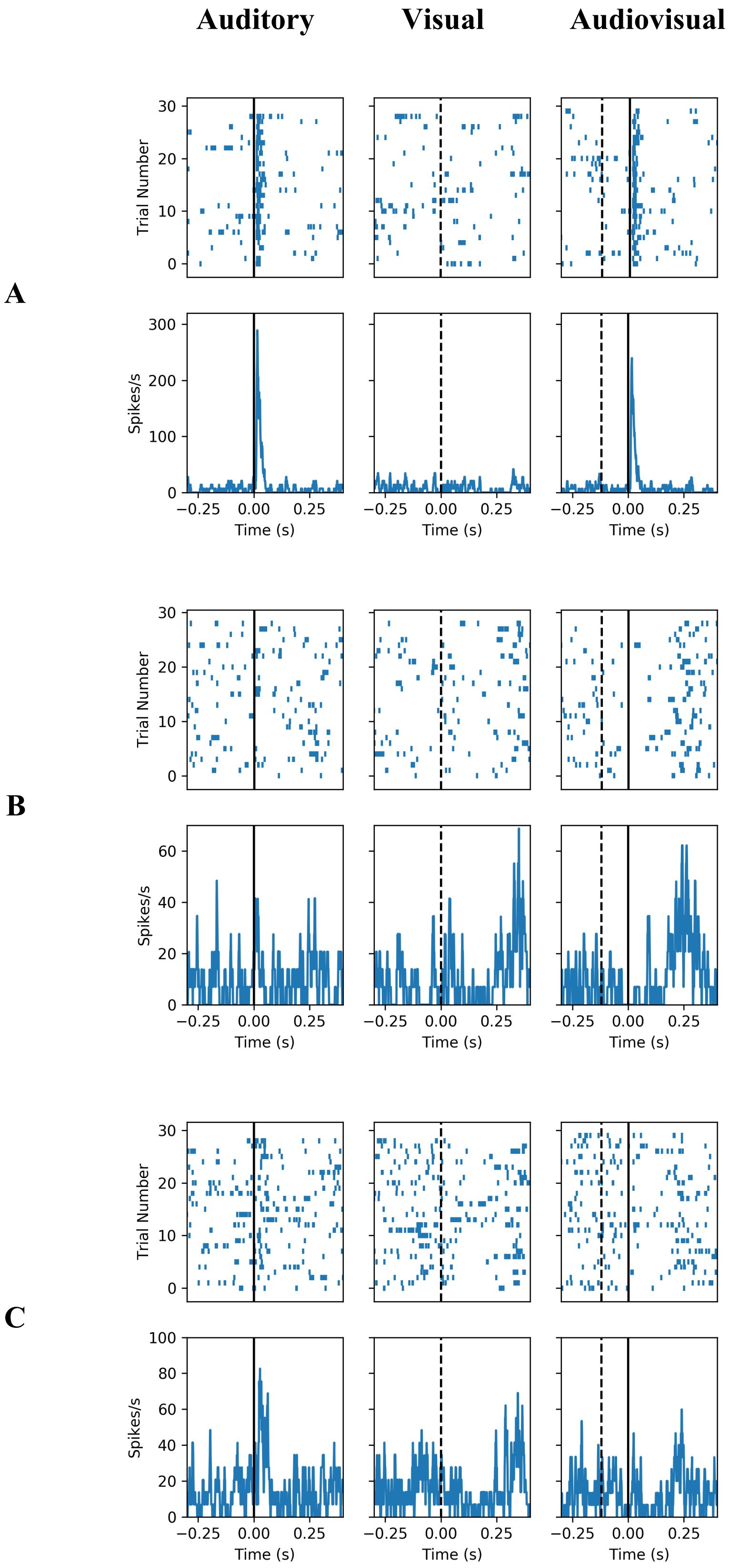


Figure 4. Instances of frequency and sound level combinations presented in A1. 37 distinct pure tones varying in frequency and sound level were presented for a total of 46 recording locations. Responses to these pure tones were used to investigate multisensory integration in A1.

Different types of sensory unit activity were found including unimodal auditory, unimodal visual, subthreshold auditory, subthreshold visual, and bimodal. Neural responses were collected using auditory pure tone stimuli, a visual flash stimulus, and audiovisual stimuli that paired the pure tone following the flash at various SOAs (1ms, 40ms, 80ms, 120ms, 160ms, 200ms, 240ms). Unit activity following an audiovisual stimulus that differed from auditory or visual unisensory responses was defined as multisensory integrative, and responses to an audiovisual stimulus that were comparable to unisensory responses were not multisensory integrative. The sensory responsiveness to auditory, visual, and audiovisual stimuli of sample units are depicted in **Figure 5** using dot rasters and PSTHs.

Unimodal unit activity (**Fig. 5A, 5B**) responded to either auditory or visual stimuli ($p < 0.05$, Wilcoxon signed-rank test) and were not multisensory integrative ($p > 0.05$, Wilcoxon signed-rank test). Bimodal unit activity (**Fig. 5C**) responded to both auditory and visual stimuli, and could be multisensory integrative ($p < 0.05$, Wilcoxon signed-rank test) or non-integrative ($p > 0.05$, Wilcoxon signed-rank test). Subthreshold auditory or visual unit activity (**Fig. 5D, 5E**) responded to either auditory or visual stimuli and were multisensory integrative ($p < 0.05$, Wilcoxon signed-rank test). Subthreshold bimodal units (**Fig. 5F**) were also discovered, where responses to either auditory or visual stimuli did not occur but a response to the audiovisual stimulus was present ($p < 0.05$, Wilcoxon signed-rank test).


Auditory
Visual
Audiovisual
A
B
C
Trial Number
Spikes/s
Time (s)
0
10
20
30
0
100
200
300
0
20
40
60
80
100
−0.25
0.00
0.25

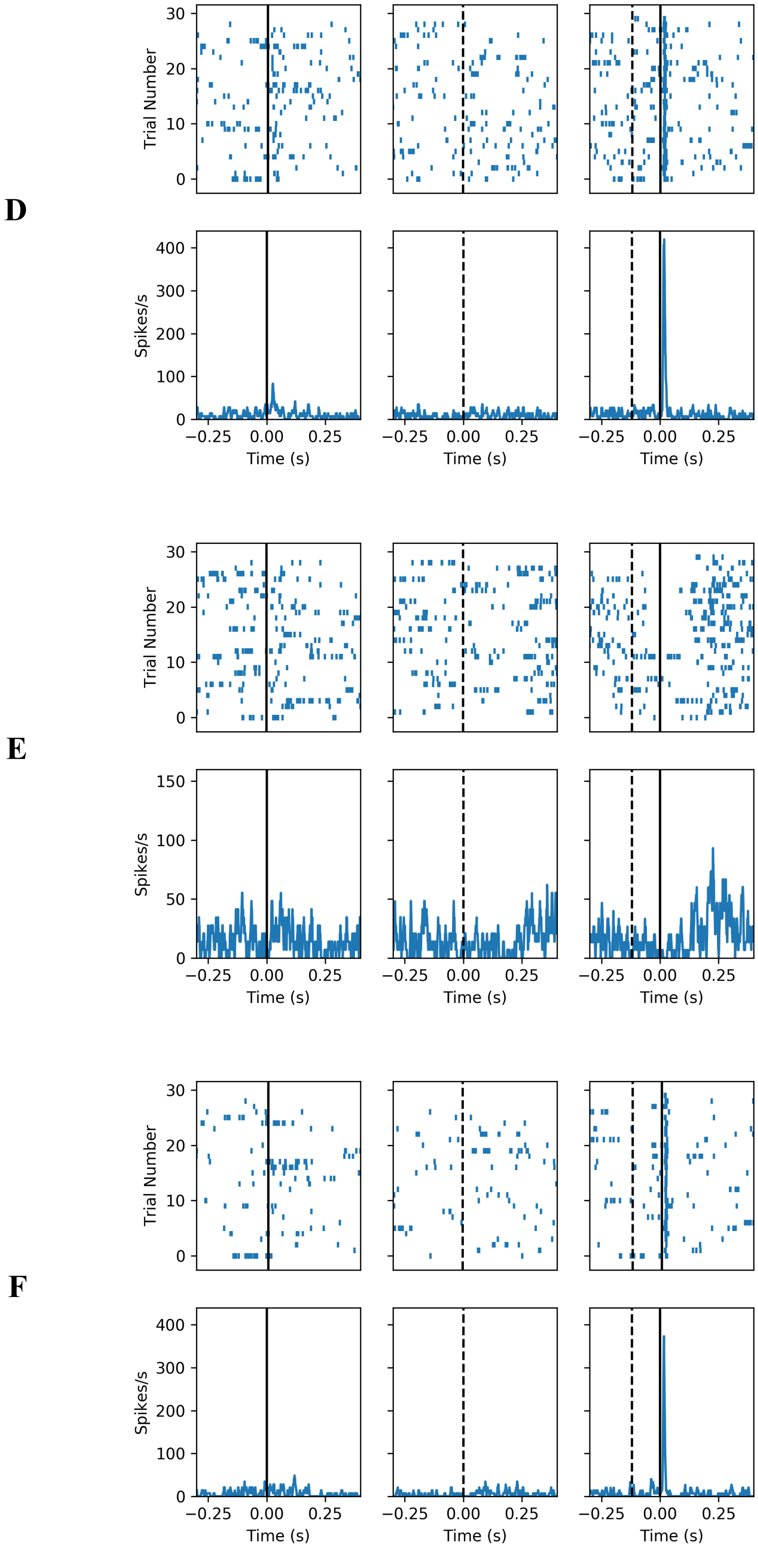


Figure 5. Sample unimodal and multisensory multi-unit activity in A1.

Responses to an auditory stimulus are in the left column, responses to a visual stimulus are in the middle column, and responses to an audiovisual stimulus are in the right column. A solid black line represents the onset of an auditory pure tone stimulus and a dashed black line represents the onset of a visual flash stimulus. The audiovisual stimulus has a SOA of 120ms, with the flash preceding the onset of the pure tone. **A)** Responses of unimodal auditory multiunit activity. The response to the auditory stimulus is equivalent to the response to the audiovisual stimulus ($p = 0.85$, Wilcoxon signed-rank test). **B)** Responses of unimodal visual multiunit activity. The response to the visual stimulus is equivalent to the response to the audiovisual stimulus ($p = 1.00$, Wilcoxon signed-rank test). **C)** Responses of bimodal suppressed multiunit activity. The response to the auditory and visual stimuli is greater than the response to the audiovisual stimulus ($p = 0.003$, Wilcoxon signed-rank test). **D)** Responses of subthreshold enhanced auditory multiunit activity. The response to the auditory stimulus is lesser than the response to the audiovisual stimulus ($p = 0.000001$, Wilcoxon signed-rank test). **E)** Responses of subthreshold enhanced visual multiunit activity. The response to the visual stimulus is lesser than the response to the audiovisual stimulus ($p = 0.002$, Wilcoxon signed-rank test). **F)** Responses of subthreshold bimodal enhanced multiunit activity. The only response occurs from the audiovisual stimulus ($p = 0.000003$, Wilcoxon signed-rank test).

The present study also revealed that the majority of neural activity in A1 is influenced by visual inputs. The proportions of all multisensory and unimodal units recorded in A1 are depicted in **Figure 6.** Unimodal auditory multiunit activity is detected less frequently than expected. Unimodal auditory and subthreshold auditory activity made up a little over half of all sensory responses. The remaining is divided up by unimodal visual, subthreshold visual, bimodal, and subthreshold bimodal unit activity.

Visual and bimodal units may be present across cortical layers and around A1 borders. Non-auditory activity was found across frequencies, and channels of the linear microelectrode array (**Fig. 7**). Channel 1 was positioned deepest within A1, and channel 12 was positioned at the surface of A1. Visual responses appeared to be more frequent at recording sites that process lower frequencies (**Fig. 7A**), however more data is needed to confirm this trend. Auditory units were mostly recorded from the central channels (5-9) of the microelectrode array, only channel 3 did not record any visual units, and most bimodal units were recorded from channels 5, 9, and 10. (**Fig. 7B**). Also, the majority of unimodal auditory and subthreshold auditory activity was sampled along central A1 (**Fig. 8**). Also, most of the visual or bimodal activity was recorded along the borders of A1. These results need to be confirmed with precise histological analysis, but they suggest that sub-regions of A1 can integrate or are modulated by visual inputs.

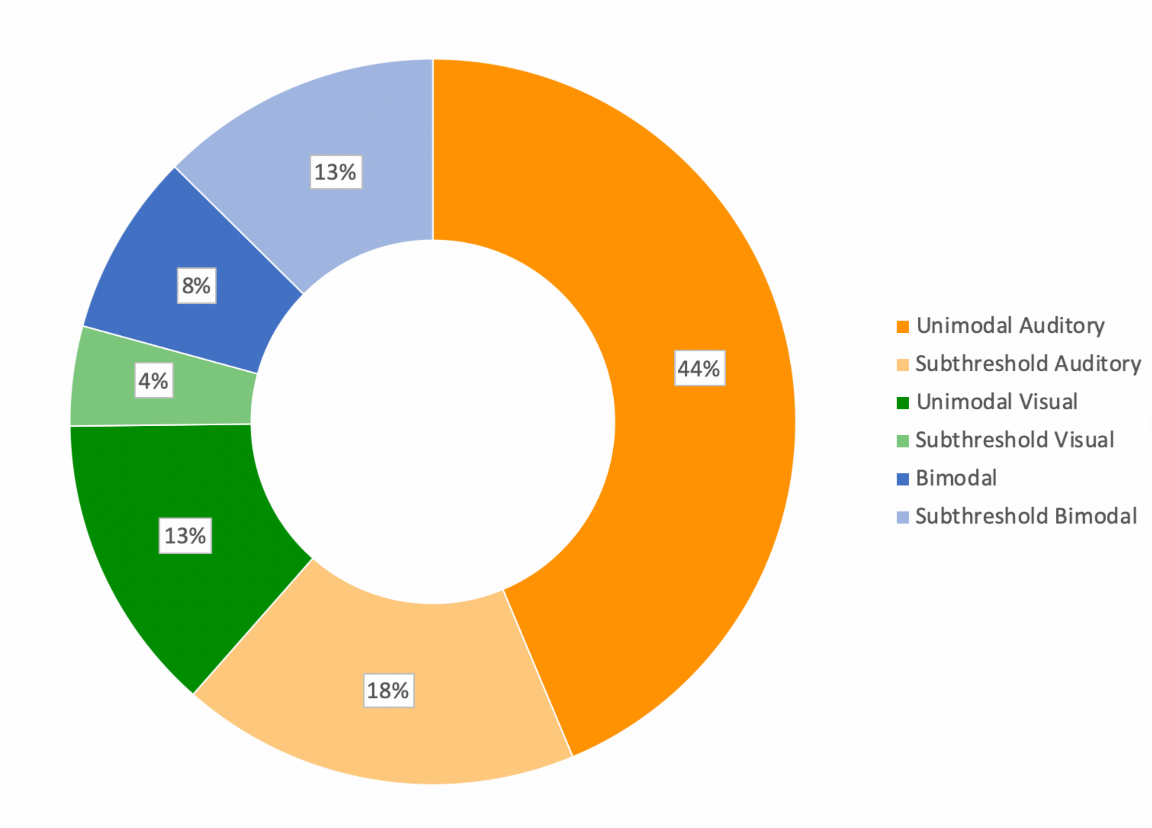


Figure 6. Proportions of unit activity in A1.

Auditory units are depicted in orange, visual units are depicted in green, and bimodal units are depicted in blue. Unimodal or bimodal units have an opaque colour and subthreshold units have a transparent colour. In total (n = 135), 59 units are classified as unimodal auditory, 18 units are unimodal visual, 24 units are subthreshold auditory, 6 units are subthreshold visual, 11 units are bimodal, and 17 units are subthreshold bimodal.

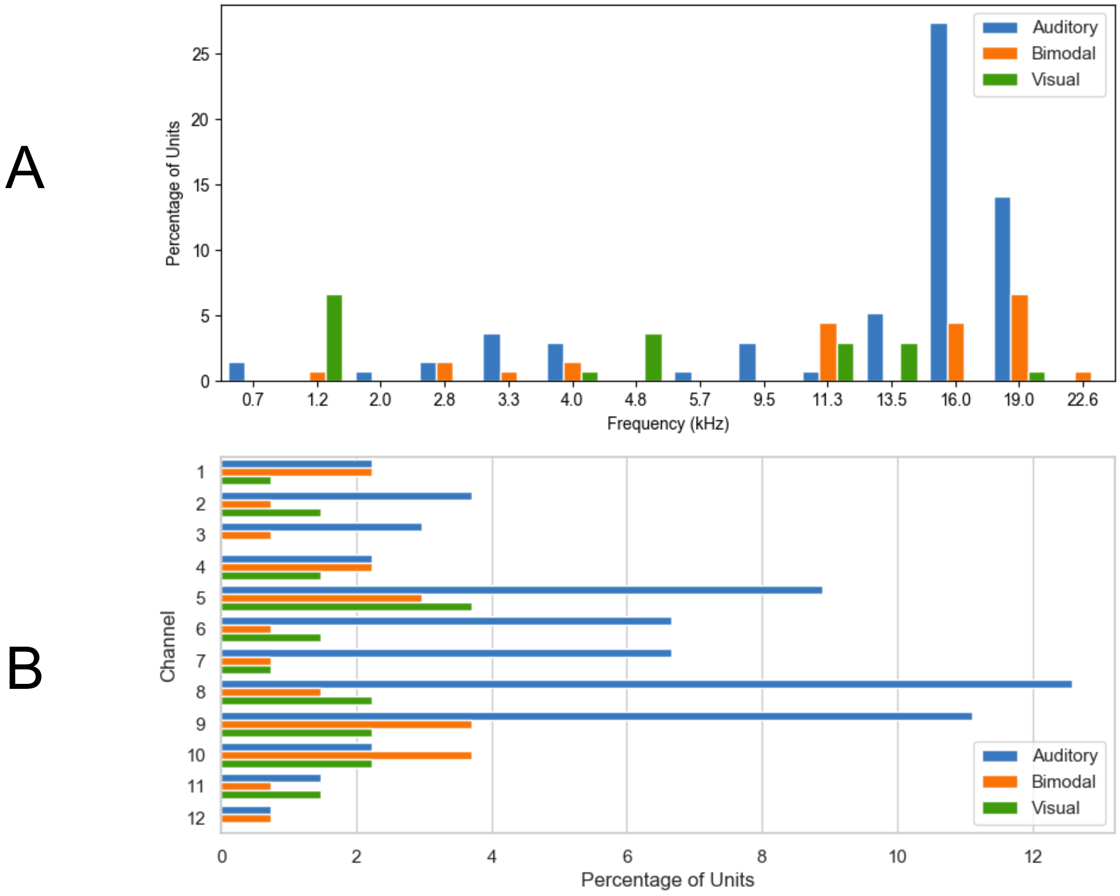


Figure 7. Unit activity across frequencies and channels.

The auditory category includes auditory unimodal and auditory subthreshold units, the visual category includes visual unimodal and visual subthreshold units, and the bimodal category includes bimodal integrative, bimodal non-integrative, and bimodal subthreshold units. **A)** The x-axis represents the frequency of pure tones, and the y-axis represents the percentage of unit activity from each of the three categories. Visual or bimodal units are found across frequencies, and most of the auditory units are found at higher frequencies. **B)** The x-axis represents the percentage of unit activity from each of the three categories and the y-axis represents the channel number. Channel 1 is positioned deepest within A1, and channel 12 is positioned at the surface of A1. Most of the auditory activity is found in the central channels.

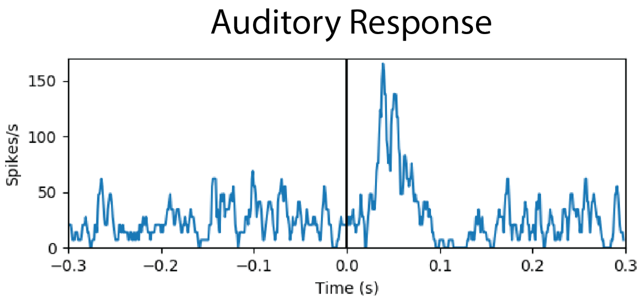
Figure 8. Illustrative model of unit activity and their location in A1.

Illustration of recording locations in A1 and the categories of sensory responsiveness found at each location. Each recording location is depicted as an ellipse, and sensory categories as colours. Unimodal or bimodal activity is represented by a more saturated colour, and subthreshold activity is represented by a more transparent colour. Most of the non-auditory activity is found along the dorsal (D) and ventral (V) borders of A1, with some bimodal activity along the posterior (P) and anterior (A) borders.

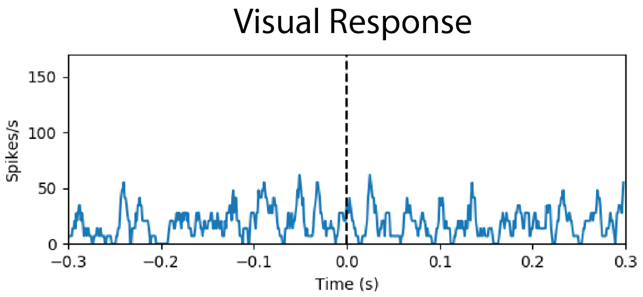
Visual modulation of pure tone processing is dependent on the SOA, which is indicative of multisensory integration. Unit activity following an audiovisual stimulus that was greater than auditory or visual unisensory responses was defined as enhanced, unit activity following an audiovisual stimulus that was lesser than unisensory responses was defined as suppressed, and unit activity following an audiovisual stimulus that was comparable to unisensory responses was unmodulated. The sensory responsiveness to auditory (**Fig. 9A**), visual (**Fig 9B**), and audiovisual stimuli (**Fig. 9C-E**) of a sample auditory subthreshold unit is depicted using PSTHs. This sample unit is unresponsive to a visual stimulus in isolation, but its auditory response is most suppressed when a flash occurs 120ms prior to the onset of a pure tone. At other SOAs of 1ms or 240ms, this unit's auditory response is unmodulated by the visual flash stimulus. Across units, multisensory enhancement or suppression was quantified using the multisensory interaction index (Meredith and Stein, 1983). The most enhancement appears between 120ms and 160ms SOA and the least suppression appears at 1ms and 40ms SOA (**Fig. 10**). Separate repeated-measures ANOVAs were conducted, one for suppression and the other for enhancement. An effect of SOA was observed for units with suppressed multisensory interaction indices ($F_{(6,453)=}2.468$, $p = 0.023$]), but no effect of SOA was observed for units with enhanced multisensory interaction indices ($F_{(6,461)=}0.605$, $p = 0.727$]). Also, post hoc Tukey's HSD tests revealed no significant differences ($p>0.05$) between SOAs for enhanced or suppressed units. Taken together, SOAs may have a greater effect on multisensory suppression rather than enhancement in A1.

The mean response profiles of auditory, visual, and bimodal populations show some key differences in sensory responsiveness. Following an auditory stimulus, auditory units respond with a firing rate higher than the response from bimodal units (**Fig. 11A**). A weak increase in firing rate occurs from visual units, and is under 20 spikes/s. All of these responses are aligned with the onset of the pure tone and have latency of 15-20ms. Bimodal units respond faintly to the onset of visual flash stimulus with a latency of approximately 40ms, while the firing rate of visual units decreases around 150ms then increases around 300ms (**Fig. 11B**). Auditory units do not respond to the flash stimulus. Following an audiovisual stimulus with a SOA of 120ms, bimodal units respond twice, first following the onset of the flash and again following the onset of the pure tone (**Fig. 11C**). Visual units respond with increase in firing rate 300ms after the onset of the flash, and the audiovisual response is much greater compared to the visual-only response. Auditory units respond to the onset of the pure tone, and the firing rate following the audiovisual stimulus is suppressed slightly compared to the response from a pure tone stimulus alone. These findings demonstrate that visual inputs exist, and multisensory modulation occurs in A1.

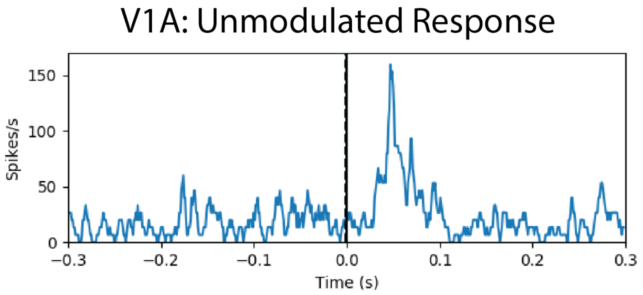
A


Auditory Response

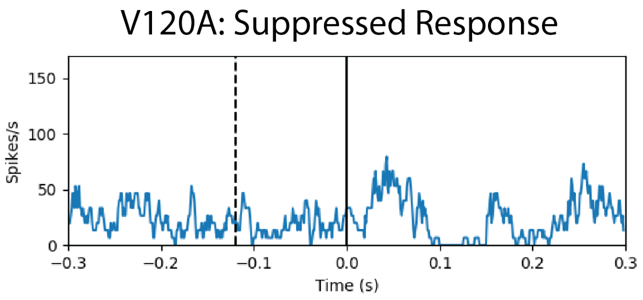
B


Visual Response

C


V1A: Unmodulated Response

D


V120A: Suppressed Response

E

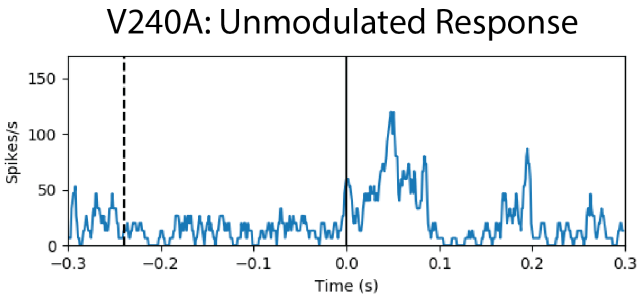

V240A: Unmodulated Response

Figure 9. Sample subthreshold unit activity across stimulus conditions.

Sample unit activity, categorized as subthreshold auditory, following an auditory pure tone stimulus, a visual flash stimulus, and audiovisual stimuli combining the pure tone and the flash at three different SOAs. **A)** The response to the auditory stimulus peaks at 148 spikes/s ($p < 0.000001$, Wilcoxon signed-rank test). **B)** The activity following the visual stimulus peaks at 65 spikes/s, and is not considered to be a neural response ($p = 0.21$, Wilcoxon signed-rank test). **C)** The response to an audiovisual stimulus, presenting the flash stimulus 1ms before the onset of the pure tone, peaks at 160 spikes/s ($p = 0.35$, Wilcoxon signed-rank test) and is considered to be unmodulated by the presence of the flash. **D)** The response to an audiovisual stimulus, presenting the flash stimulus 120ms before the onset of the pure tone, peaks at 62 spikes/s ($p = 0.01$, Wilcoxon signed-rank test) and is considered to be suppressed by the presence of the flash. **E)** The response to an audiovisual stimulus, presenting the flash stimulus 240ms before the onset of the pure tone, peaks at 101 spikes/s ($p = 0.35$, Wilcoxon signed-rank test) and is considered to be unmodulated by the presence of the flash.

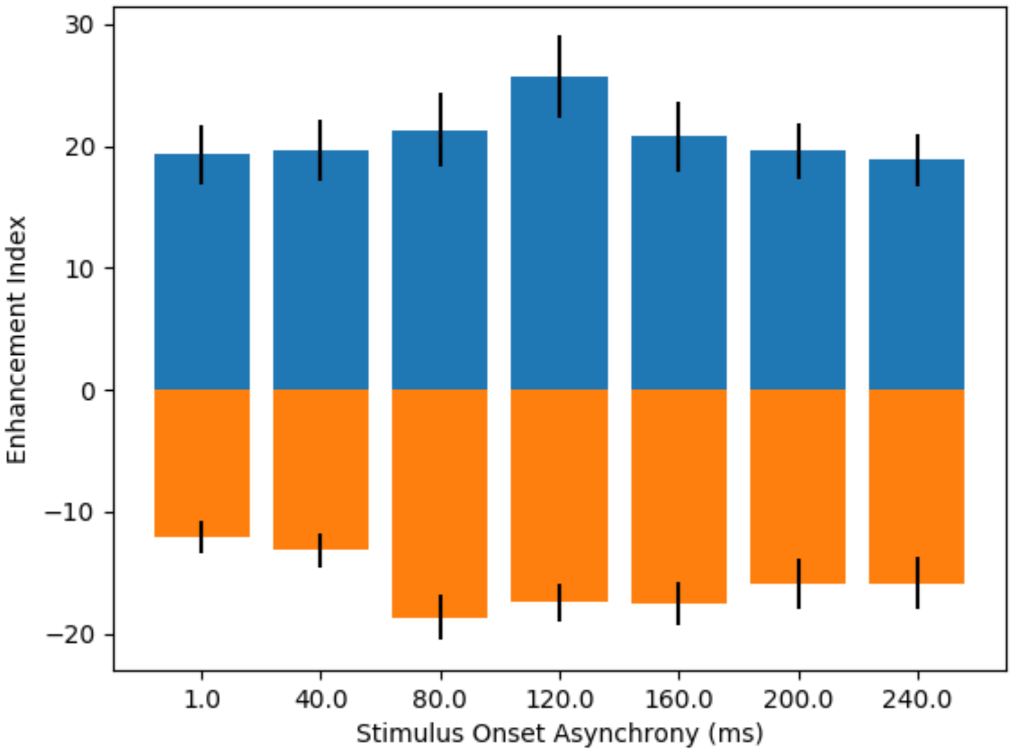
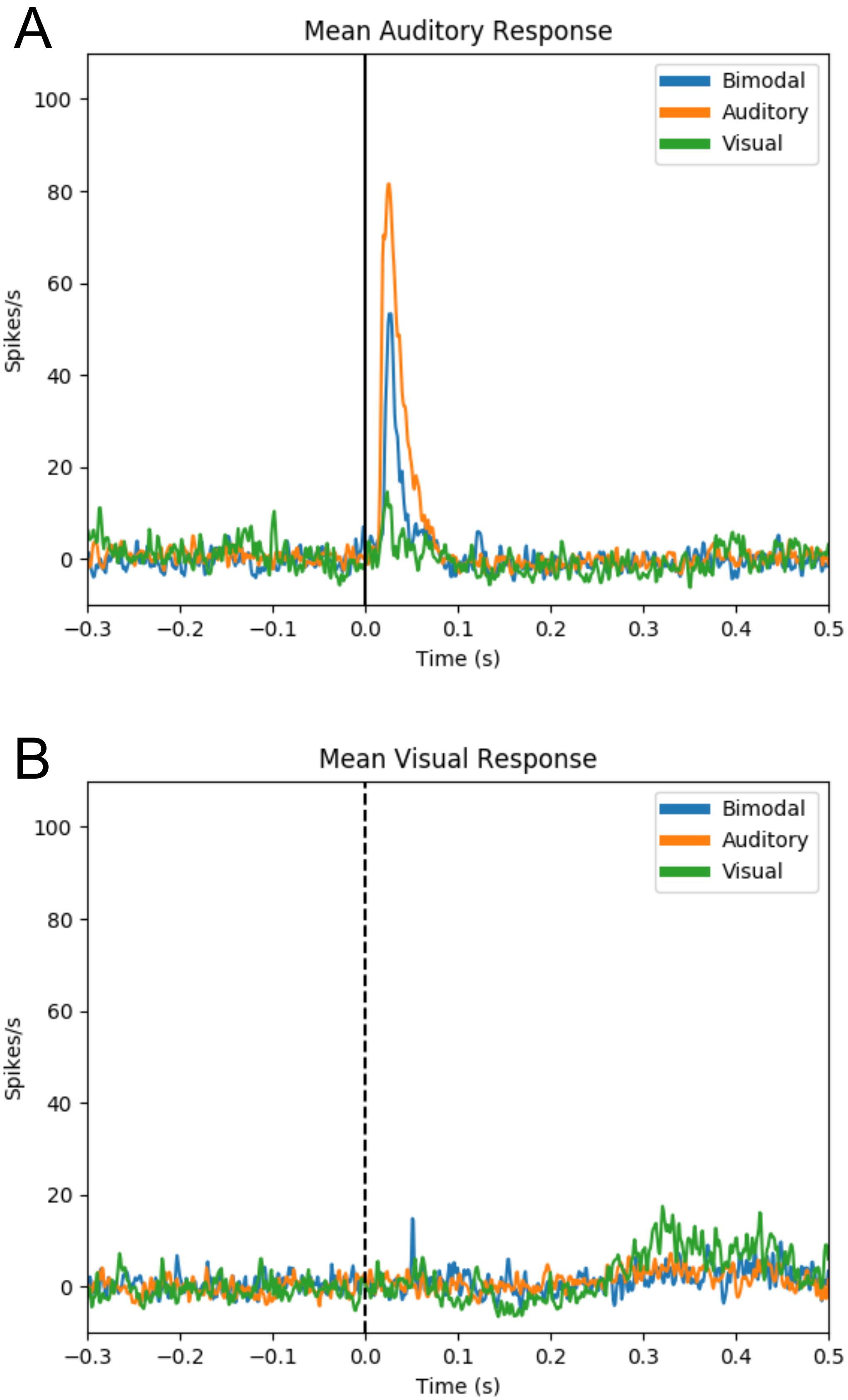


Figure 10. Visual modulation following an audiovisual stimulus is dependent on the stimulus onset asynchrony.

The x-axis represents the SOA of the audiovisual stimuli, and the y-axis represents the multisensory interaction index from unimodal and multisensory units. A positive index indicates enhancement and a negative index indicates suppression. The blue bars depict the mean index of enhanced units across SOAs, and the orange bars depict the mean index of suppressed units across SOAs. The error bars depict the standard error of the mean.

Enhanced units appear to be modulated the most at 120ms SOA and suppressed units appear to be modulated the least at 1ms and 40ms SOA. Statistical comparisons are based on separate one-way repeated measures ANOVAs and Tukey's post hoc tests to account for the multiple comparisons.


A
Mean Auditory Response
Spikes/s
100
80
60
40
20
0
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4
0.5
Time (s)
Bimodal
Auditory
Visual
B
Mean Visual Response
Spikes/s
100
80
60
40
20
0
−0.3
−0.2
−0.1
0.0
0.1
0.2
0.3
0.4
0.5
Time (s)
Bimodal
Auditory
Visual

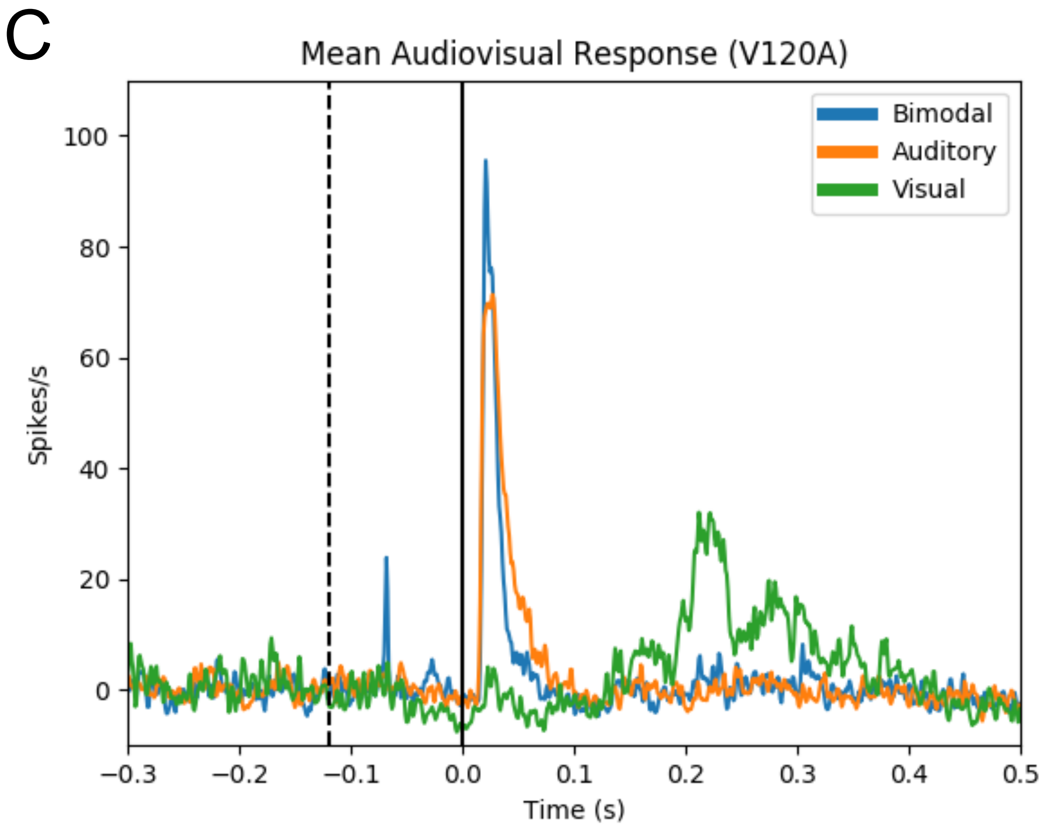


Figure 11. Average responses of auditory, visual, and bimodal populations.

The mean response from bimodal units (integrative, non-integrative, and subthreshold) are depicted in blue, the mean response from auditory units (unimodal and subthreshold) are depicted in orange, and the mean response from visual units (unimodal and subthreshold) are depicted in green. A solid black line represents the onset of an auditory pure tone stimulus and a dashed black line represents the onset of a visual flash stimulus. **A)** The response to the auditory stimulus across sensory categories. **B)** The response to the visual stimulus across sensory categories. **C)** The response to an audiovisual stimulus at 120ms SOA across sensory categories.

Chapter 4

4 Discussion

This study presents evidence of visual modulation of auditory responses, and visual responses in A1 of the cat. Most of the non-auditory unit activity is subthreshold, rather than unimodal visual or bimodal. Also, the presence of a visual stimulus can affect the response to a pure tone, where auditory-visual interactions either suppressed or enhanced neuronal activity. These results are supported by findings from other species, demonstrating multisensory integration or non-auditory modulation in A1 (Cahill et al., 1996; Werner-Reiss et al., 2003; Fu et al., 2004; Wallace et al., 2004; Hunt et al., 2006; Bizley et al., 2007; Lakatos et al., 2007; Kayser et al., 2008; Meredith and Allman, 2015). It is currently difficult to determine the significance of visual inputs to A1, but it is possible that visual influence and multisensory modulation could influence A1 function such as frequency tuning or sound localization.

Higher-order regions of auditory cortex demonstrate a greater capacity for multisensory integration compared to the core auditory cortex (Ghazanfar and Schroeder, 2006; Kayser et al., 2008; Bizley and King, 2009). In the cat, A1 is strongly connected to, and borders, the dorsal zone of auditory cortex (DZ) (Barone et al., 2013). This auditory area is multisensory integrative and auditory responses can be modulated by visual input (Kok, 2015). Additionally, transitional regions bordering other cortical areas tend to be more multisensory integrative (Wallace et al., 2004). Since DZ is multisensory integrative and strongly connected to A1, auditory processing in the dorsal subregion of A1 could be visually modulated via corticocortical connections. To elucidate this hypothesis, recording from a greater number of units across A1 along with the histological reconstruction of those recording penetrations is required.

The timing (Meredith et al., 1987), location (Meredith and Stein, 1986), and efficacy (Meredith and Stein, 1983) of a stimulus are known to influence multisensory integration. The presence and magnitude of multisensory enhancement and suppression are temporally sensitive (Meredith and Stein, 1985, 1986; Meredith et al., 1987), even in A1 (Kayser et al., 2008). The present study confirmed that varying the SOA has differential effects on enhancement and suppression in A1. The spatial location of a stimulus can also affect multisensory integration, such that stimuli presented contralaterally tend to be enhanced and stimuli presented ipsilaterally tend to be suppressed (Meredith and Stein, 1986; Lakatos et al., 2007). Auditory stimuli were presented contralaterally, and full-field visual stimuli were presented to the contralateral eye in the current study. Ipsilateral auditory or visual stimulation may have differential multisensory effects in A1. Manipulating the auditory source location and visual receptive field may also affect multisensory integration. Lastly, stimuli with weaker effectiveness or intensity will yield greater multisensory enhancement or suppression (Meredith and Stein, 1983). In the present study, only one sound level was selected for each recording site and one visual luminance was selected for all recordings. The stimulus effectiveness principle could be examined in the future to investigate if auditory stimulus type (noise bursts, pure tones, FM sweeps, vocalizations, etc.), frequency tuning, sound level, and visual luminance affect multisensory integration in A1.

Multisensory enhancement and suppression of auditory responses in A1 vary between macaques and cats. In macaques, multisensory interaction effects were assessed with SOAs of ±320ms, 160ms, 40ms, and 20ms, and the SOA where the visual stimulus preceded the auditory stimulus by 40ms had the greatest effect on the auditory response (Kayser et al., 2008). The present study showed that the visually modulated response to a pure tone is affected by an interval of 10ms in the SOA and is non-significantly greatest at a SOA where the visual stimulus preceded the auditory stimulus by 120ms. The disparity in the most effective SOA between results suggests that multisensory integration in A1 may have different mechanisms in the macaque and the cat, or originate from different sensory pathways.

Previous studies of multisensory integration in A1 reported a smaller proportion of bimodal and subthreshold neurons (Hunt et al., 2006; Bizley et al., 2007; Meredith and Allman, 2015). Only 7.5% of neurons in the core auditory cortex of mice (Hunt et al., 2006) and 2% (Bizley et al., 2007) to 4% (Meredith and Allman, 2015) of neurons in A1 of ferrets were bimodal and responded to visual stimulation. Yet, this investigation reports 17% of A1 activity in the cat responds to visual stimulation and were classified as unimodal visual, subthreshold visual, or bimodal. It may be that visual responses in A1 are more frequently detected from multiunit activity than from single-units, similarly to findings reported in DZ (Kok, 2015). It is also possible that visually classified units could respond to an auditory stimulus other than a pure tone, thus being bimodal in nature. Additionally, 7% (Bizley et al., 2007) to 8% of neurons in A1 of ferrets are subthreshold auditory (Meredith and Allman, 2015), while our study found that 18% of A1 unit activity in the cat is subthreshold auditory. These previous studies presented auditory noise burst stimuli, while the current investigation presented auditory pure tone stimuli. Since A1 maximally responds to pure tone stimuli (Hall and Lomber, 2015; Hall et al., 2016), it is possible that a larger number of subthreshold connections are formed with neurons that respond to pure tones instead of noise bursts. A future experiment could compare the extent of auditory subthreshold responses in A1 using noise burst and pure tone stimuli.

The multisensory modulation observed in A1 may arise from visual corticocortical connections, or visual input along the ascending auditory pathway (Allman et al., 2009). Neurons in the dorsal cochlear nucleus and inferior colliculus of normally developed brains of cats and guinea pigs have been shown to respond to somatosensory or trigeminal ganglion stimulation (Aitkin et al., 1981; Kanold & Young, 2001; Shore & Zhou, 2006; Shore et al., 2000). Additionally, neurons in the inferior colliculus (IC) can respond or be modulated by a visual stimulus (Syka and Radil-Weiss, 1973; Tawil et al., 1983; Mascetti and Strozzi, 1988; Porter et al., 2007; Bulkin and Groh, 2012). The proportion of neurons within the IC that display responses to visual stimuli or exhibited saccade-related activity varies between studies. Earlier investigations using anesthetized and paralyzed cats reported almost a tenth of neural activity in the IC is visually responsive (Tawil et al., 1983; Mascetti and Strozzi, 1988), while a more recent experiment conducted in awake, behaving macaques found that almost two thirds of responses in the IC exhibited visual activity (Porter et al., 2007). Taken together, it is possible that non-auditory responses in subcortical areas underlies the multisensory integration observed in A1, to the extent that projections originating from the IC and the ventral MGN may carry information from the visual or somatosensory systems.

Findings from the IC may elucidate the anatomical sources of visual input in A1 which are currently unknown. The IC of the cat receives direct projections from the retina (Paloff et al., 1985) and from the visual cortex (Cooper and Young, 1976). These retinal projections may cause visual activation of the IC as Tawil et al. (1983) has reported visually responsive cells with response latencies ranging from 20ms to 30ms. These responses are consistent with the ~20ms latency recorded from the optic tract of the cat (Freund et al., 1972). Our findings demonstrate that the population response curve of bimodal units have a response latency of ~40ms following the onset of a visual stimulus. Direct retinal innervation to the IC may be the source of these visual responses in A1. Additionally, in non-human primates, visual responses with latencies ranging between 60 and 115ms were recorded in the IC (Porter et al., 2007). These visual inputs may originate from visual cortex or the superior colliculus (Porter et al., 2007), since the visual cortex has latencies around 100ms in the cat (Ouellette and Casanova, 2006) and the superior colliculus has latencies up to 100ms in the macaque (Bell et al., 2006). A latency of 120ms was measured from the visual and subthreshold activity from our current study, suggesting that these visual inputs could originate from the IC or from corticocortical connections.

Studies of multisensory integration in hearing animals may inform the potential for crossmodal plasticity following deafness, which occurs when a sensory brain region deprived of normal input is reorganized to subserve a remaining sensory modality. Higher-order regions of auditory cortex that are multisensory integrative are expected to undergo greater functional change following deafness, while low-level core areas such as A1 would be expected to undergo less functional change. Currently, evidence for crossmodal plasticity in the core auditory cortex of the cat is conflicting. None of the studies that demonstrate enhanced visual performance in congenitally deaf cats have shown evidence of behavioral or perceptual enhancement arising from reorganization of the auditory core, despite examining the contributions of A1 and AAF to the perception of a wide variety of visual stimulus features (Lomber et al., 2010). Additionally, some studies in congenitally deaf white and hearing cats report minimal output of A1 neurons in response to visual (Stewart and Starr, 1970; Kral et al., 2003) or somatosensory stimuli (Kral et al., 2003). However, one study of deaf cats (congenital and early-cochleectomized) showed the presence of visual evoked potentials in A1 (Rebillard et al., 1977), and AAF of early-deaf cats showed robust somatosensory- and visual crossmodal reorganization that is populated by somatosensory (47.6%), visual (12%), and somatosensory-visual (bimodal, 35%) neurons (Meredith and Lomber, 2011). Additionally, the mechanism of action of the selected anesthetic agents may influence neural activity in the core auditory cortex of anesthetized animals. For example, studies using gaseous halothane have found no visually-evoked activity in A1 of hearing and deaf cats (Kral et al., 2003), while our study performed under ketamine anesthesia has reported visually-evoked activity in A1 of hearing cats. A future electrophysiological study performed under ketamine could yield evidence of crossmodal activity in the primary auditory cortex of early-deaf cats. Since A1 remains structurally coupled to higher-order auditory regions (Chabot et al., 2015), responds to visual stimulation, and given evidence in support of plasticity within other core areas (e.g. AAF), it would be quite surprising to find that A1 is not subject to crossmodal reorganization to some degree.

4.1 Summary

The current study is the first to examine multisensory integration in A1 of the cat and confirms findings from other species. Also, the multisensory interactions discussed depend on the relative audiovisual timing. Since previous studies of multisensory integration in A1 of other species typically presented auditory noise burst stimuli and A1 maximally responds to pure tones, visual modulation of pure tone processing should be further investigated. The anatomical source of visual inputs remain unclear, as only sparse non-auditory connections are present in A1. The role of multisensory integration and visual influence in A1 for auditory processing, behaviour, and cognition is also uncertain. Overall, this study supports the notion that multisensory integration extends to primary sensory cortices and is not a function exclusive to higher-order association areas of the cortex.

References

Allman BL, Keniston LP, Meredith MA (2009) Adult deafness induces somatosensory conversion of ferret auditory cortex. Proc Natl Acad Sci 106:5925–5930.

Banks M, Smith P (2011) Thalamocortical relations. In: The Auditory Cortex (Winer JA, Schreiner CE, eds), pp 75–97. New York: NY: Springer.

Barone P, Lacassagne L, Kral A (2013) Reorganization of the connectivity of cortical field DZ in congenitally deaf cat. PLoS One 8:1–21.

Baumann S, Petkov CI, Griffiths TD (2013) A unified framework for the organisation of the primate auditory cortex. Front Syst Neurosci 7:1–19.

Bell AH, Meredith MA, Van Opstal AJ, Munoz DP (2006) Stimulus intensity modifies saccadic reaction time and visual response latency in the superior colliculus. Exp Brain Res 174:53–59.

Bizley JK, King AJ (2009) Visual influences on ferret auditory cortex. Hear Res 258:55–63.

Bizley JK, Nodal FR, Bajo VM, Nelken I, King AJ (2007) Physiological and anatomical evidence for multisensory interactions in auditory cortex. Cereb Cortex 17:2172–2189.

Bizley JK, Nodal FR, Nelken I, King AJ (2005) Functional organization of ferret auditory cortex. Cereb Cortex 15:1637–1653.

Brodmann K (1909) Vergleichende Lokalisationslehre der Großhirnrinde. In: Ihren Prinzipien Dargestellt Aufgrund des Zellaufbaus. Leipzig.

Bulkin DA, Groh JM (2012) Distribution of visual and saccade related information in the monkey inferior colliculus. Front Neural Circuits 6:1–12.

Cahill L, Ohl F, Scheich H (1996) Alteration of auditory cortex activity with a visual stimulus through conditioning: A 2-deoxyglucose analysis. Neurobiol Learn Mem 65:213–222.

Cappe C, Barone P (2005) Heteromodal connections supporting multisensory integration at low levels of cortical processing in the monkey. Eur J Neurosci 22:2886–2902.

Carrasco A, Lomber SG (2009a) Differential modulatory influences between primary auditory cortex and the anterior auditory field. J Neurosci 29:8350–8362.

Carrasco A, Lomber SG (2009b) Evidence for hierarchical processing in cat auditory cortex: Nonreciprocal influence of primary auditory cortex on the posterior auditory field. J Neurosci 29:14323–14333.

Chabot N, Butler BE, Lomber SG (2015) Differential modification of cortical and thalamic projections to cat primary auditory cortex following early- and late-onset deafness. J Comp Neurol 523:2297–2320.

Clemo HR, Keniston LP, Meredith MA (2012) Structural Basis of Multisensory Processing. In: The Neural Bases of Multisensory Processes (Murray MM, Wallace MT, eds), pp 3–12. Boca Raton, FL: CRC Press/Taylor & Francis.

Code RA, Winer JA (1985) Commissural neurons in layer III of cat primary auditory cortex (AI): Pyramidal and non-pyramidal cell input. J Comp Neurol 242:485–510.

Cooper MH, Young PA (1976) Cortical projections to the inferior colliculus of the cat. Exp Neurol 51:488–502.

Driver J, Noesselt T (2008) Multisensory interplay reveals crossmodal influences on ‘sensory-specific’ brain regions, neural responses, and judgments. Neuron 57:11–23.

Dubner R, Zeki SM (1971) Response properties and receptive fields of cells in an anatomically defined region of the superior temporal sulcus in the monkey. Brain Res 35:528–532.

Falchier A, Clavagnier S, Barone P, Kennedy H (2002) Anatomical evidence of multimodal integration in primate striate cortex. J Neurosci 22:5749–5759.

Freund HJ, Wita CW, Brüstle R (1972) Latency differences between inhibitory and excitatory responses of cat optic tract units. Exp Brain Res 16:60–74.

Fu KG, Shah AS, Connell MNO, Mcginnis T, Eckholdt H, Lakatos P, Smiley J, Schroeder CE (2004) Timing and laminar profile of eye-position effects on auditory responses in primate auditory cortex. J Neurophysiol 92:3522–3531.

Fuster JM, Bodner M, Kroger JK (2000) Cross-modal and cross-temporal association in neurons of frontal cortex. Nature 405:347–351.

Ghazanfar AA, Maier JX, Hoffman KL, Logothetis NK (2005) Multisensory integration of dynamic faces and voices in rhesus monkey auditory cortex. J Neurosci 25:5004–5012.

Ghazanfar AA, Schroeder CE (2006) Is neocortex essentially multisensory? Trends Cogn Sci 10:278–285.

Greicius MD, Supekar K, Menon V, Dougherty RF (2009) Resting-state functional connectivity reflects structural connectivity in the default mode network. Cereb Cortex 19:72–78.

Gross CG, Rocha-Miranda CE, Bender DB (1972) Visual properties of neurons in inferotemporal cortex of the Macaque. J Neurophysiol 35:96–111.

Hackett TA, Stepniewska I, Kaas JH (1998) Thalamocortical connections of the parabelt auditory cortex in macaque monkeys. J Comp Neurol 400:271–286.

Hall AJ, Butler BE, Lomber SG (2016) The cat’s meow: A high-field fMRI assessment of cortical activity in response to vocalizations and complex auditory stimuli. Neuroimage 127:44–57.

Hall AJ, Lomber SG (2015) High-field fMRI reveals tonotopically-organized and core auditory cortex in the cat. Hear Res 325:1–11.

Hershenson M (1962) Reaction time as a measure of intersensory facilitation. J Exp Psychol 63:289–293.

Horsley V, Clarke RH (1908) The structure and functions of the cerebellum examined by a new method. Brain 31:45–124.

Huang CL, Winer JA (2000) Auditory thalamocortical projections in the cat: Laminar and areal patterns of input. J Comp Neurol 427:302–331.

Hunt DL, Yamoah EN, Krubitzer L (2006) Multisensory plasticity in congenitally deaf mice: How are cortical areas functionally specified? Neuroscience 139:1507–1524.

Innocenti GM, Berbel P, Clarke S (1988) Development of projections from auditory to visual areas in the cat. J Comp Neurol 272:242–259.

Jiang W, Jiang H, Stein BE (2002) Two corticotectal areas facilitate multisensory orientation behavior. J Cogn Neurosci 14:1240–1255.

Kaas JH, Collins CE (2001) The organization of sensory cortex. Curr Opin Neurobiol 11:498–504.

Kaas JH, Hackett TA (2000) Subdivisions of auditory cortex and processing streams in primates. Proc Natl Acad Sci 97:11793–11799.

Kayser C, Petkov CI, Logothetis NK (2008) Visual modulation of neurons in auditory cortex. Cereb Cortex 18:1560–1574.

Kelly JB, Judge PW, Phillips DP (1986) Representation of the cochlea in primary auditory cortex of the ferret (Mustela putorius). Hear Res 24:111–115.

Knight PL (1977) Representation of the cochlea within the anterior auditory field (AAF) of the cat. Brain Res 130:447–467.

Kok MA (2015) Exploring the Structural and Functional Organization of the Dorsal Zone of Auditory Cortex in Hearing and Deafness.

Kowalski N, Versnel H, Shamma SA (1995) Comparison of responses in the anterior and primary auditory fields of the ferret cortex. J Neurophysiol 73:1513–1523.

Kral A, Schröder JH, Klinke R, Engel AK (2003) Absence of cross-modal reorganization in the primary auditory cortex of congenitally deaf cats. Exp Brain Res 153:605–613.

Lakatos P, Chen CM, O'Connell MN, Mills A, Schroeder CE (2007) Neuronal oscillations and multisensory interaction in primary auditory cortex. Neuron 53:279–292.

Lee CC, Winer JA (2005) Principles governing auditory cortex connections. Cereb Cortex 15:1804–1814.

Lomber SG, Meredith MA, Kral A (2010) Cross-modal plasticity in specific auditory cortices underlies visual compensations in the deaf. Nat Neurosci 13:1421–1427.

Lovelace CT, Stein BE, Wallace MT (2003) An irrelevant light enhances auditory detection in humans: A psychophysical analysis of multisensory integration in stimulus detection. Cogn Brain Res 17:447–453.

Luethke LE, Krubitzer LA, Kaas JH (1989) Connections of primary auditory cortex in the new world monkey, Saguinus. J Comp Neurol 285:487–513.

Malhotra S, Stecker GC, Middlebrooks JC, Lomber SG (2008) Sound localization deficits during reversible deactivation of primary auditory cortex and/or the dorsal zone. J Neurophysiol 99:1628–1642.

Mascetti GG, Strozzi L (1988) Visual cells in the inferior colliculus of the cat. Brain Res 442:387–390.

McDonald JJ, Teder-Saälejärvi WA, Hillyard SA (2000) Involuntary orienting to sound improves visual perception. Nature 407:906–908.

Meredith MA, Allman BL (2009) Subthreshold multisensory processing in cat auditory cortex. Neuroreport 20:126–131.

Meredith MA, Allman BL (2015) Single-unit analysis of somatosensory processing in the core auditory cortex of hearing ferrets. Eur J Neurosci 41:686–698.

Meredith MA, Lomber SG (2011) Somatosensory and visual crossmodal plasticity in the anterior auditory field of early-deaf cats. Hear Res 280:38–47.

Meredith MA, Nemitz JW, Stein BE (1987) Determinants of multisensory integration neurons . I . Temporal factors. J Neurosci 7:3215–3229.

Meredith MA, Stein BE (1983) Interactions among converging sensory inputs in the superior colliculus. Science (80-) 221:389–391.

Meredith MA, Stein BE (1985) Descending efferents from the superior colliculus relay integrated multisensory information. Science (80-) 227:657–659.

Meredith MA, Stein BE (1986) Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. J Neurophysiol 56:640–662.

Merzenich MM, Brugge JF (1973) Representation of the cochlear partition on the superior temporal plane of the macaque monkey. Brain Res 50:275–296.

Merzenich MM, Knight PL, Roth GL (1975) Representation of cochlea within primarv auditory cortex in the cat. J Neurophysiol:231–249.

Morel A, Garraghty PE, Kaas JH (1993) Tonotopic organization, architectonic fields, and connections of auditory cortex in macaque monkeys. J Comp Neurol 459:437–459.

Olfert ED, Cross BM, McWilliam AA (1993) Guide to the care and use of experimental animals.

Ouellette BG, Casanova C (2006) Overlapping visual response latency distributions in visual cortices and LP-pulvinar complex of the cat. Exp Brain Res 175:332–341.

Paloff AM, Usunoff KG, Hinova-Palova D V., Ivanov DP (1985) Retinal innervation of the inferior colliculus in adult cats: Electron microscopic observations. Neurosci Lett 54:339–344.

Perrett DI, Rolls ET, Caan W (1982) Visual neurones responsive to faces in the monkey temporal cortex. Exp Brain Res 47:329–342.

Phillips DP, Judge PW, Kelly JB (1988) Primary auditory cortex in the ferret (Mustela putorius): neural response properties and topographic organization. Brain Res 443:281–294.

Porter KK, Metzger RR, Groh JM (2007) Visual- and saccade-related signals in the primate inferior colliculus. Proc Natl Acad Sci U S A 104:17855–17860.

Reale RA, Imig TJ (1980) Tonotopic organization in auditory cortex of the cat. J Comp Neurol 291:265–291.

Rebillard G, Carlier E, Rebillard M, Pujol R (1977) Enhancement of visual responses on the primary auditory cortex of the cat after an early destruction of cochlear receptors. Brain Res 129:162–164.

Schreiner CE, Mendelson JR, Sutter ML (1992) Functional topography of cat primary auditory cortex: representation of tone intensity. Exp Brain Res 92:105–122.

Schreiner CE, Sutter ML (1992) Topography of excitatory bandwidth in cat primary auditory cortex: single-neuron versus multiple-neuron recordings. J Neurophysiol 68:1487–1502.

Schreiner CE, Urbas J V (1988) Representation of amplitude modulation in the auditory cortex of the cat. II. Comparison between cortical fields. Hear Res 32:49–63.

Spence C, Nicholls MER, Gillespie N, Driver J (1998) Cross-modal links in exogenous covert spatial orienting between touch, audition, and vision. Percept Psychophys 60:544–557.

Stein BE, Meredith MA, Huneycutt WS, McDade L (1989) Behavioral indices of multisensory integration: Orientation to visual cues is affected by auditory stimuli. J Cogn Neurosci 1:12–24.

Stewart DL, Starr A (1970) Absence of visually influenced cells in auditory cortex of normal and congenitally deaf cats. Exp Neurol 28:525–528.

Stiebler I, Neulist R, Fichtel I, Ehret G (1997) The auditory cortex of the house mouse: left-right differences, tonotopic organization and quantitative analysis of frequency representation. J Comp Physiol A Sensory, Neural, Behav Physiol 181:559–571.

Stolzberg D, Butler BE, Lomber SG (2018) Effects of neonatal deafness on resting-state functional network connectivity. Neuroimage 165:69–82.

Syka J, Radil-Weiss T (1973) Acoustical responses of inferior colliculus neurons in rats influenced by sciatic nerve stimulation and light flashes. Int J Neurosci 5:201–206.

Tawil RN, Saadé NE, Bitar M, Jabbur SJ (1983) Polysensory interactions on single neurons of cat inferior colliculus. Brain Res 269:149–152.

Tsao DY, Freiwald WA, Knutsen TA, Mandeville JB, Tootell RBH (2003) Faces and objects in macaque cerebral cortex. Nat Neurosci 6:989–995.

Wallace MT, Meredith MA, Stein BE (1992) Integration of multiple sensory modalities in cat cortex.

Wallace MT, Ramachandran R, Stein BE (2004) A revised view of sensory cortical parcellation.

Werner-Reiss U, Kelly KA, Trause AS, Underhill AM, Groh JM (2003) Eye position affects activity in primary auditory cortex of primates. Curr Biol 13:554–562.

Winer JA (1992) The Functional Architecture of the Medial Geniculate Body and the Primary Auditory Cortex. In: The Mammalian Auditory Pathway: Neuroanatomy (Douglas B. Webster, Arthur N. Popper RRF, ed), pp 222–409. New York: Springer, New York, NY.

Winer JA, Miller LM, Lee CC, Schreiner CE (2005) Auditory thalamocortical transformation: Structure and function. Trends Neurosci 28:255–263.

Curriculum Vitae

Name:	Catherine Boucher
Post-secondary Education and Degrees:	Carleton University Ottawa, Ontario, Canada 2012-2017 B.Cog.Sc The University of Western Ontario London, Ontario, Canada 2017-2019 M.Sc.
Honours and Awards:	Trainee Professional Development Award Society for Neuroscience 2019 Western Graduate Research Scholarship The University of Western Ontario 2017-2019 Dean’s List Carleton University 2016 Entrance Scholarship Carleton University 2012
Related Work Experience	Teaching Assistant The University of Western Ontario 2017-2019

Publications:

Boucher, C., Butler, B.E., Meredith, M.A. & Lomber, S.G. *Crossmodal Plasticity in Core Auditory Cortex Following Deafness: Evidence For and Against.* Neuroscience and Biobehavioral Reviews (Submitted).

Conference Presentations:

Boucher, C., Bao, X., Merrikhi, Y., Meredith, M.A. & Lomber, S.G. *Multisensory Responses in Primary Auditory Cortex of the Cat.* Poster session presented at Neuroscience Research Day, London, ON. May 9-10, 2019.

Boucher, C., Bao, X., Merrikhi, Y., Meredith, M.A. & Lomber, S.G. *Multisensory Responses in Primary Auditory Cortex of the Cat.* Poster session presented at the International Hearing Loss Conference, Niagara-On-The-Lake, ON. May 5-9, 2019.

Boucher, C., Butler, B.E. & Lomber, S.G. *Multisensory Responses in the Primary Auditory Cortex of the Cat.* Poster session presented at the International Multisensory Research Forum, Toronto, ON. June 14-17, 2018.