Inducing Neural Plasticity and Modulation Using Multisensory Stimulation: Techniques for Sensory Disorder Treatment

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Summary

Sensory systems are vastly connected in the brain through multisensory integration, in which neurons from different sensory systems can modulate each other to lead to a unified perceptual experience. Previous studies have shown that repeated stimulation of one sensory system can induce neural plasticity in another sensory system, and that stimulation timing may be important for plasticity induction. In the work of this dissertation, we characterized the modulatory and plasticity effects of paired multisensory stimulation on neural firing in sensory systems across the brain, investigating the timing effects of stimulation between modalities and the effects of different sensory stimulation combinations. In the auditory system, we discovered that electrical somatosensory stimulation can either suppress or facilitate neural firing in the inferior colliculus (IC; auditory nucleus in the midbrain) and primary auditory cortex (A1) depending on what somatosensory location is stimulated. After observing these results, we tested plasticity effects in A1 in response to paired somatosensory and acoustic stimulation with different inter-stimulus delays in anesthetized guinea pigs. In general, we observed that contralateral somatosensory stimulation induces more facilitation while ipsilateral stimulation induces more suppression. When looking at paired stimulation with different somatosensory locations and varying inter-stimulus delays, we found that plasticity induced by paired acoustic stimulation with right mastoid electrical stimulation was consistently suppressive regardless of delay, but paired acoustic stimulation with electrical stimulation of either pinna was timing-dependent, where one inter-stimulus

delay was consistently suppressive while other delays induced random changes. To ensure that these effects could also be observed in awake animals, we performed the pinna stimulation experiments again in two groups of chronic awake-recording animals with different stress levels to also assess stress effects on modulation and plasticity in A1. We found that in low-stress animals, the same inter-stimulus delay was suppressive and a neighboring delay was consistently facilitative, which matches previous invasive spike-timing dependent plasticity studies (anesthesia may have affected these trends). Meanwhile, the high-stress animal group showed results that were inconsistent with expected time dependence, with no trends across inter-stimulus delays, indicating that stress can have adverse or confounding effects on expected neuromodulation plasticity outcomes. After establishing the ability to induce controlled plasticity in the auditory system, we tested paired stimulation in the other four primary sensory cortices, and found that differential effects can be induced such that the location, amount, type, and timing of plasticity can be controlled by strategically choosing which sensory systems to pair and what stimulation parameters to use for each sensory cortex. We also investigated the ability to target subpopulations of neurons within a given brain region by varying stimulation parameters. We found that by stimulating at levels near activation thresholds, specific subpopulations of IC neurons can be targeted by varying somatosensory stimulation location. Furthermore, acoustic stimulation can excite or modulate specific areas of primary somatosensory cortex, and we mapped the guinea pig homunculus in somatosensory cortex to characterize this. Overall, these findings further confirm the immense interconnectivity between sensory systems, and multisensory stimulation may

provide a novel noninvasive approach for inducing controlled and differential plasticity to disrupt pathogenic neural activity and treat neural sensory disorders, such as tinnitus and pain.

Abbreviations

A1: primary auditory cortex

DBS: deep brain stimulation

DCN: dorsal cochlear nucleus

FRM: frequency response map

FSL: first spike latency

GC: gustatory cortex

HTA: Healing Touch for Animals

IC: inferior colliculus

ICC: central nucleus of the inferior colliculus

ICD: dorsal cortex of the inferior colliculus

ICX: external nucleus of the inferior colliculus

L: lateral

MGV: ventral division of the medial geniculate body

mSync: Multimodal Synchronization Therapy

OC: piriform olfactory cortex

PSTH: post-stimulus time histogram

R: rostral

S1: primary somatosensory cortex

SC: superior colliculus

tDCS: transcranial direct current stimulation

TMS: transcranial magnetic stimulation

V1: primary visual cortex

VCN: ventral cochlear nucleus

Chapter 1: Introduction

Multisensory Integration

Multisensory integration is a term describing the anatomical and functional interconnectedness of sensory systems in the brain. These connections allow complex organisms to evaluate their surrounding environment as one complete perception as opposed to separate individual sensory viewpoints. They also aid in making sense of multiple sensory inputs from the same source by combining them in a way that is easily understood. For example, the spatial map of the visual field is overlaid and aligned with the spatial map of the auditory field in the superior colliculus (Drager and Hubel 1975; King and Palmer 1985; Meredith and Stein 1986; Wallace et al. 1998) in such a way that we can correlate the visual perception of an object with the sound that it makes, even when other unrelated visual/auditory inputs are simultaneously coming from other locations. In another example, there is evidence that neurons in primary auditory cortex (**A1**) respond with different firing rates/patterns to the sound of a voice when it is combined with visual perception of someone speaking, helping us make better sense of the speech (Ghazanfar et al. 2005), and auditory cortex is also activated during silent lip-reading when no sound stimulus is present (Calvert et al. 1997). Such integration allows us to better interact with our environment for a more complete experience. We know that all of this is possible because our sensory systems are connected through direct and indirect sensory projections in the brain (Ghazanfar and Schroeder 2006; Murray and

Wallace 2011), but it is not completely clear how these relationships are precisely coded in cortical areas leading to perceptual and behavioral effects (Stein and Stanford 2008). Even in situations where we have mapped primary and multisensory stimulation effects in cortical and subcortical brain regions, we have a limited understanding of the mechanisms related to how one sensory system modulates the neural firing of another, or how multisensory integration can lead to plasticity.

Previous studies have briefly investigated the effects of multisensory integration on neural firing in sensory areas, both through stimulation of a single sensory system and through multisensory stimulation. In the auditory system, excitatory activity can be elicited by somatosensory stimulation in A1 (Foxe et al. 2002; Murray et al. 2005) and in subcortical auditory areas such as the cochlear nucleus (Kanold et al. 2011) and inferior colliculus (**IC**) (Aitkin et al. 1978; Aitkin et al. 1981b). In addition to these responses, plasticity has been induced in A1 using solely electrical stimulation of somatosensory receptors (Gloeckner et al. 2013; Kayser et al. 2005; Markovitz et al. 2015) or direct electrical stimulation of somatosensory cortex (Ma and Suga 2003). Olfactory interactions have also been shown to naturally induce plasticity through the reorganization of maps in auditory cortex (Cohen et al. 2011), and visual/auditory interactions in A1 have been well-documented as well (Calvert et al. 1997; Ghazanfar et al. 2005).

Other sensory systems have shown similar results. For example, primary somatosensory cortex (**S1**) activity modulation has been elicited using auditory

stimulation (Foxe et al. 2000). Similarly, primary visual cortex (**V1**) has been modulated by auditory stimuli (Watkins et al. 2006), and it has been shown that visual cortex receives direct projections to and from various regions with the auditory and somatosensory systems (Dehay et al. 1988; Innocenti et al. 1988), especially cortical regions (Miller and Vogt 1984). Such projections to the visual system often result in excitatory activity (Giraud et al. 2001; Yaka et al. 1999), and in fact, somatosensory inputs from brail reading activates V1 consistently in blind patients (Sadato et al. 1996). In gustatory cortex (**GC**), which gives and receives inputs to and from the auditory (Yan and Dando 2015), visual (Rolls and Baylis 1994; Spence 2013; Zampini et al. 2007), somatosensory (de Araujo and Simon 2009; Simon et al. 2006; Simon et al. 2008), and olfactory (de Araujo and Simon 2009; Rolls and Baylis 1994) systems, interactions between all five sensory systems are directly involved in gustation and flavor perception in the brain (Auvray and Spence 2008). Piriform olfactory cortex (**OC**) is already considered to be a highly multisensory brain region and functions much like associative cortex with a high density of neighboring axonal outputs and a lack of columnar organization (Johnson et al. 2000). This cortical area is directly modulated by the auditory (Seo and Hummel 2011; Wesson and Wilson 2010), somatosensory (Demattè et al. 2006; Fiore 1993), and visual (Gottfried and Dolan 2003; Leonard and Masek 2014) systems, and it has a well-documented direct relationship with gustatory cortex through many direct network projections (Maier et al. 2015) and modulatory features (Rolls and Baylis 1994). It should be noted that in all cases where stimulation was used to show changes in neural firing, the stimulation of only one sensory system was used, showing

the simple modulatory effects of cross-sensory inputs, and none of the studies listed have combined the stimulation of multiple sensory systems in a systematic way to induce controlled plasticity, with the exception of the already published studies presented in this dissertation.

Outside of traditional central sensory pathways, there are also areas of the brain that have been classified as primarily multisensory. The superior colliculus was already mentioned as a multisensory hub, and the occipital temporal cortex also receives projections from the visual, somatosensory, and auditory systems (Amedi et al. 2001; Beauchamp 2005). There is even a provocative hypothesis that the entire neocortex is multisensory, since traditional sensory cortical areas receive so many projections from other sensory systems (Ghazanfar and Schroeder 2006). This is an interesting theory, as while there are certainly many cortical multisensory interactions, traditional primary sensory cortices are still highly optimized for coding one primary sensory input. Nonetheless, multisensory integration plays an important role in the perception of any sensory observation given the modulatory nature of cross-sensory connections in the brain.

Relevant Sensory Brain Regions

Primary Somatosensory Cortex

S1 contains a somatotopic representation of the body, and this map has been characterized in humans (Aminoff et al. 1985; Baumgartner et al. 1991; Hari et al. 1993;

Itomi et al. 2000; Kakigi et al. 1995; Liguori et al. 1991; Mogilner et al. 1994; Nakamura et al. 1998; Narici et al. 1991; Nobre 2001; Penfield and Boldrey 1937; Woolsey et al. 1979; Yang et al. 1994b), rats (Cho et al. 2007; Godde et al. 2002; Petersen et al. 2001; Welker 1976), cats (Celesia 1963; Davenport et al. 2010; Dykes et al. 1980; Iwamura and Tanaka 1978; Shigenaga et al. 1989), pigs (Craner and Ray 1991), monkeys (Pons et al. 1985), and other mammals (Schott 1993). To our knowledge, no one has characterized a map of auditory projections to S1, or the relationship between the somatotopic map and auditory inputs. However, previous studies have investigated common mechanisms of plasticity in S1 (Feldman and Brecht 2005; Foeller and Feldman 2004; Jones 2000; Schlaggar et al. 1993), including long-term potentiation and depression (Buonomano and Merzenich 1998; Feldman et al. 1999; Wolters et al. 2005), cortical reorganization (Borsook et al. 1998; Diamond et al. 1994; Jones and Pons 1998; Mogilner et al. 1993; Xerri et al. 1996; Yang et al. 1994a), alteration of GABA inhibitory circuits (Dykes 1997; Foeller and Feldman 2004), interhemispheric interactions (Clarey et al. 1996), NMDA receptor activation (Buonomano and Merzenich 1998; Garraghty and Muja 1996), and timing-dependent plasticity (Florence et al. 1997; Wolters et al. 2005).

Primary Auditory Cortex

A1 is characterized by a tonotopic representation of sound frequencies across neurons (Galaburda and Sanides 1980; Hackett et al. 1998; Rees and Palmer 2010; Stiebler et al. 1997; Wallace et al. 2000), with some neurons coding for onset and offset (Wehr and Zador 2003). Auditory cortex projects and receives information to and from

lower auditory areas such as the inferior colliculus (Markovitz et al. 2013; Straka et al. 2014) and the medial geniculate body in the thalamus (Barth and MacDonald 1996; Diamond et al. 1969). The descending projections have been shown to be involved with auditory plasticity and learning along the auditory pathway from the brainstem up to the auditory cortex (Bajo and King 2012; Suga 2008; Winer 2005). One common form of plasticity that has been demonstrated in A1 and throughout the auditory system is Hebbian plasticity, which generally involves timing dependent mechanisms that can either lead to facilitation or suppression in neural firing (Tzounopoulos and Kraus 2009; Wu et al. 2015), and this plasticity form has been found in other brain regions as well (Tzounopoulos et al. 2007). In fact, multiple studies have shown that spike-timing dependent plasticity is achievable throughout the auditory system using invasive bimodal stimulation, including within A1 (Basura et al. 2015; Basura et al. 2012; Koehler and Shore 2013a; Wu et al. 2015).

Inferior Colliculus

The inferior colliculus (**IC**) is an auditory structure within the midbrain acting as a relay between the cochlear nucleus and the thalamus (Aitkin and Phillips 1984; Casseday et al. 2002; Ehret 1997). It consists of several sub-regions, including the central nucleus (**ICC**), the dorsal cortex (**ICD**), and the external nucleus/region (**ICX**), all of which exhibit unique properties. The ICC, like auditory cortex, is characterized by tonotopy (Malmierca et al. 2008; Oliver 2005; Snyder et al. 2004) and short acoustic-driven latencies (Lumani and Zhang 2010), while the ICD and ICX have broader tuning,

longer latencies, and larger latency jitters (Barnstedt et al. 2015; Lumani and Zhang 2010). External IC regions like ICD and ICX are important in sound localization (Binns et al. 1992; Huffman and Henson 1990; Knudsen and Knudsen 1983) and attention (Jane et al. 1965), and are innervated by both ascending and descending auditory pathways (Coleman and Clerici 1987; Oliver 2005). In general, ICX neurons have shorter latencies than ICD (Syka et al. 2000), and lateral IC neurons have shorter latencies than other non-ICC areas in general (Langner et al. 2002; Schreiner and Langner 1988). Maps of acoustic-driven threshold (Stiebler 1986), latency (Hattori and Suga 1997) and duration and latency jitter (Straka et al. 2014) have all been discovered throughout the IC. Additionally, the IC is a multisensory hub in the auditory system (Aitkin et al. 1978; Aitkin et al. 1981b), receiving inputs from the somatosensory, visual, and limbic systems (Coles and Aitkin 1979; Gruters and Groh 2012; Schofield et al. 2011b; Winer 2005).

Neural Sensory Disorders

While some neural sensory disorders are more directly related to problems in the peripheral nervous system, such as hearing loss being connected to the death of auditory hair cells, other sensory disorders result from abnormal firing patterns in the central nervous system. This dissertation will focus on central nervous system sensory disorders, including tinnitus and some forms of pain.

Tinnitus

Tinnitus is a neural disorder characterized by a phantom sound percept generated within the brain in the absence of an external sound source (Møller et al. 2010). It affects

over 250 million people worldwide and is debilitating for about 1% of the world population (ATA 2010). Tinnitus is thought to be caused by abnormal neural plasticity throughout the auditory system, and the tinnitus percept has been linked to hyperactivity, hyper-synchrony across neurons, tonotopic reorganization, and altered neural firing patterns (Eggermont and Roberts 2004; Henry et al. 2014; Kaltenbach 2011; Lanting et al. 2008; Lanting et al. 2009; Møller et al. 2010). Neuromodulation techniques have been used to attempt to treat tinnitus (Vanneste and De Ridder 2012), including noninvasive treatments such transcranial magnetic stimulation (De Ridder et al. 2011b). However, these treatments have had only limited success with inconsistent results (Møller et al. 2010). Invasive treatments like deep brain stimulation have been used with some success (Cheung and Larson 2010), but such treatments are available to only a small portion of the tinnitus population due to high costs and surgical risks. No single treatment seems to work for all patients due in part to a high pathological variance across tinnitus patients.

Pain

Phantom limb pain, like tinnitus, is a neural disorder characterized by a phantom sensory perception despite a lack of sensory stimulus. In this case, an amputee continues to feel pain in a missing limb, even though he/she knows the limb is no longer present. This percept has been connected with abnormal firing patterns in the somatosensory system, in which neurons fire without an actual somatosensory pain input (Smith et al. 1999). One current treatment for phantom limb pain is called mirror therapy, in which a mirror is placed down the center of the body such that a reflection of the non-amputated

limb can be seen in a way that creates the illusion that the limb still exists (Chan et al. 2007). This use of multisensory integration, where a visual input informs the somatosensory system (in this case a deceptive input), is able to reduce unnecessary cortical firing and eliminate the pain percept.

Chronic pain has many different causes and pathogeneses, so it is impossible to completely characterize it effectively by specific neural biomarkers. However, some forms of chronic pain exhibit specific abnormal neural patterns, such as alterations in neural opioid systems (Millan et al. 1987), increases in neurotransmitter release (Zhao et al. 2006), S1 reorganization (Flor et al. 1997), and increased gain in pain-related neural activity (Woolf and Salter 2000). Current chronic pain neuromodulation treatments include peripheral stimulation (Gildenberg 2006), spinal cord stimulation (Alo and Holsheimer 2002), and direct brain stimulation via epidural motor cortex stimulation, deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), and transcranial direct current stimulation (tDCS) (Charleston et al. 2010; Fregni et al. 2007). Like tinnitus, neuropathic pain is highly patient specific (Diatchenko et al. 2005; Whyte and Niven 2001), where each patient has a unique set of symptoms that may originate from unique groups of neurons and firing patterns.

Effects of Stress and Anesthesia on Neural Activity

Stress has a thoroughly documented effect on neural activity and plasticity induction. First and foremost, it has been shown to increase levels of several neurotransmitters, including dopamine (Abercrombie et al. 1989; Del Arco et al. 2007;

Finlay et al. 1995), norepinephrine (Finlay et al. 1995), and acetylcholine (Del Arco et al. 2007), all of which have many important roles at synapses. Stress has also been shown to decrease apical dendritic length (Bose et al. 2010) and branch quantity (Bloss et al. 2010), which can affect plasticity. NMDA receptors can be activated more frequently with stress (Kim et al. 1996b; Shors et al. 2004), which may be related to blocks in long-term potentiation (Kavushansky and Richter-Levin 2006; Shakesby et al. 2002), and general studies have shown that stress can impair plasticity at the synaptic level (Jay et al. 2004). These modifications can be critically obstructive to the study of neural firing and plasticity in an awake animal, and care must be taken to control for the effects of stress in chronic awake studies.

Central Goals of this Dissertation

In the work of this dissertation, we attempt to induce controlled plasticity in sensory systems using noninvasive sensory stimulation through a new neuromodulation treatment called Multimodal Synchronization Therapy (**mSync**). mSync combines various sensory stimuli at specific inter-stimulus delays to induce timing-dependent plasticity (Gloeckner et al. 2013; Markovitz et al. 2015). Multiple sensory system stimulation has not typically or routinely been used for neural disorder treatment. Some examples of single-sense stimulation for neuromodulation include trigeminal nerve stimulation, which has been used to treat epilepsy (DeGiorgio et al. 2013) and depression (Cook et al. 2013), and somatosensory stimulation, which has been attempted for tinnitus treatment (Dehmel et al. 2008b; Levine et al. 2003). Assuming multisensory stimulation

can induce controlled and differential plasticity in sensory regions, we believe that this approach could be an effective form of neuromodulation for treating neural sensory disorders, and this dissertation characterizes the effects of such stimulation on the brain.

While previous work has investigated multisensory interactions and their effects on neural firing, the novelty of the studies in this dissertation lies in systematic multisensory stimulation to take advantage of such interactions for the induction on controlled plasticity. This work may lead to a new form of noninvasive neuromodulation for the treatment of sensory disorders, which can easily be implemented in patients. Specifically, the studies performed in this work attempt to address four major questions:

1. Can paired multisensory stimulation induce controllable suppressive and facilitative changes in the auditory system, which might be useful in the treatment of tinnitus?

2. What are the plasticity effects of using different somatosensory stimulation locations or the stimulation of different combinations of sensory systems in different sensory cortical areas?

3. What are the effects of inter-stimulus timing on modulation and plasticity induction in the auditory system, where timing-dependent plasticity has been previously studied?

4. Is it possible to target specific subpopulations of neurons in sensory brain regions, which might be useful for treating patient specific symptoms in neural sensory disorders?

All of these questions are addressed in this dissertation, in which some questions are spread out across multiple chapters as different studies addressed different parts of each question. Each chapter will be presented independently, with a fresh introduction and explanation of methods, so that it is not necessary to have to read previous chapters in order to fully understand a given chapter of interest. Additionally, an appendix representing a related study has also been included for reference.

Chapter 2: A New Concept for Noninvasive Tinnitus Treatment Utilizing Multimodal Pathways

In this preliminary study, we introduced the mSync concept and investigated whether or not somatosensory stimulation can induce modulatory changes in the auditory system to assess if paired somatosensory and sound stimulation may have potential for tinnitus treatment. This study was previously published as an IEEE EMBS Conference Proceedings paper (Gloeckner et al. 2013).

Summary

Current noninvasive treatments for tinnitus have shown mixed results. There have been encouraging developments in using invasive brain or vagal nerve stimulation to modulate neural populations driving the tinnitus percept; however, these invasive treatments can only be used in a small patient population with severe conditions. In this preliminary study, we investigated a new treatment option we call Multimodal Synchronization Therapy (mSync), which attempts to achieve synchronized and localized brain activation without invasive neural stimulation. mSync combines multiple sensory, motor, limbic, and cognitive inputs to elicit activation of multimodal neurons and modulate specific neurons driving the tinnitus percept. We used a guinea pig model to show that mSync with somatosensory and auditory stimulation is able to alter neural activity within the inferior colliculus, a multimodal integration center in the midbrain that has shown pathological changes in animals and patients with tinnitus. Electrical somatosensory stimulation of different body locations induced excitatory activity in the

inferior colliculus, eliciting responses in up to 41% of all recording sites for a given somatosensory stimulation location. Paired somatosensory and acoustic stimulation resulted in facilitated or suppressed acoustic-driven neural activity that varied depending on stimulation and recording location. Similar types of modulation effects were observed in auditory cortex, which may relate to changes in auditory perception and could potentially treat tinnitus symptoms in patients.

Introduction

Tinnitus, a neurological disorder resulting in a phantom sound generated within the brain in the absence of an external sound source, affects about 250 million people worldwide and is debilitating for about 1% of the world population (ATA 2010; Moller et al. 2011). It is caused by abnormal neural plasticity that occurs throughout the auditory system, and has been linked to hyperactivity, hyper-synchrony across neurons, tonotopic reorganization, and altered spike patterns (Bauer et al. 2008; Eggermont and Roberts 2004; Lanting et al. 2009; Moller et al. 2011). Currently, noninvasive treatments show mixed results across patients (ATA 2010; Hobson et al. 2012; Moller et al. 2011; Tass et al. 2012; Vanneste and De Ridder 2012; Vanneste et al. 2013). There have been recent developments in using invasive cortical, deep brain, and vagal nerve stimulation to directly modulate the neurons driving the tinnitus percept (Cheung and Larson 2010; De Ridder et al. 2011b; Engineer et al. 2011; Vanneste and De Ridder 2012). However, these treatments are available only to a limited subset of patients due to the surgical risks and costs associated with device implantation.

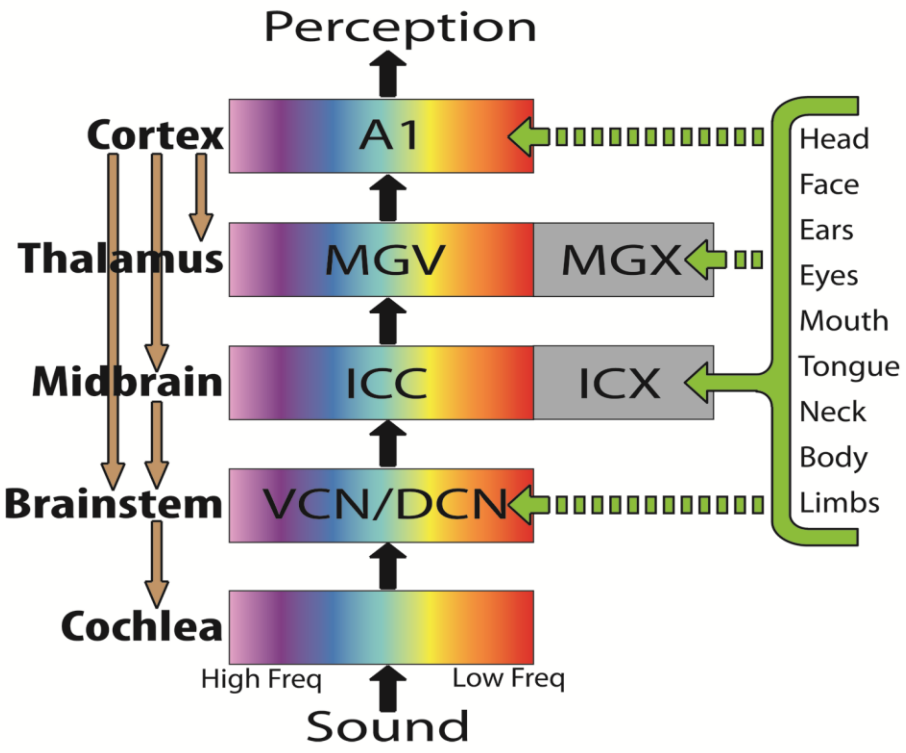


Figure 1: Somatosensory projections into the auditory system

A simplified schematic of the ascending and descending auditory system is shown (only some relevant regions and projections are included). The VCN (ventral cochlear nucleus), DCN (dorsal cochlear nucleus), ICC (central nucleus of inferior colliculus), and MGV (ventral division of medial geniculate nucleus) are ascending tonotopic core nuclei leading to A1 (primary auditory cortex). The ICX and MGX are external regions involved with multimodal processing, and each has projections to its corresponding core auditory pathway nucleus. mSync will take advantage of the somatosensory pathways that project to the auditory midbrain and other regions of the auditory system based on the information presented in this schematic.

We propose a new tinnitus treatment using noninvasive brain activation that targets specific neural populations in the auditory system through multimodal integration. Previous studies have reported that tinnitus can be modulated through manipulations of the eyes, head, neck, jaw, and shoulders (Levine et al. 2007; Moller et al. 2011; Simmons et al. 2008), which is consistent with the existence of multimodal integration across motor and sensory pathways (Dehmel et al. 2008a; Huffman and Henson 1990). Other studies have identified somatosensory, visual, motor, limbic, and cognitive inputs to the auditory system, including the inferior colliculus (**IC**) (Aitkin et al. 1981a; Basura et al. 2012; Gruters and Groh 2012; Hurley and Sullivan 2012; Ledoux et al. 1987; Marsh et al. 2002; Schofield et al. 2011a; Winer 2006). Figure 1 shows a simplified schematic of somatosensory projections to the external nucleus of the inferior colliculus (**ICX**) that interact with neurons in the central nucleus of the inferior colliculus (**ICC**), part of the core auditory pathway. Additionally, auditory plasticity has been achieved when co-activating auditory and somatosensory pathways with specific timing (Basura et al. 2012; Dehmel et al. 2012).

We hypothesize that artificially activating different multimodal pathways with the appropriate timing will elicit synchronized activation and neural plasticity within specific neural populations of the auditory system. With this approach, which we call Multimodal Synchronization Therapy (**mSync**), we will attempt to disrupt abnormal firing patterns that are driving the tinnitus percept using paired somatosensory and auditory stimulation. We used a guinea pig model to investigate the multimodal interactions between the

somatosensory and auditory pathways, particularly the effects of electrical stimulation of different locations across the body on auditory responses within the IC and primary auditory cortex (**A1**).

Materials and Methods

Overview

Basic surgical and analysis procedures have been described in detail in previous work (Lim and Anderson 2006; Markovitz et al. 2012) and are only briefly described here. Electrophysiology experiments were performed on three young female Hartley guinea pigs (300–400 g; Elm Hill Breeding Labs, Chelmsford, MA) anesthetized with an initial intramuscular injection of a ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL) cocktail, with varying supplements every 45–60 minutes to maintain an areflexive state. Neural recordings were performed inside an electrically-shielded and acoustic-attenuating room using hardware from Tucker-Davis Technology (Alachua, FL), and neural signals were processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Surgery and Neural Recordings

A craniotomy was performed revealing the visual cortex and auditory cortex on the right side of the guinea pig brain. A 32-site Michigan-style recording electrode array (NeuroNexus Technologies, Ann Arbor, MI) was inserted through the visual cortex and

into the IC. This array consisted of two 10-mm long shanks (site area approximately 413 μm^2) with 16 recording sites per shank and a site spacing of 100 µm. A second 32-site Michigan-style recording electrode array was inserted into the A1 of the animal, and this array consisted of four 5-mm long shanks with eight recording sites per shank at a spacing of 200 µm. The recording electrode ground was inserted into the upper neck of the animal. Heart rate and blood oxygen content were monitored using an H100 pulse oximeter from EdanUSA (San Diego, CA), and body temperature was monitored using an Oakton Acorn series JKT thermocouple rectal probe (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN). For surgery, the animal was fixed into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars. Recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine wave. Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of neural spike activity. A detection threshold of 3.5 times the standard deviation of the voltage noise floor was used to detect when spikes occurred, and spike voltage waveforms were visually inspected to ensure that background noise was not falsely detected.

Recording Electrode Placement

IC recording electrode arrays were inserted through the visual cortex and into the IC (approximately 5-6 mm deep, depending on the animal). Broadband noise acoustic

stimulation (50 ms duration, 0.5 ms rise/fall time, 70 dB SPL, equal energy between 625 Hz and 40 kHz) was performed using a speaker (Tucker-Davis Technology, Alachua, FL) coupled to the left ear bar, and functional responses to this stimulus were used to confirm that all electrode sites resided in the IC. The speaker-ear bar system was calibrated using a 0.25 in. condenser microphone (ACO Pacific, Belmont, CA). For IC placements, the functional location of each recording site was determined using frequency response maps (FRMs), similar to previous studies (Lim and Anderson 2007a; Markovitz et al. 2013; Offutt et al. 2014; Straka et al. 2014). For each FRM, pure tone stimulation (1-40 kHz with 8 tones/octave, 0-70 dB-SPL in 10 dB steps, 4 trials each, 2/second in a random order) was presented to the animal's left ear to map the tuning and thresholds at each recording site. ICC locations exhibited a tonotopic gradient with sharp tuning (Snyder et al. 2004), while ICX locations exhibited broad tuning with no tonotopic gradient.

A1 recording electrode arrays were placed between the pseudosylvian sulcus and the lateral suture line near its intersection with the Bregma suture line. These arrays were inserted at a depth such that the main input Layer IV could be observed near the middle of the eight recording sites on each shank (determined by locating the initial sink using current-source density) (Lim and Anderson 2007a; Markovitz et al. 2013; Straka et al. 2014). This usually resulted in the tip sites residing 1.2-1.3 mm below the surface of the cortex.

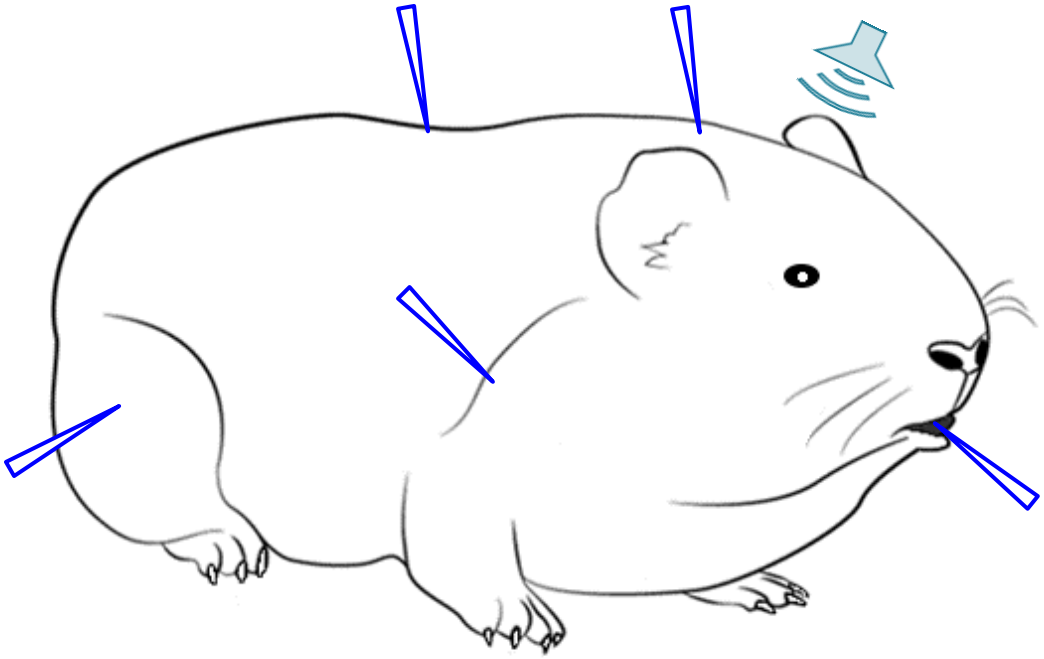


Figure 2: Somatosensory stimulation methods

Subdermal needle electrodes were used to electrically stimulate the somatosensory system at locations that spanned the entire animal, including the tongue, neck, back, left and right shoulders, left and right hind legs, and genitals. These locations were stimulated independently during recordings. Contralateral (left side) somatosensory stimulation locations in the shoulder and leg, along with the genitals, are not shown. Broadband noise was played into the left ear for auditory stimulation.

Sensory Stimulation

Electrical (biphasic, 205 µs per phase, 50 trials, 2/second, 560 µA) somatosensory simulation locations are shown in Figure 2 and include the tongue, neck, left and right shoulders, back, left and right legs, and genitals (for all somatosensory locations that

were performed with stimulation on both sides of the body, the left sides are not shown in the figure, and the genitals are also omitted). Subcutaneous needle electrodes (Rhythmlink International LLC, Columbia, SC) were used to stimulate all somatosensory locations except the tongue and genitals, which were stimulated using a surface electrode and ball electrode, respectively. The neck electrode was inserted subcutaneously halfway between the ears and the shoulder joints centrally, and the shoulder electrodes were inserted dorsal of the shoulder joints. The back electrode was inserted along the spine halfway between the neck electrode and the end of the spine, and the hind leg electrodes were placed laterally halfway between the hip joint and the knee joint. For all somatosensory stimulation locations, the stimulation ground was distributed between four other stimulation electrode locations, as spreading the ground across four separate locations mitigated unintended activation of ground areas. For all cases of electrical somatosensory stimulation alone, stimulation locations were randomized across trials to mitigate cumulative effects. Post-stimulus time histograms (**PSTH**s) were plotted for each of the ICC and ICX recording sites in response to somatosensory stimulation for further analysis.

Paired Stimulation Protocol

Paired acoustic and somatosensory stimulation was comprised of simultaneous somatosensory stimulation of one body location (same parameters as above) and acoustic broadband noise stimulation (50 dB SPL). Before paired stimulation, IC and A1 responses to 100 trials of broadband noise were recorded. Next, 500-1000 trials of paired

stimulation were performed, depending on the experiment, and ICC, ICX, and A1 responses to paired stimulation were compared to responses to the preceding broadband noise stimulation to assess the modulatory effects of paired stimulation. For all cases, an unequal variance two-tailed ranked t-test ($P<0.05$) was used to confirm a significant change in activity between paired and acoustic-only stimulation based on total spike rate (Ruxton 2006). PSTHs including both paired stimulation responses and the preceding broadband noise responses were plotted for a visual representation of differences in activity.

Results

Somatosensory stimulation alone elicited excitatory responses for many recording sites in the IC, as shown in Figure 3. Most sites of activation resided in the ICX (black bars), while only a few were found in the ICC (grey bars). Some somatosensory stimulation locations yielded responses in a large fraction of ICX recording sites, with neck stimulation having the highest percentage (41%). Differences in activation thresholds for a given IC location were found depending on the somatosensory location being stimulated, and different IC locations had different thresholds for a given somatosensory stimulation location. Some areas of the ICX were activated by more than one somatosensory location, as the black bars in Figure 3 sum to over 100%.

Paired stimulation resulted in a wide range of response patterns across recording sites and somatosensory stimulation locations. PSTH examples of both paired and acoustic-only stimulation for two different locations in the ICX, ICC, and A1 are

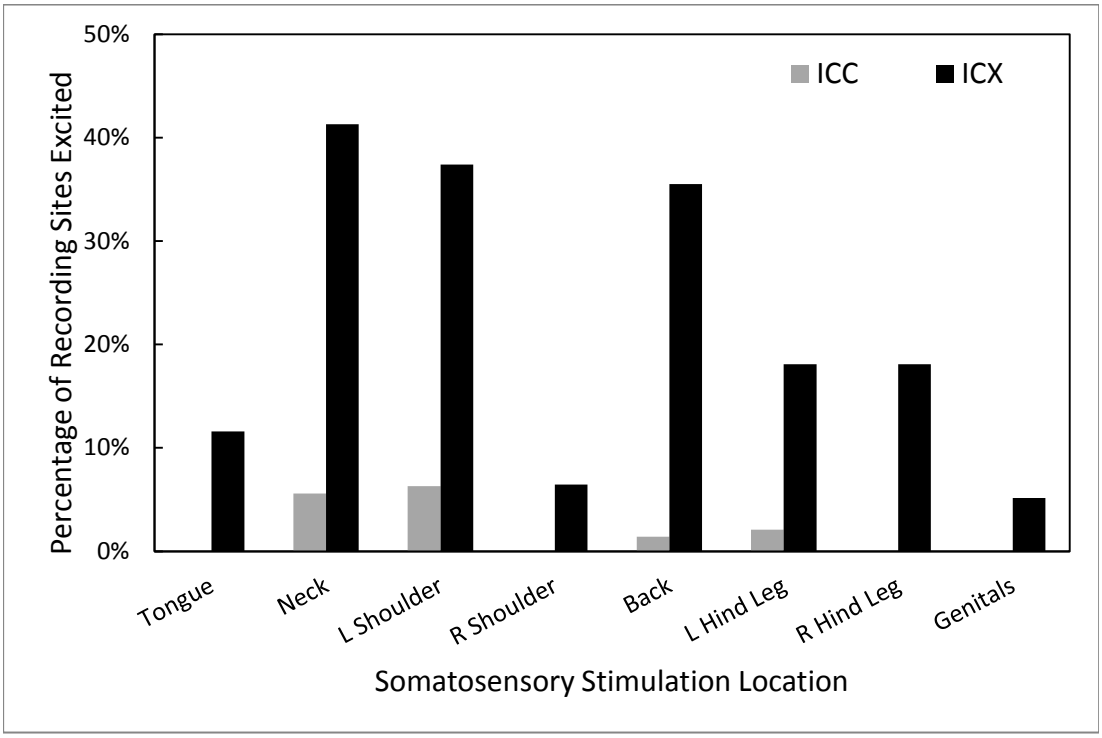


Figure 3: Changes in spike activity in the inferior colliculus to somatosensory stimulation alone

The percentages of ICC (n=143) and ICX (n=155) recording sites that showed excitatory activity in response to each stimulated somatosensory location at 560 μA are shown. For all stimulation locations, a higher percentage of ICX recording sites showed excitatory responses than ICC recording sites. All percentages represent the number of recording sites in a given recording region that showed activity divided by the total number of recording sites in that region.

presented in Figure 4, where blue PSTHs represent preceding broadband noise stimulation responses and red PSTHs represent paired stimulation responses (for each of these examples, no excitatory responses were observed for somatosensory stimulation

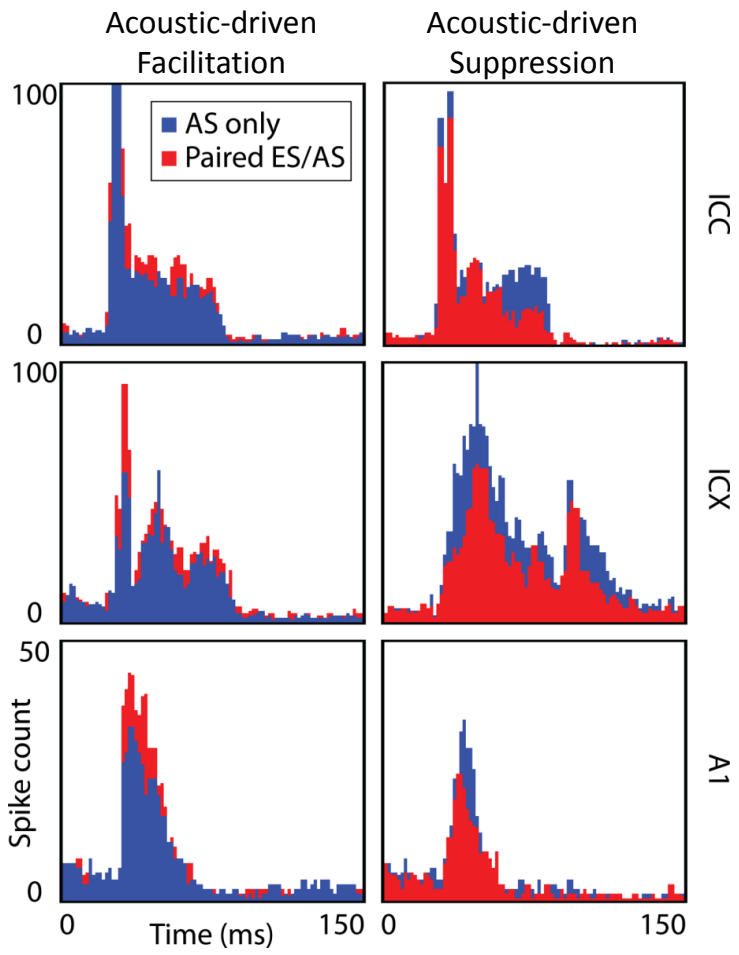


Figure 4: Facilitation and suppression in the auditory system

A comparison of broadband noise stimulation (blue, abbreviated AS for acoustic stimulation) and paired stimulation (red, abbreviated ES/AS for electrical stimulation and acoustic stimulation) responses for six PSTHs (1 ms bins) in the ICC, ICX, and A1 are shown. An unequal variance two-tailed ranked t-test was used to test for significance. Paired stimulation resulted in facilitation (left) and suppression (right) of acoustic-driven activity in all three regions. The ICC examples show a 16% increase in total spike rate for facilitation and a 27% decrease for suppression of acoustic-driven activity. The ICX and A1 examples show differences of 25% and 31% for facilitation, and 33% and 24% for suppression, respectively.

alone, as this would complicate analysis due to unknown trends in response summation). Paired stimulation induced suppression and facilitation of acoustic-driven activity at various recording sites in both the ICC and ICX. Similar to the effects in the midbrain, we observed facilitated and suppressed activity in A1 depending on the recording and stimulation locations.

Discussion

The results in Figure 3 are consistent with previous studies (Aitkin et al. 1981a; Jain and Shore 2006), illustrating that somatosensory stimulation can excite neurons in the auditory midbrain, including the ICC within the core auditory pathway. It is possible that somatosensory activation of the ICC occurs through polysynaptic pathways via the ICX or through other auditory regions that then project to the ICC (see Figure 1). It is also possible that somatosensory activation of the ICC occurs through non-auditory cortical regions that project to the auditory cortex and down to the ICC (King and Walker 2012; Lemus et al. 2010; Winer 2006). By investigating additional current levels closer to the activation threshold for each recording site, we can further assess if it is possible to locally activate regions within the IC, which could enable mSync to target specific neural populations driving the tinnitus percept (this is investigated in Chapter 7 of this dissertation).

The modulatory results for paired stimulation demonstrate that somatosensory stimulation not only elicits excitatory responses in the IC, but can also modulate acoustic-driven activity in the IC and A1 either via the ascending auditory pathway or through

other non-auditory pathways. The suppression results in Figure 4 are encouraging for mSync implementation, considering that tinnitus has been linked to hyperactivity across the auditory midbrain. Somatosensory stimulation combined with acoustic stimulation could potentially suppress this hyperactivity and reduce or eliminate the tinnitus percept. In addition, because some recording sites were facilitated while others were suppressed, mSync could be used to increase firing rates of some neurons while decreasing those of others. By affecting the firing rates of each neuron differently, mSync could break up hyper-synchrony by desynchronizing firing rates across a large population of neurons, which would also be useful for tinnitus treatment.

There are still several questions that need to be investigated to assess if mSync can actually suppress pathological neural activity related to the tinnitus percept. The preliminary results presented here provide initial neurophysiological evidence that somatosensory stimulation across the body can modulate and suppress activity within the auditory system. However, tinnitus can be associated with different types of abnormal patterns that only occur in a subset of neurons, including varying temporal/bursting patterns (Bauer et al. 2008; Eggermont and Roberts 2004; Lanting et al. 2009; Moller et al. 2011). The ability to appropriately modulate specific neurons in the IC and A1 would be required of mSync in order to target particular neural populations with abnormal firing patterns driving the tinnitus percept. An investigation of latencies for different somatosensory stimulation locations and for different IC and A1 recording areas would reveal appropriate stimulation locations and timing parameters to use for mSync.

Incorporating visual, motor, limbic, and cognitive inputs as additional multimodal integration sources could also be useful by widening the parameter space, potentially making mSync more patient-adaptable. Following these investigations, we must demonstrate that this alteration of neural activity directly corresponds to the elimination of the tinnitus percept. This demonstration can be achieved through mSync experiments in tinnitus animal models, utilizing behavioral testing for changes in tinnitus perception (Dehmel et al. 2012; Turner 2007), or through human studies. Since mSync is noninvasive and can utilize electrical and acoustic stimulators already approved for human use, clinical trials can be conducted with tinnitus patients in the future. Finally, mSync could also be considered as a potential treatment for other neurological disorders linked with abnormal but reversible brain patterns, such as chronic pain.

Chapter 3: Effects of Somatosensory Stimulation Location and Timing on Multimodal Synchronization Therapy Plasticity Outcomes

Based on the encouraging preliminary results in Chapter 2, we performed a more detailed and systematic study on the effects of different mSync parameters on plasticity effects in A1. We studied the effects of timing and somatosensory stimulation location on paired acoustic and somatosensory stimulation in A1, as these parameters could be readily adjusted in animal experiments and in tinnitus patients to optimize treatment.

Summary

Paired acoustic stimulation and electrical somatosensory stimulation was used to induce plasticity in primary auditory cortex in anesthetized guinea pigs as a form of noninvasive neuromodulation. Spike responses before and after paired stimulation were compared to determine if spike activity had been suppressed or facilitated for different somatosensory stimulation locations and inter-stimulus delays. Contralateral somatosensory locations relative to the recorded brain region tended to be more facilitative of spike activity, while ipsilateral locations were more suppressive. For both contralateral and ipsilateral pinna stimulation, an inter-stimulus delay of 15 ms (acoustic-leading) consistently induced suppression of spike activity at significantly more recording sites than facilitation, while all other delays showed no clear trends. Meanwhile, mastoid stimulation was suppressive across all inter-stimulus delays. The ability to noninvasively control plasticity induction in sensory systems by strategically choosing parameters such

as somatosensory stimulation location and inter-stimulus delays may be useful in treating complicated neural sensory disorders such as tinnitus.

Introduction

Neuromodulation is a field of neural disorder treatment in which neural activity is changed either constantly during stimulation or permanently through plasticity induction, and its use has significantly grown for various brain disorders over the past few decades. Many neuromodulation approaches have been successful in treating neurological conditions using various invasive and noninvasive brain stimulation modality, with positive outcomes in a variety of patients with various neural disorders (Johnson et al. 2013). Some treatments involve the targeting of plasticity in specific brain regions (Engineer et al. 2011) in order to alter firing in neurons whose abnormal firing patterns are a key component of a pathogenesis. The use of simple sensory stimulation for the neuromodulation treatment of brain disorders has been attempted by several research groups, including the stimulation of the trigeminal nerve for epilepsy and depression (Cook et al. 2013; DeGiorgio et al. 2013) and visual stimulation for phantom limb pain (Chan et al. 2007). However, few have attempted to combine multiple sensory stimuli to induce plasticity through multisensory integration.

Human sensory systems are complexly integrated in the brain through multisensory integration, which allows us to perceive our surroundings as one complete experience as opposed to separate sensory observations. Multisensory integration also

helps us evaluate the combinations of multiple sensory inputs from one source by integrating them in such a way that we can understand how they interact. For example, the spatial maps of the visual and auditory systems are overlapped and integrated in the superior colliculus (Drager and Hubel 1975), such that we can understand how the visual perception of an object in motion correlates with the sound that the object makes. Despite our understanding of this concept, it is not well understood how such integrative interactions are coded in sensory systems (Murray and Wallace 2011; Stein and Stanford 2008), and while we can observe a spatial organization between the visual and auditory systems, the relationship between the somatosensory and auditory systems has not been as thoroughly studied. Some researchers have briefly explored the modulatory and plasticity effects of somatosensory and auditory interactions in the brain (Aitkin et al. 1978; Aitkin et al. 1981b; Foxe et al. 2000; Foxe et al. 2002; Meredith and Stein 1986; Murray et al. 2005), especially in primary auditory cortex (**A1**) (Dehmel et al. 2008b; Gloeckner et al. 2013; Kanold et al. 2011; Levine et al. 2003; Ma and Suga 2003; Markovitz et al. 2015), but these previous studies have only touched the surface of what these interactions are capable of doing to neural activity. This study further investigates how the combination of somatosensory and auditory activation at specific timing delays can modulate neural activity and/or induce plasticity in auditory cortex through existing multisensory integration pathways, and this concept could potentially be used as a form of neuromodulation for treatments of neural sensory disorders within the auditory system, like tinnitus.

Tinnitus is a neural disorder characterized by a phantom sound percept generated within the brain in the absence of an external sound source, affecting over 250 million people worldwide and debilitating for about 1% of the world population (ATA 2010; Møller et al. 2010). It is caused by abnormal neural plasticity that occurs throughout the auditory system and has been linked to hyperactivity, hyper-synchrony across neurons, tonotopic reorganization, and altered spike patterns (Eggermont and Roberts 2004; Henry et al. 2014; Kaltenbach 2011; Lanting et al. 2008; Lanting et al. 2009; Møller et al. 2010). Neuromodulation techniques have been used to treat tinnitus (Vanneste and De Ridder 2012), including invasive treatments like deep brain stimulation (Cheung and Larson 2010) and noninvasive treatments like transcranial magnetic stimulation (De Ridder et al. 2011b), but these treatments have had only limited success with inconsistent results (Møller et al. 2010). Another recent approach has been to take advantage of spike timing-dependent plasticity using invasive bimodal brain stimulation (Caporale and Dan 2008; Tzounopoulos et al. 2007; Wu et al. 2015), which can induce Hebbian-like plasticity in the dorsal cochlear nucleus (Basura et al. 2012) and auditory cortex (Basura et al. 2015), but invasive stimulation can only be available to a small sub-population of patients due to surgical risks. In this study, we attempt to induce similar controlled plasticity in auditory cortex noninvasively using sensory stimulation through a new neuromodulation treatment called Multimodal Synchronization Therapy (**mSync**), which combines various sensory stimuli at specific inter-stimulus delays to induce timing-dependent plasticity. For this study, mSync is implemented with just somatosensory and acoustic stimulation, but other

sensory inputs can be incorporated in future studies (investigated in Chapter 6 of this dissertation).

Materials and Methods

Overview

Electrophysiology experiments were performed on 22 young female Hartley guinea pigs (400–450 g; Elm Hill Breeding Labs, Chelmsford, MA), each anesthetized with an initial intramuscular injection of a ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL) mixture, with supplemental injections every 45–60 minutes to maintain an areflexive state. Neural recordings were performed inside an acoustic-attenuating and electrically-shielded booth using hardware from Tucker-Davis Technology (Alachua, FL), and data was processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Surgery and Neural Recordings

A craniotomy revealing the right primary auditory cortex of each animal was performed. Following the completion of the surgery, the animal's heart rate and blood oxygen content were continuously monitored using an H100 pulse oximeter from EdanUSA (San Diego, CA), and body temperature was monitored using an Oakton Acorn series JKT thermocouple rectal probe (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN).

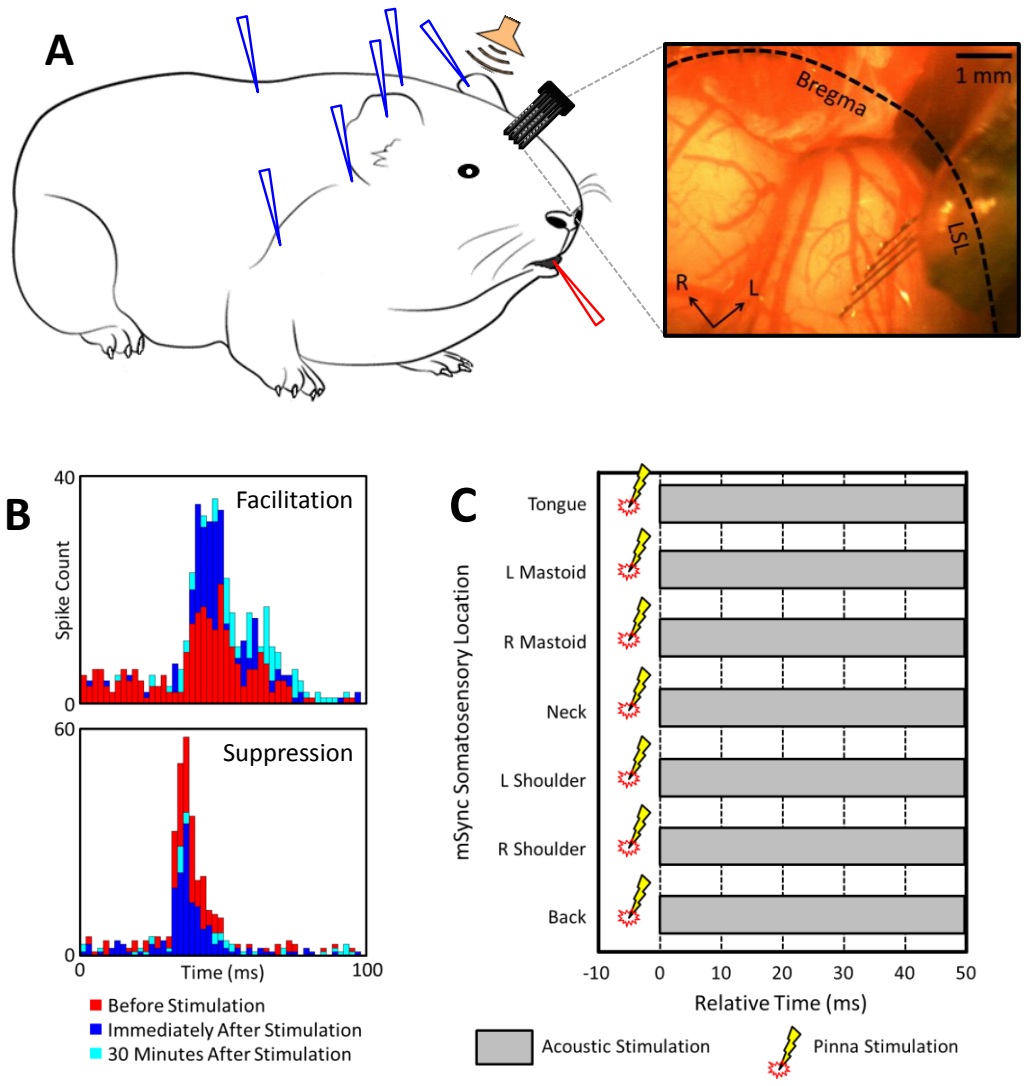

A
Bregma
1 mm
LSL
R
L
B
Facilitation
Suppression
Spike Count
40
0
60
0
0
Time (ms)
100
Before Stimulation
Immediately After Stimulation
30 Minutes After Stimulation
C
mSync Somatosensory Location
Tongue
L Mastoid
R Mastoid
Neck
L Shoulder
R Shoulder
Back
-10
0
10
20
30
40
50
Relative Time (ms)
Acoustic Stimulation
Pinna Stimulation
D
Delay (ms)
-25
-15
-5
+5
+15
+25
-30
-20
-10
0
10
20
30
40
50
Relative Time (ms)

Figure 5: Recording and stimulation methods

A: Surface electrodes were used to electrically stimulate the tongue of the animal (marked with a red triangle), and needle electrodes were used to electrically stimulate the left and right pinna, left and right mastoid region, left and right shoulder, neck, and back of the animal (marked with blue triangles). The broadband noise auditory stimulus was presented to the contralateral (left) ear. Multiunit neural activity was recorded from A1 using a 32-site recording electrode array (sample placement shown). LSL indicates the location of the lateral suture line. **B**: Examples of PSTH comparisons in cases where spike activity was significantly facilitated (top PSTH) or suppressed (bottom PSTH) at a given recording site. For each example, three PSTHs are compiled, including activity before stimulation (red), activity immediately after stimulation (blue), and activity 30 minutes after stimulation (cyan). A two-tailed, unequal variance, ranked t-test ($P<0.01$) was used to determine significance. **C**: Seven somatosensory stimulation locations were used for the initial stimulation location study, and in all cases, the somatosensory stimulus preceded the acoustic stimulus by 5 ms. **D**: Six inter-stimulus delays were used for the inter-stimulus delay study, ranging from the electrical stimulus preceding the acoustic stimulus by 25 ms (-25) to the acoustic stimulus preceding the electrical stimulus by 25 ms (+25) in 10 ms increments.

The animal's head was secured into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars. A 32-site Michigan-style recording electrode array (NeuroNexus Technologies, Ann Arbor, MI) was inserted into the right A1 of the guinea pig brain spanning neurons that

responded mainly to frequencies 1-20 kHz (Figure 5-A). A1 was located based on the position of the pseudosylvian sulcus, the lateral suture line, and the Bregma suture line, based on previous studies (Wallace et al. 2000). The electrode recording array was comprised of four 5-mm long shanks separated by 500 μm with eight iridium sites linearly spaced at 200 μm along each shank (site area = 413 μm^2) and agarose was used to cover and protect the cortex and array after insertion for the remainder of the experiment. This array was placed at a depth such that the main input Layer IV could be observed near the middle of the eight recording sites on each shank (determined by locating the initial sink using current-source density; (Lim and Anderson 2007a; Markovitz et al. 2013; Straka et al. 2014)), which generally resulted in the tip sites being inserted 1.2-1.3 mm below the surface of the cortex. Recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine wave. The recording ground for the electrode array was inserted into the brain in the visual cortex near the intersection of the lambda suture line and the midline.

Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of neural spike activity. A detection threshold of 3.5 times the standard deviation of the voltage noise floor was used to determine when spikes occurred, and spike voltage waveforms were visually inspected to ensure that no noise was falsely detected as spike activity.

General Protocol

For each experiment, 100 trials (2 per second) of responses to broadband noise (50 ms duration, 0.5 ms rise/fall time, 70 dB SPL, equal energy between 625 Hz and 40 kHz) were recorded. Afterward, 1000 trials of mSync (2 per second) were performed, and this was followed by another 100 trials of broadband noise response recordings, 30 minutes of rest, and then one final session of 100 trials of broadband noise response recordings. mSync stimulation consisted of broadband noise stimulation (70 dB) paired with electrical somatosensory stimulation of a specific body location depending on the experiment (350 μA,biphasic, 205 μs per phase), using needle electrodes (Rhythmlink International LLC, Columbia, SC). Stimulation ground electrodes were distributed in each arm and each leg of the animal in order to prevent activation of the somatosensory system with ground electrodes. For the initial study investigating the effects of mSync with different somatosensory stimulation locations, seven animals were used. For the study on inter-stimulus delays, three animals were tested for mSync with right pinna stimulation, three animals with left pinna stimulation, six animals with right mastoid stimulation, and three control animals did not receive mSync at all.

Responses before mSync were compared to those immediately after and 30 minutes after to determine if paired stimulation had induced significant changes in spike activity (two-tailed, unequal variance, ranked t-test, $P<0.01$), and activity on a given recording site was only counted as changed if both the recordings immediately after and 30 minutes after paired stimulation were significantly different from recordings before

stimulation (post-stimulus time histogram examples shown in Figure 5-B). Afterward, the next experiment was performed with a different parameter, and the order of parameters was randomized to mitigate cumulative effects. Care was taken to ensure that each animal received each parameter the same number of times to reduce bias due to animal variability.

mSync Stimulation Parameters

For the initial somatosensory stimulation location experiment, mSync stimulation consisted of 1000 trials (2/s) of acoustic broadband noise stimulation paired with electrical stimulation of either the tongue (n=108 recording sites across 5 animals), left mastoid (n=90 across 4 animals), right mastoid (n=486 across 6 animals), neck (n=79 across 5 animals), left shoulder (n=58 across 3 animals), right shoulder (n=86 across 3 animals), or back (n=57 across 2 animals). In all cases, electrical somatosensory stimulation preceded acoustic stimulation by 5 ms (Figure 5-C), and this was repeated in 13 animals. For the inter-stimulus delay experiment, acoustic broadband noise stimulation was paired with electrical stimulation of either the right pinna (n=1386 across 3 animals), left pinna (n=1167 across 3 animals), or right mastoid (n=2916 across 6 animals). In each case, one of six specific inter-stimulus delays was used, which spanned from -25 to +25 ms in 10 ms increments, where negative delays indicate that the somatosensory stimulus preceded the acoustic stimulus (Figure 5-D). Additionally, control experiments were performed (n=882 across 3 animals), where mSync was replaced with no stimulation in the same protocol.

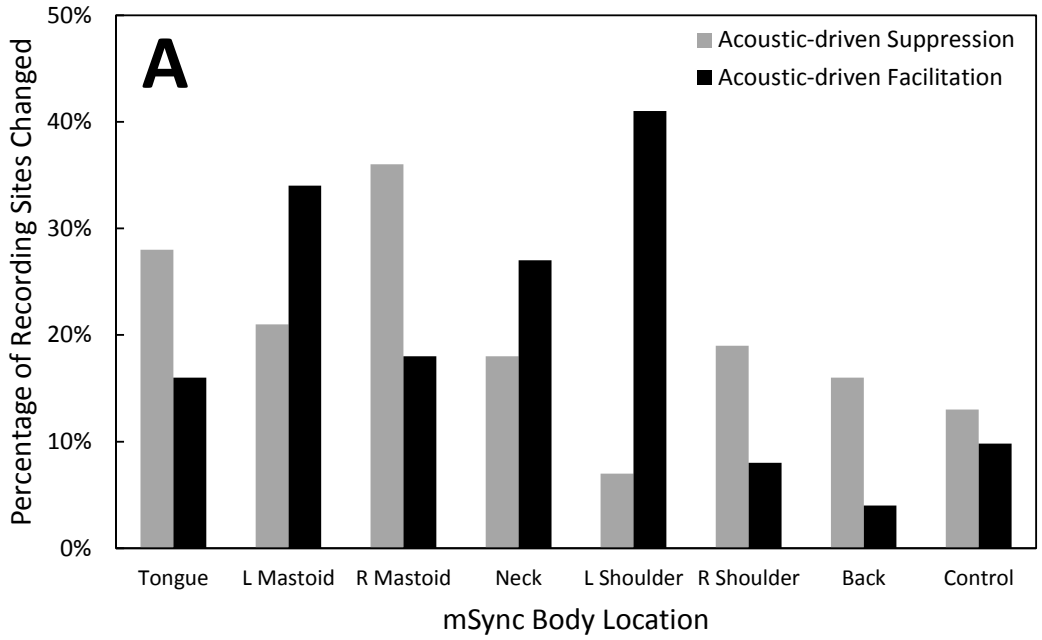
A1 Reconstructions

For each animal, a photograph of the recording electrode placement location was taken perpendicular to the cortical surface using a Moticam 2300 3-megapixel USB microscope camera (Motic, Kowloon, Hong Kong) through a Carl Zeiss surgical microscope (Oberkochen, Germany) at 25x magnification. These photographs captured the individual electrode shanks after insertion, the lateral suture line, the Bregma suture line, the pseudosylvian sulcus, and a 2-D scale bar measuring 5 mm by 5 mm with markings every 1 mm. Using the scale bar, the location of Bregma, and the location of the intersection between the pseudosylvian sulcus and the lateral suture line, electrode placement locations were normalized onto the same image for each animal using Rhinoceros software (Seattle, WA) such that we could confirm that electrode placements were relatively consistent across animals.

Results

Somatosensory Stimulation Location Effects

In the portion of the study investigating somatosensory stimulation location, we found that different somatosensory stimulation locations yield different results for mSync stimulation (Figure 6-A). For example, when pairing tongue stimulation with acoustic stimulation, we observed a greater percentage of recorded A1 sites that exhibited suppressive changes in neural firing compared to facilitative changes; yet when pairing left shoulder stimulation with acoustic stimulation, we observed the opposite trend. In


A
Acoustic-driven Suppression
Acoustic-driven Facilitation
Percentage of Recording Sites Changed
50%
40%
30%
20%
10%
0%
Tongue
L Mastoid
R Mastoid
Neck
L Shoulder
R Shoulder
Back
Control
mSync Body Location

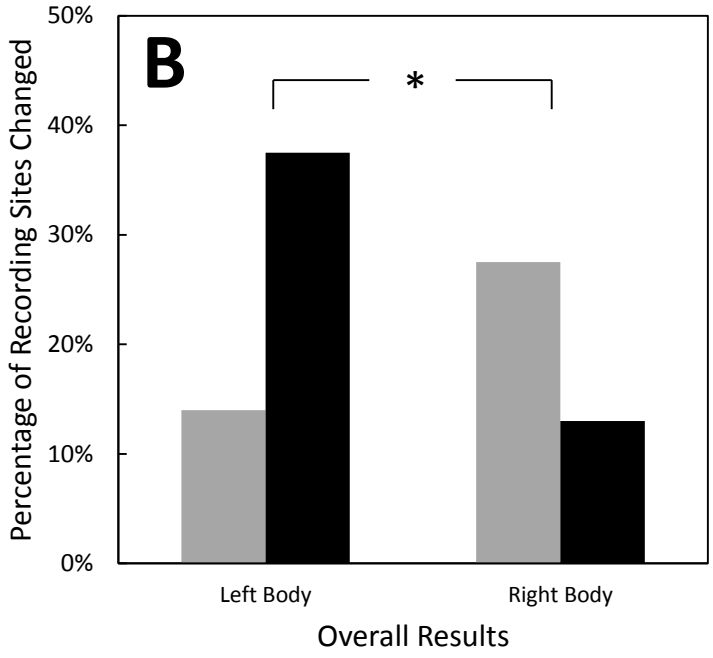
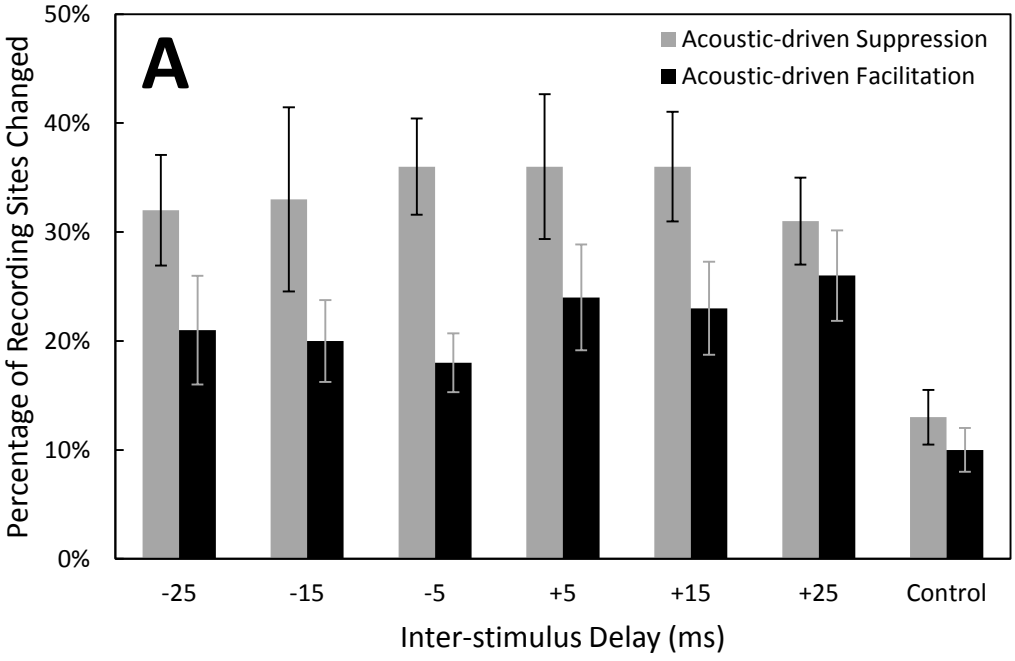

B
*
Percentage of Recording Sites Changed
50%
40%
30%
20%
10%
0%
Left Body
Right Body
Overall Results

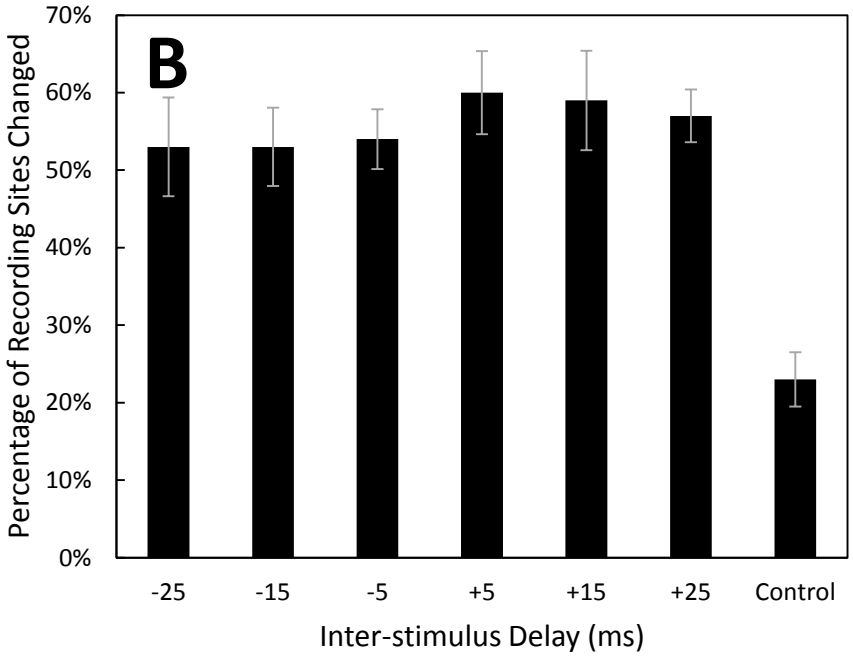
Figure 6: Plasticity induced by mSync with various somatosensory locations.

The percentage of total recording sites in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) immediately after stimulation is shown for mSync with different somatosensory stimulation locations (**A**). In all cases, somatosensory stimulation preceded acoustic stimulation by 5 ms. Tested somatosensory locations included the tongue (n=108 across 5 animals), left mastoid (n=90 across 4 animals), right mastoid (n=486 across 6 animals), neck (n=79 across 5 animals), left shoulder (n=58 across 3 animals), right shoulder (n=86 across 3 animals), and back (n=57 across 2 animals). For classifying a recording site as suppressed or facilitated, significance was determined using a two-tailed, unequal variance, ranked t-test ($P<0.01$) when comparing post-stimulus activity immediately after and 30 minutes after stimulation to pre-stimulus activity. In three control animals (n=882), mSync was replaced with no stimulation, but recordings were performed at the same times. In general, contralateral (left) somatosensory locations induced more facilitated recording sites than suppressed, while ipsilateral (right) locations induced more suppressed than facilitated sites (summary in **B**), and this was statistically significant across somatosensory stimulation locations ($P<0.05$, standard t-test).

fact, left shoulder stimulation elicited mostly facilitative effects. Overall, a different percentage of changed A1 recording sites and a different ratio between suppressed and facilitated sites was elicited depending on somatosensory location stimulated.

Interestingly, one major trend was observed from the data: stimulation locations contralateral to the recording location (left shoulder and mastoid) were generally more facilitative than suppressive, while ipsilateral locations (right shoulder and mastoid) were


A
Acoustic-driven Suppression
Acoustic-driven Facilitation
Percentage of Recording Sites Changed
50%
40%
30%
20%
10%
0%
-25
-15
-5
+5
+15
+25
Control
Inter-stimulus Delay (ms)


B
Percentage of Recording Sites Changed
70%
60%
50%
40%
30%
20%
10%
0%
-25
-15
-5
+5
+15
+25
Control
Inter-stimulus Delay (ms)

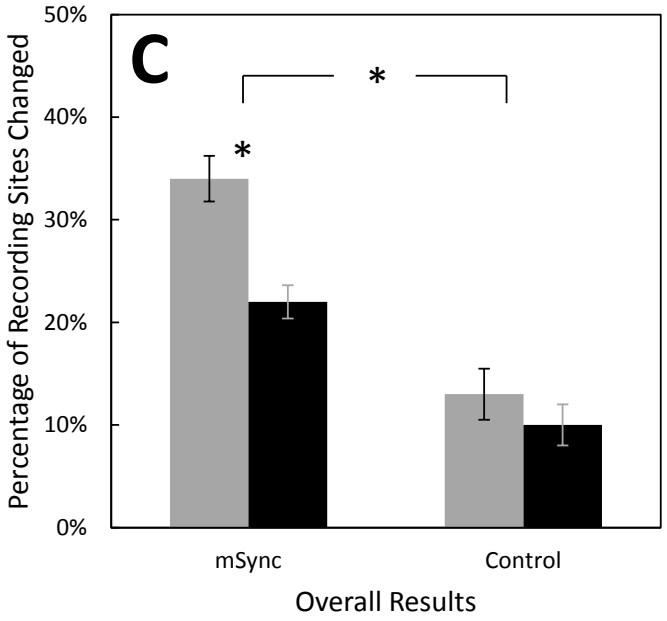


Figure 7: Plasticity induced by mSync with right mastoid stimulation.

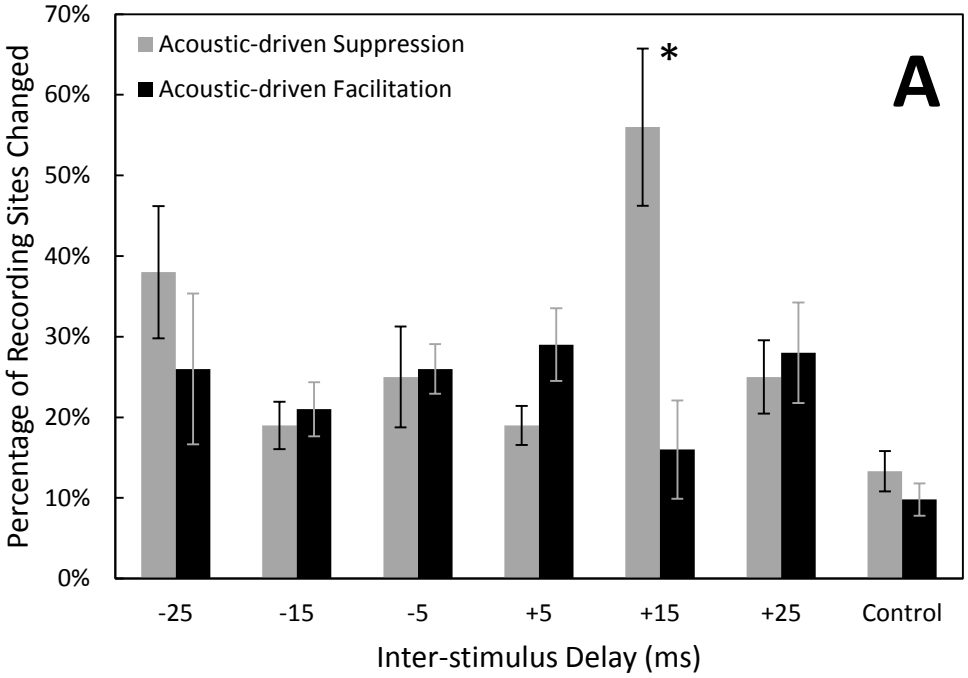
The percentage of total recording sites (n=2916 across 6 animals) in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) both immediately after and 30 minutes after stimulation for mSync with right mastoid stimulation is shown for different inter-stimulus delays (**A**). An inter-stimulus delay of -25 indicates that electrical pinna stimulation preceded acoustic stimulation by 25 ms. For classifying a recording site as suppressed or facilitated, significance was determined using a two-tailed, unequal variance, ranked t-test ($P<0.01$) when comparing post-stimulus activity to pre-stimulus activity. For three control animals (n=882), no stimulation was used, but recordings were performed at the same times. Error bars show standard error across animals. mSync was suppressive regardless of the delay used, although some delays were more suppressive than others (there is no statistical significance when comparing one delay to another for either suppression or facilitation). All delays induced changes in significantly more total recording sites

(facilitation plus suppression) than control (**B**). **C** shows a summary of mSync with data from all delays combined compared to control. In this plot, the percent of sites suppressed was significantly greater than the percent of sites facilitated ($P<0.05$), and both suppression and facilitation were significantly greater than that of control ($P<0.05$).

more suppressive than facilitative (Figure 6-B), and the difference in suppression/facilitation ratio was statistically significant across somatosensory stimulation locations (standard two-tailed, unequal variance t-test, $P<0.05$). Some of the data shown in Figure 6 was published in a previous paper in which data was collected and analyzed by multiple authors, including the author of this dissertation (Markovitz et al. 2015).

Inter-stimulus Delay Effects

When pairing right mastoid stimulation with acoustic stimulation in our inter-stimulus delay study, mSync tended to have a suppressive effect on neural firing in A1 regardless of inter-stimulus delay, and although different delays could induce more suppression than others on average, there was no statistically significant difference in suppression or facilitation between any two delays (Figure 7-A). All delays resulted in more total sites changed (regardless of whether they were facilitated or suppressed) than control (Figure 7-B), and if all of the mSync data is combined across all delays (Figure 7-C), statistical analysis shows that mSync with right mastoid stimulation was statistically significantly more suppressive than facilitative ($P<0.05$), and induced both suppression and facilitation in a greater percentage of sites than no stimulation ($P<0.05$).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Percentage of Recording Sites Changed
Acoustic-driven Suppression
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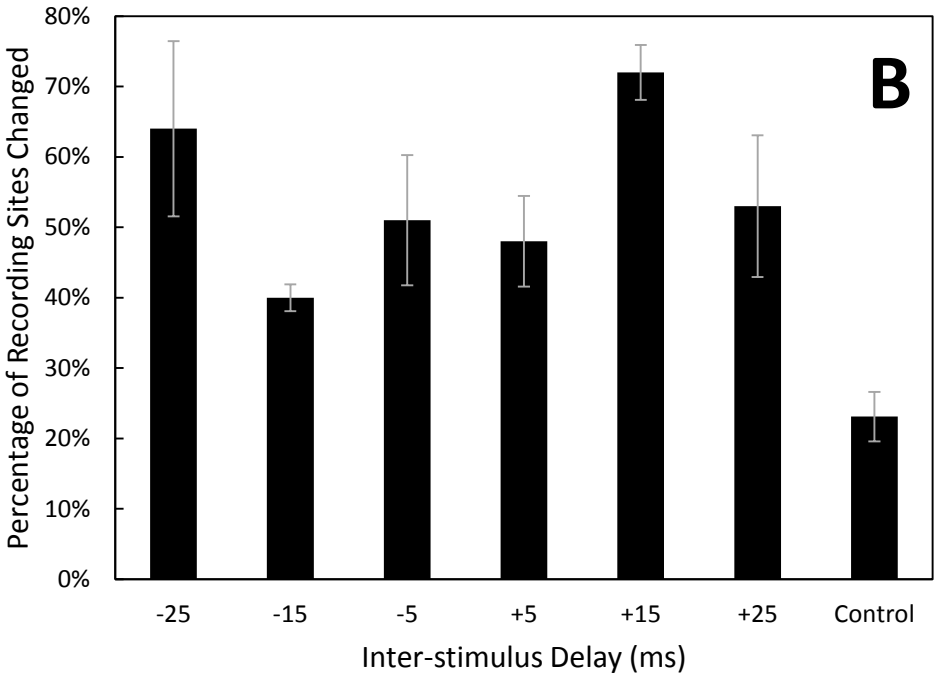
Control
Inter-stimulus Delay (ms)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Percentage of Recording Sites Changed
B
-25
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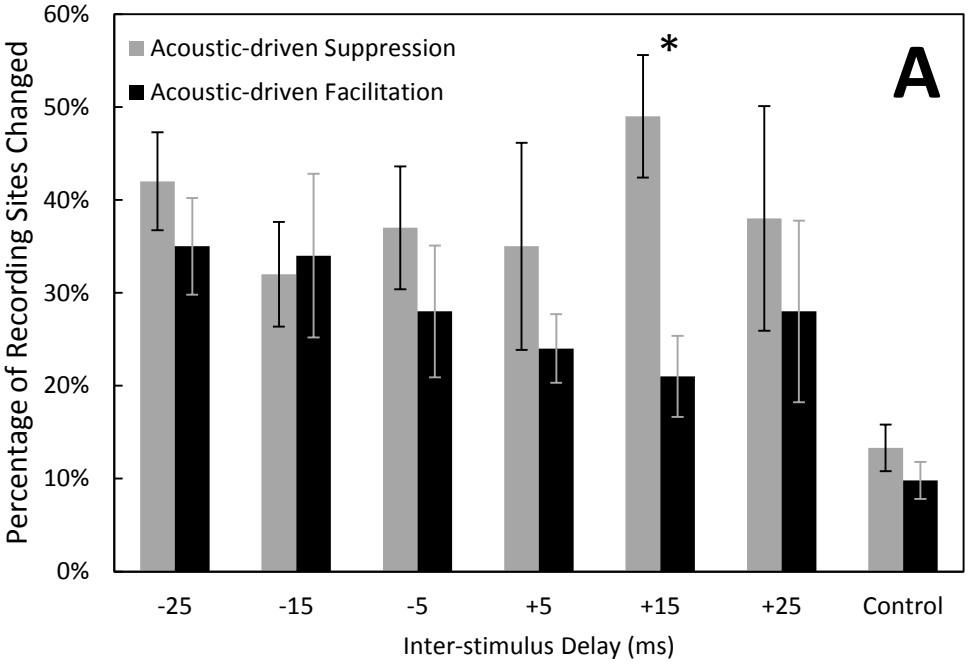
+5
+15
+25
Control
Inter-stimulus Delay (ms)

Figure 8: Plasticity induced by mSync with left pinna stimulation.

The percentage of total recording sites in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) both immediately after and 30 minutes after stimulation for mSync with left pinna stimulation is shown for different inter-stimulus delays (**A**, n=1167 recording sites across 3 animals) . The same statistical methods as in Figure 7 were used, and error bars show standard error across animals. For three control animals (n=882), no stimulation was used, but recordings were performed at the same times. Only the inter-stimulus delay of +15 yielded statistically significantly more suppressed recording sites than facilitated recording sites ($P<0.05$). All delays induced changes in more total sites (facilitated sites plus suppressed sites) than control (**B**).

For mSync with pinna stimulation, similar results were observed for left pinna (Figure 8-A) and right pinna (Figure 9-A). In both cases, for inter-stimulus delays of -25, -15, -5, +5, and +25 ms, there were no significant differences between the percentages of sites suppressed and facilitated. However, the inter-stimulus delay of +15 ms induced a significantly higher percentage of suppressed sites than facilitated sites ($P<0.05$), and this phenomenon was consistent across all six animals for both pinnas. All delays yielded more total recording sites changed (regardless of whether they were facilitated or suppressed) than no stimulation (Figures 8-B and 9-B), and when the total percentage of sites across delays is compared to control, paired stimulation resulted in changes in significantly more sites than control for both left and right pinnas (Figure 10, p-values on figure).


60%
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Percentage of Recording Sites Changed
Acoustic-driven Suppression
Acoustic-driven Facilitation
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+5
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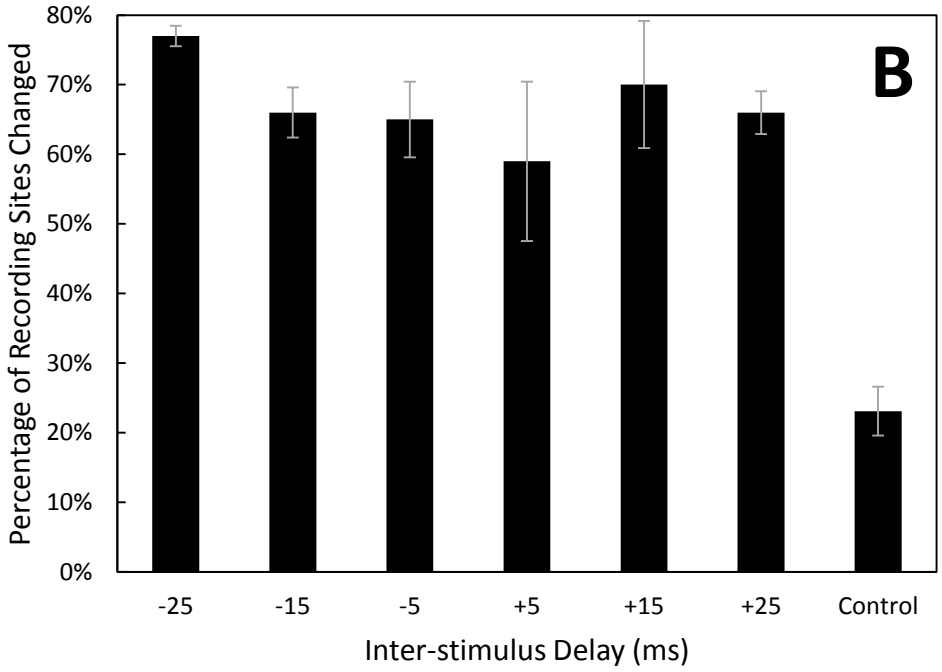
+25
Control
Inter-stimulus Delay (ms)


80%
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Percentage of Recording Sites Changed
B
-25
-15
-5
+5
+15
+25
Control
Inter-stimulus Delay (ms)

Figure 9: Plasticity induced by mSync with right pinna stimulation.

The percentage of total recording sites in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) both immediately after and 30 minutes after stimulation for mSync with right pinna stimulation is shown for different inter-stimulus delays (**A**, n=1167 recording sites across 3 animals) . The same statistical methods as in Figure 7 were used, and error bars show standard error across animals. For three control animals (n=882), no stimulation was used, but recordings were performed at the same times. Only the inter-stimulus delay of +15 yielded statistically significantly more suppressed recording sites than facilitated recording sites ($P<0.05$). All delays induced changes in more total sites (facilitated sites plus suppressed sites) than control (**B**).

Discussion

Influence of mSync Parameters

The results of these two studies may be promising for neuromodulation treatments of neural sensory disorders such as tinnitus. Because hyperactivity in A1 is an important biomarker of tinnitus (Eggermont and Roberts 2004; Lanting et al. 2008; Lanting et al. 2009), suppression may be a key goal in using mSync for tinnitus treatment, as well as the treatment of other sensory disorders. Therefore, the suppressive nature of mSync with right mastoid stimulation and mSync with pinna stimulation at an inter-stimulus delay of +15 might be useful in neuromodulation treatments. Additionally, hyper-synchrony is also an important biomarker for tinnitus (Eggermont and Roberts 2004), and it may be possible to break up hyper-synchrony by increasing the firing rates of some neurons

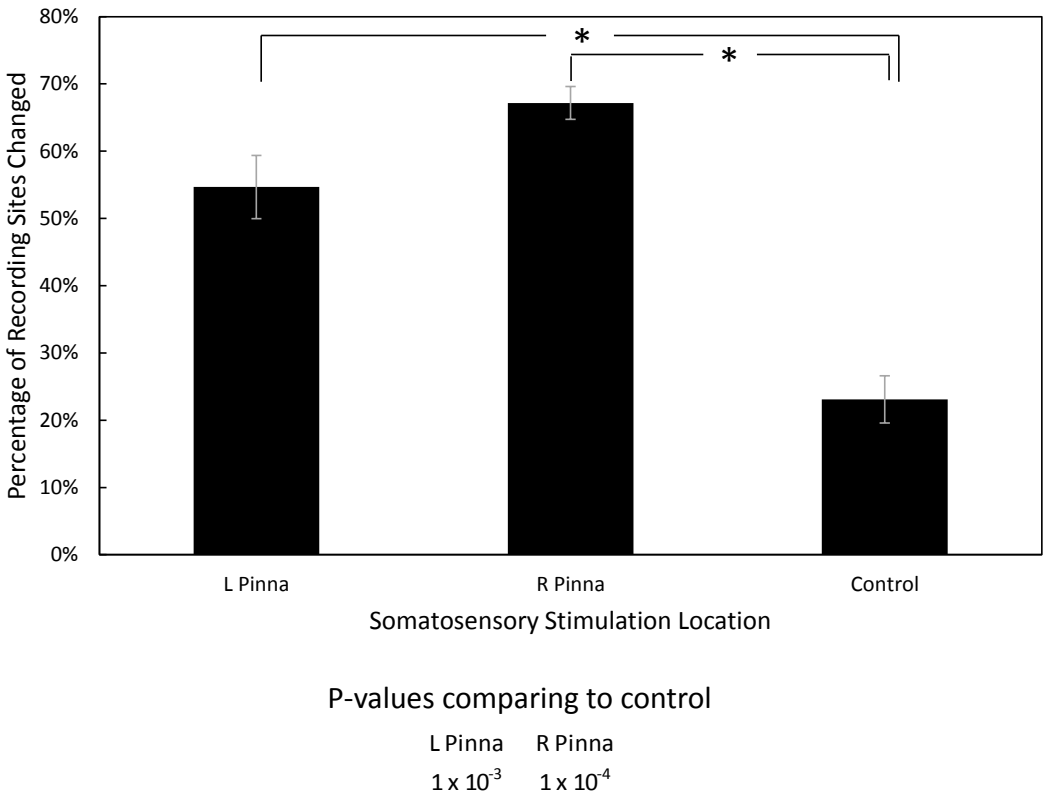


Figure 10: Comparing pinna stimulation with control

The percentage of total recording sites in which acoustic-driven spike activity was changed both immediately after and 30 minutes after stimulation across all inter-stimulus delays is shown for mSync with left pinna stimulation, right pinna stimulation, and control. Both left and right pinna stimulation induced changes in significantly more recording sites than control (standard t-test, p-values are shown on figure).

while decreasing firing rates of others. In our data, even with our most suppressive paradigms, there are still some recording sites that are facilitated, and there are some somatosensory stimulation locations that are naturally facilitative as well. The right combination of stimulation parameters may be useful in addressing problems with

synchrony across neurons, as increasing firing rates of some neurons while decreasing firing rates of others will cause them to desynchronize. Unfortunately, it is difficult to measure changes in synchrony in healthy animals that don't have tinnitus, since these animals already have low synchrony levels, but if a reliable tinnitus animal model is used, and high synchrony levels can be established in A1, the ability to combat hyper-synchrony with mSync could be further investigated.

Furthermore, the diverse results as different somatosensory stimulation locations are used might be helpful for treating tinnitus and other neural disorders in which symptoms vary greatly across patients. It may be inferred that because every tinnitus patient experiences different phantom sounds, each patient might need a slightly different treatment to address a different population of pathogenic neurons with different abnormal firing patterns. Our ability to target different forms of plasticity (through facilitation/suppression) and potentially target different groups of neurons might be useful for optimizing treatment for each patient individually. Finally, our inter-stimulus delay results are similar to previous studies aimed at treating tinnitus with invasive bimodal stimulation, but we are able to achieve these results noninvasively with mSync. Safe, noninvasive stimulation would open this treatment up to a much larger patient population if such a treatment is successful in humans.

The observation that mSync with contralateral somatosensory stimulation locations yields different results than that with ipsilateral locations in our initial experiments (Figure 6) isn't surprising. If we assume that the integration of

somatosensory activity with other sensory systems happens after the pyramidal decussation in the caudal portion of the medulla, then any projections leading to multisensory integration would occur on the side of the brain that is opposite of stimulation. This means that contralateral somatosensory stimulation would actually project to the same side of the brain as our A1 recordings, while ipsilateral stimulation would have to travel to the contralateral side of the brain before either crossing back over or modulating the auditory system contralaterally (which could induce inhibition on the opposite side of the brain). Regardless of the true mechanism, if somatosensory projections are either excitatory or inhibitory to the auditory system, then contralateral somatosensory stimulation should have the opposite effect compared to ipsilateral stimulation. Previous studies have shown excitatory activity in the auditory midbrain induced by somatosensory stimulation (Aitkin et al. 1981b; Gloeckner et al. 2013), so it not surprising to observe facilitative contralateral stimulation locations and suppressive ipsilateral locations.

In Figure 6, mSync with right mastoid stimulation is the most suppressive of all somatosensory stimulation locations, so it isn't surprising to see that all inter-stimulus delays are suppressive in Figure 7. However, in Figures 8 and 9, we see that mSync with both left and right pinna stimulation leads to nearly identical facilitation and suppression for all delays but one. We expected to observe an importance in timing based on previous studies of invasive bimodal stimulation (Basura et al. 2015; Basura et al. 2012; Tzounopoulos et al. 2007; Wu et al. 2015), so the consistent suppressive result for the

+15 delay isn't surprising; however, it is surprising to see that inter-stimulus delay played an important role for pinna stimulation and not mastoid stimulation. There are two possible explanations for this. First, it is possible that mSync with right mastoid stimulation is naturally so suppressive that a significant difference across delays could not be observed. This is plausible because while all delays were suppressive, some delays had more suppressed sites and fewer facilitated sites than others, indicating that inter-stimulus delay could still be a factor, albeit a smaller factor than somatosensory stimulation location in this case. Another possible explanation is differences in onset delays. Our measure of inter-stimulus delay is based on the time difference between the beginning of the two stimuli, but because somatosensory stimulation likely has a different latency than acoustic stimulation in A1, the delay between the onsets of the two stimulus responses in A1 is likely different. Furthermore, different somatosensory locations have different latencies, which means the onset latency for mSync at a particular inter-stimulus delay is different for two different somatosensory stimulation locations. If the difference in latency between pinna and mastoid stimulation is not a multiple of 10 ms, then the ideal onset delay for mastoid (observed at +15 for pinna) might fall in between the delays that were tested in this study. Because of this, we can't be certain whether or not inter-stimulus delay plays an important role for mSync with right mastoid stimulation.

Previous studies on invasive bimodal stimulation for timing-dependent plasticity induction show that in the auditory cortex and dorsal cochlear nucleus, facilitative inter-stimulus delays often neighbor suppressive delays, and these two delays are usually about

8-15 ms apart (Basura et al. 2015; Basura et al. 2012; Tzounopoulos et al. 2007; Wu et al. 2015). In our study, we did not observe any facilitative delays, even in our pinna stimulation experiments. There are two possible explanations for this. First, we used ketamine to anesthetize our animals, and ketamine is known to inhibit N-Methyl-D-Aspartate (NMDA) receptor activation (Gonzales et al. 1995; Kim et al. 1996a; Silva et al. 1997), which is closely linked with plasticity induction. Previous studies have shown that ketamine can specifically inhibit the excitation of spike activity in brain slices (Hu and Davies 1997) and in the auditory system in vivo (Kaltenbach et al. 2000). This would explain why we were unable to achieve facilitation at any particular delay while still observing suppression at a delay of +15. This same phenomenon was observed in a previous Hebbian plasticity study, where the facilitative inter-stimulus delay had much smaller effects than the suppressive delay (Tzounopoulos et al. 2007). Second, it is possible that the true facilitative delay for mSync with pinna stimulation falls between delays that we tested. The timespan between facilitative and suppressive delays varied across previous studies, so it is difficult to calculate what delay actually should be facilitative solely based on our observation of a suppressive +15 delay. If the facilitative delay is not exactly 10 ms away from the suppressive delay in our experiment, then we would not observe it in our data.

Future Studies

In the data presented here, inter-stimulus delay played a role for some somatosensory locations and not others. Based on the narrowness of the time-span for

inter-stimulus delays that have specific facilitative or suppressive effects in previous studies (Basura et al. 2015; Basura et al. 2012; Tzounopoulos et al. 2007; Wu et al. 2015), it is possible that differences in latencies for different somatosensory locations could allow for the ideal facilitative/suppressive delays to fall in between the delays we chose for some locations and not others. A future study could test delays in finer increments to better investigate this possibility. Additionally, these delay experiments could be repeated for other somatosensory stimulation locations to see if there are trends when comparing locations that are affected by inter-stimulus delay to those that are not.

Chapter 4: The Effects of Stress on Neuromodulation and Neuroplasticity

The experiments in Chapter 3 were performed using an anesthetized animal preparation. We know that anesthesia can potentially affect or limit plasticity effects, so we repeated these experiments in awake animals. Furthermore, we investigated the effects of stress on mSync outcomes, since it is expected that stress could confound or limit plasticity effects, especially in neural disorder patients who have varying degrees of anxiety and stress on a daily basis.

Summary

Various types of invasive and noninvasive neuromodulation approaches are being used to induce plasticity in the brain and treat different neurological and psychiatric disorders. In general, neuromodulation outcomes can vary substantially across subjects, even for the same stimulation method. Although stress has been shown to affect the brain in many ways, it is not typically addressed in neuromodulation studies. To address this issue, we investigated the effects of stress on timing-dependent plasticity in auditory cortex when applying a new multisensory neuromodulation approach for tinnitus treatment that combines somatosensory and acoustic stimulation with specific inter-stimulus delays. Low-stress animals exhibited consistent timing-dependent plasticity patterns while high-stress animals exhibited patterns inconsistent with the expected time-dependence and fewer neurons changed. Furthermore, timing-dependent neuromodulatory effects were observed in low-stress animals, which we expect based on

results in anesthetized animals, while high-stress animals exhibited no such effects. These findings reveal the critical importance of monitoring or controlling for stress that can potentially confound or limit neuromodulation outcomes.

Introduction

The effects of stress on the brain have been studied in animals and in humans. Stress has been shown to increase levels of several neurotransmitters, including dopamine (Abercrombie et al. 1989; Del Arco et al. 2007; Finlay et al. 1995), norepinephrine (Finlay et al. 1995), and acetylcholine (Del Arco et al. 2007), while also decreasing apical dendritic length (Bose et al. 2010) and branch quantity (Bloss et al. 2010), all of which have implications on plasticity and the modulation of spike activity. Stress has also been shown to block long-term potentiation (Kavushansky and Richter-Levin 2006; Shakesby et al. 2002), which is an increase in synaptic strength through plasticity induction, and changes in serotonin levels/uptake may play an important role in this phenomenon (Shakesby et al. 2002). NMDA receptors are also shown to be activated more frequently with stress (Kim et al. 1996b; Shors et al. 2004). More generally, previous studies have investigated how stress affects plasticity in various brain structures (Lupien et al. 2009; Sapolsky 2003), especially the cortex (Arnsten 2009; McEwen and Morrison 2013), with evidence that stress can impair plasticity at the synaptic level (Jay et al. 2004). Given how these studies demonstrating the effects of stress on neurotransmitters, neural firing, and plasticity, it is also possible that stress could confound or limit various

neuromodulation approaches attempting to induce neural plasticity and/or modulate spike activity for the treatment of brain disorders.

Neuromodulation is a field of treatment that focuses on altering neural firing, which can lead to acute changes or more long-term plasticity for the treatment of symptoms associated with an abnormal brain state. A broad range of neuromodulation approaches have emerged over the past few decades with a recent surge in their use for numerous neurological and psychiatric disorders. For example, deep brain stimulation is being used for improving symptoms in motor tremors, obsessive compulsive disorder, depression, and memory loss (Aouizerate et al. 2004; Johnson et al. 2008; Laxton et al. 2010; Sturm et al. 2003), while noninvasive transcranial magnetic stimulation is being used to treat depression, pain, tinnitus, and motor recovery (De Ridder et al. 2011b; George et al. 2000; Kanda et al. 2003; Lefaucheur 2006; Londero et al. 2006). However, while many neuromodulation approaches have shown initial promise in treating various brain conditions, outcomes remain inconsistent across patients (Johnson et al. 2013).

Given the effects stress can have on the brain, we investigated how stress may lead to inconsistencies in neuromodulation outcomes and plasticity induction. To test these effects, we used a new noninvasive form of neuromodulation called Multimodal Synchronization Therapy (mSync), which takes advantage of multisensory integration by combining the stimulation of multiple sensory systems at different inter-stimulus delays to induce timing-dependent plasticity in sensory cortices for the treatment of neural sensory disorders (Gloeckner et al. 2013; Markovitz et al. 2015). Multisensory integration

enables our ability to perceive the surrounding environment as one complete experience as opposed to separate sensory inputs or cues. For example, the spatial maps of the visual and auditory space are integrated within the superior colliculus (Drager and Hubel 1975), such that we can understand how a visual experience of an action correlates with the sound of that action. Previous studies have shown interactions between the somatosensory system and auditory system (Aitkin et al. 1981b; Foxe et al. 2000; Meredith and Stein 1986; Stein and Stanford 2008), and combinations of somatosensory and auditory stimulation can modulate activity and induce plasticity in the auditory system (Foxe et al. 2002; Levine et al. 2003; Ma and Suga 2003; Murray et al. 2005), which can also be used to modulate tinnitus (Levine et al. 2003). mSync will leverage multisensory convergence in the brain to induce plasticity for the disruption of abnormal firing patterns. Recent studies have explored the ability to combine invasive trigeminal nerve and sound stimulation to treat tinnitus (Basura et al. 2015; Latifpour et al. 2009; Markovitz et al. 2015), but mSync attempts to do this noninvasively.

In this study, we used mSync consisting of paired electrical somatosensory stimulation of the pinnas and acoustic broadband noise stimulation to induce timing-dependent plasticity in auditory cortex, a brain region implicated for tinnitus in which controllable suppression or enhancement of neural firing could potentially disrupt the pathogenic activity driving the phantom and potentially debilitating sound percept. Plasticity effects were assessed in anesthetized animals alongside high-stress and low-stress awake animals to evaluate the effects of stress and anesthesia on neuromodulation outcomes.

Materials and Methods

Anesthetized Study Preparation

Nine young female Hartley guinea pigs (400–450 g; Elm Hill Breeding Labs, Chelmsford, MA) were anesthetized with an initial intramuscular injection of a ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL) cocktail, with 0.1 mL supplements every 45–60 minutes to maintain an areflexive state. Three of these animals were used for the control, while the remaining six received mSync stimulation. Neural recordings were performed inside an acoustic-attenuating and electrically-shielded booth using hardware from Tucker-Davis Technology (Alachua, FL), and neural data was processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

A craniotomy was performed revealing the right primary auditory cortex of each animal. The animal's heart rate and blood oxygen content were continuously monitored using an EdanUSA H100 pulse oximeter (San Diego, CA), and body temperature was recorded using an Acorn series JKT thermocouple rectal probe by Oakton (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN). The animal was held into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars. A 32-site Michigan-style recording electrode array (NeuroNexus Technologies, Ann Arbor, MI), comprised of four 5-mm long shanks separated by 500

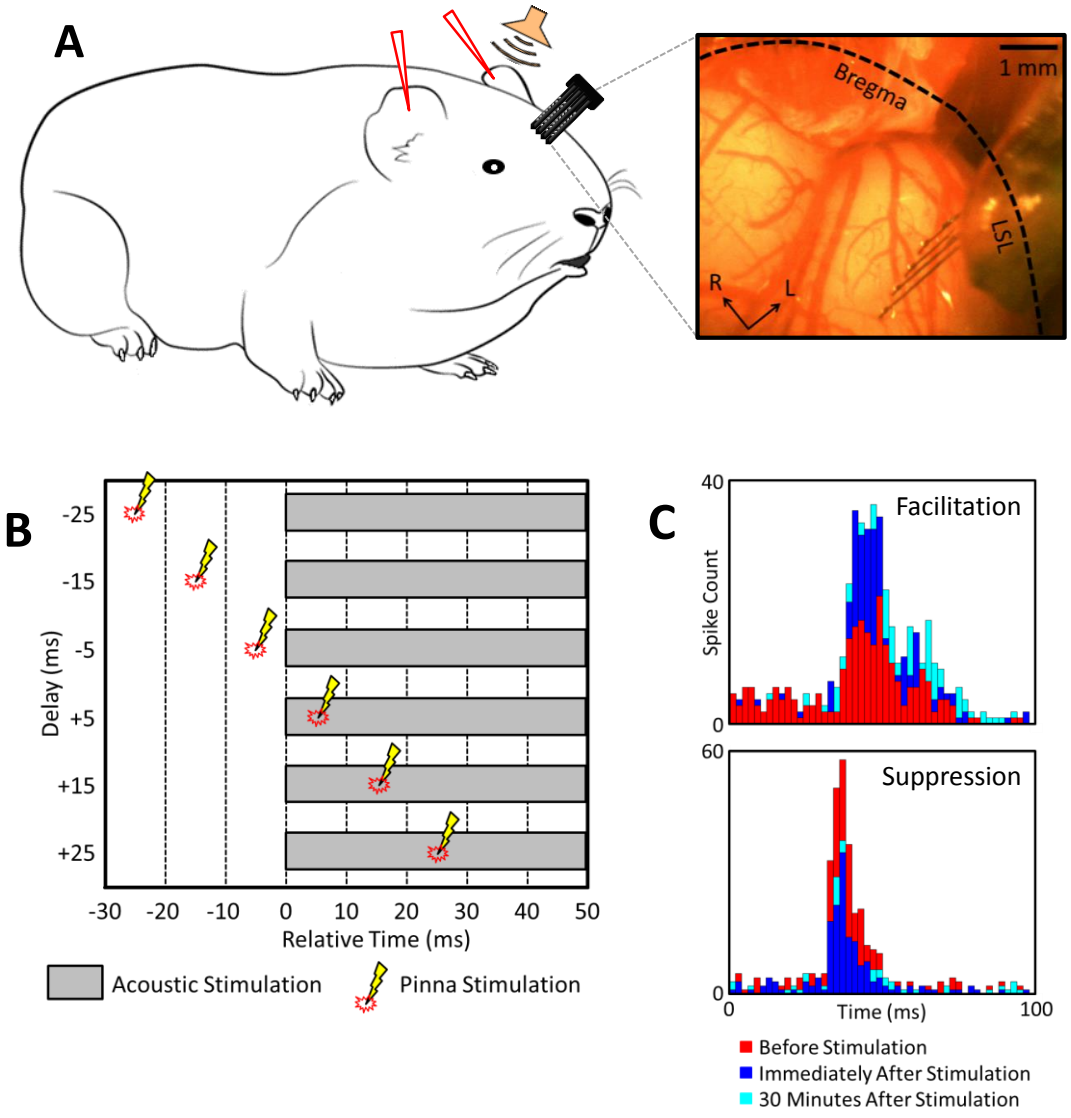


Figure 11: Recording and stimulation methods

A: Surface electrodes were used to electrically stimulate the pinnas of the animal (marked by red triangles). The broadband noise auditory stimulus was presented to the contralateral (left) ear in anesthetized animals and both ears in awake animals. Multiunit neural activity was recorded from A1using a 32-site recording electrode array (sample placement shown, where LSL indicates the location of the lateral suture line). **B**: Six inter-stimulus delays were used,

ranging from the electrical stimulus preceding the acoustic stimulus by 25 ms (-25) to the acoustic stimulus preceding the electrical stimulus by 25 ms (+25) in 10 ms increments. **C**: Examples of PSTH comparisons in cases where spike activity was significantly facilitated (example shown in top PSTH) or suppressed (bottom PSTH) at a given recording site are shown. For each example, three PSTHs are compiled, including activity before stimulation (red), activity immediately after stimulation (blue), and activity 30 minutes after stimulation (cyan). A two-tailed, unequal variance, ranked t-test ($P<0.01$) was used to determine if recording sites had significantly different spike activity before and after mSync stimulation.

μm with eight iridium sites linearly spaced at 200 μm along each shank (site area = 413 μm^2), was inserted into the right A1 of the guinea pig brain spanning neurons that responded roughly to frequencies 1-20 kHz (Figure 11-A). The array was inserted to a depth such that the main input Layer IV could be observed at approximately the middle of the eight recording sites on each shank (determined based on locating the initial sink using current-source density; (Lim and Anderson 2007a; Markovitz et al. 2013; Straka et al. 2014)), usually around 1.2-1.3 mm below the cortical surface. Recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine wave. The recording ground for the electrode array was inserted into the visual cortex near the intersection of the lambda suture line and the midline. Saline was routinely administered to the cortex after the probe was placed to limit the effects of drying.

Awake Study Preparation

Craniotomies were performed on 15 female Hartley guinea pigs (700-800 g) under one intramuscular injection of a ketamine (40 mg/kg) and xylazine (10 mg/kg) cocktail anesthetic. A 16-site Michigan-style recording electrode array, comprised of four 4-mm long shanks separated by 200 μm with four iridium sites linearly spaced at 200 μm along each shank (site area = 1250 μm^2), was inserted into the right A1 of the guinea pig brain near the main input Layer IV using a NeuroNexus d-Drive disposable drive. This array was grounded to bone screws placed in the animal's skull, and Coltene Permanent Resin bone cement (Hygenic, Cuyahoga Falls, OH) was used to seal the surgical opening. The animal was then allowed to wake up and was placed under a heat lamp until sternal. After surgery, all animals were given three days to fully recover, receiving daily intramuscular injections of Baytril 100 enrofloxacin (100 mg/mL, Bayer HealthCare LLC, Shawnee Mission, KS) and ketoprofen (10 mg/mL, Pfizer Inc., New York, NY) for infection prevention and pain relief.

Following surgery, animals were randomly split into three groups of 5. The first group received daily treatments of Healing Touch for Animals (HTA) by a certified HTA practitioner for stress relief, while the second group received only minimal necessary handling for experiments and basic animal care according to the IACUC protocol. Healing Touch is a popular form of clinical complimentary medicine (Hover-Kramer and Mentgen 2002; Wardell and Weymouth 2004) and is used to supplement clinical treatments for various diseases/disorders (Eschiti 2007; Post-White et al. 2003;

Wilkinson et al. 2002) and reduce stress in patients (Maville et al. 2008; Shore 2004). Each Healing Touch session lasted 15-45 minutes at the discretion of the practitioner. These two groups each received multiple sessions of mSync with varying inter-stimulus delays, which is further described later in this chapter. The third group of animals served as the control, in which the animals did not receive mSync or HTA.

Awake Study Behavioral Stress Testing

To test for stress, we used an elevated plus maze behavioral test, which is a method used in numerous previous studies for various rodents (Carobrez and Bertoglio 2005; Hogg 1996; Pellow et al. 1985; Varty et al. 2002), including guinea pigs (Rex et al. 1994; Rex et al. 1993a; Rex et al. 1997; Rex et al. 1993b), and is validated for stress and anxiety measurement (Walf and Frye 2007). A separate lab technician performed a behavioral plus maze experiment to determine stress levels in the animals. Each animal was placed on a custom built elevated plus maze (1 m above ground, 70 cm arm length, 20 cm arm width with two arms guarded by 30 cm tall walls and two arms completely open), and given three minutes to roam freely on the maze. The percent of time that each animal spent in the open areas of the maze was recorded, and this was repeated three separate times on alternating days in an empty room with no human presence, (animal whereabouts were recorded using an HD video camera, Sharx Security Inc., Derry, NH). The study was double blinded, and careful planning ensured that the behavioral trial technician did not come into contact with the Healing Touch practitioner such that the technician had no way of knowing which animals received treatment and the practitioner

did not know the results of the behavioral trials until after the completion of the entire study. Afterward, times in open areas between the two groups were compared using a standard t-test ($P<0.05$) to determine if stress levels were significantly different between them.

Additional Behavioral Testing for Non-Implanted Animals

Ten young (400-450 g) female Hartley guinea pigs were used for awake behavioral studies on an elevated plus maze. These animals did not receive neural implants. Animals were tested for stress in three separate sessions under the same protocol as those in the Awake Study Preparation section above. Afterward, the animals were split into two groups of five based on stress levels, such that the mean percentage of time spent in open areas was similar for both groups. During the following two weeks, one group received five sessions of HTA, with each session lasting 15-45 minutes at the discretion of the practitioner, while the other group was only handled minimally. After two weeks, animals were retested on the plus maze in three trials, and the percentage of time spent in open areas for the two groups was compared using a standard two-tailed unequal variance t-test ($P<0.05$). Once again, the study was double blinded, such that the plus maze technician had no way of knowing which animals received treatment and the practitioner did not know the results of the behavioral trials until after the completion of the entire study.

Neural Recordings and mSync Paired Stimulation

Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of neural spike activity. A detection threshold of 3.5 times the standard deviation of the voltage noise floor was used to determine when spikes occurred, and spike voltage waveforms were visually inspected to ensure that no noise was falsely detected. Responses to broadband noise acoustic stimulation (50 ms duration, 0.5 ms rise/fall time, 70 dB SPL, equal energy between 625 Hz and 40 kHz) were used to measure changes in spike activity in A1 before and after mSync stimulation. For anesthetized animals, broadband noise was presented to the animal's left ear using a speaker (Tucker-Davis Technology, Alachua, FL) coupled to the left ear bar. The speaker-ear bar system was calibrated using a 0.25 in condenser microphone (ACO Pacific, Belmont, CA). For awake animals, a free-field speaker placed about 0.65 meters away from the animal was used to present sounds to both ears. The free-field speaker was calibrated using a 0.5 in. free-field microphone.

mSync stimulation consisted of broadband noise stimulation (70 dB) paired with electrical somatosensory stimulation of the pinnas (350 μA, biphasic, 205 μs per phase) using needle electrodes (Rhythmlink International LLC, Columbia, SC) for anesthetized animals and surface electrodes for awake animals, with the stimulation electrode on the left pinna and the ground electrode on the right pinna.

General Protocol

For each experiment, 100 trials (2 per second) of responses to broadband noise were recorded. Afterward, 1000 trials of mSync (2 per second) were performed with one of six specific inter-stimulus delays, which spanned from -25 to +25 ms in 10 ms increments, where negative delays indicate that the somatosensory stimulus preceded the acoustic stimulus (Figure 11-B). This was followed by another 100 trials of broadband noise response recordings, 30 minutes of rest, and then one final session of 100 trials of broadband noise response recordings. Responses before mSync were compared to those immediately after and 30 minutes after to determine if mSync had induced significant changes in spike activity (two-tailed, unequal variance, ranked t-test, $P<0.01$), and activity was only counted as changed if both the recordings immediately after and 30 minutes after stimulation were significantly different from recordings before stimulation (post-stimulus time histogram examples are shown in Figure 11-C). Afterward, the next experiment was performed with a different inter-stimulus delay, and the order of delays was randomized to mitigate cumulative effects. Care was taken to ensure that each animal received each delay the same number of times to reduce bias due to animal variability.

Six anesthetized animals received mSync (2553 total recording sites, n=247 recording sites for the -25 ms inter-stimulus delay, 250 for +15 ms, and 214 for all other delays) while three anesthetized animals (n=882 recording sites) were used as controls, where mSync was replaced with no stimulation within the same protocol. In both the high-stress and low-stress animal groups, two chronic recording electrode arrays broke

soon after implantation such that insufficient mSync delays could be tested. In order to prevent animal variability from biasing comparisons between delays, the recordings from these animals were excluded. Since we were still able to collect sufficient data in each of the three remaining animals in both groups for the replication of the anesthesia study, it was not necessary to re-implant additional animals. Also, if additional animals were implanted later, they could experience a different environment in their housing at a different time, making it difficult to draw comparisons in stress. Therefore three high-stress and three low-stress awake animals (n=1728 recording sites for each of the groups, with 288 recording sites per delay) received mSync, while five awake animals (n=1728 recording sites) were used as controls.

Results

Plasticity Effects Under Anesthesia

In anesthetized animals, a total of 2553 sites were positioned across A1 from six animals to identify cortical locations that exhibited significant ($P<0.01$) long-term facilitation or suppression in spike activity induced by mSync with different inter-stimulus delays between pinna and acoustic stimulation. Percentages of total recording sites with significant long-term suppressed (grey) or facilitated (black) spike activity are shown in Figure 12. Negative inter-stimulus delays indicate that electrical pinna stimulation preceded acoustic stimulation, while positive delays indicate that acoustic stimulation came first. While most delays showed no difference between facilitation and suppression, the +15 delay induced significantly more suppressed recording sites than

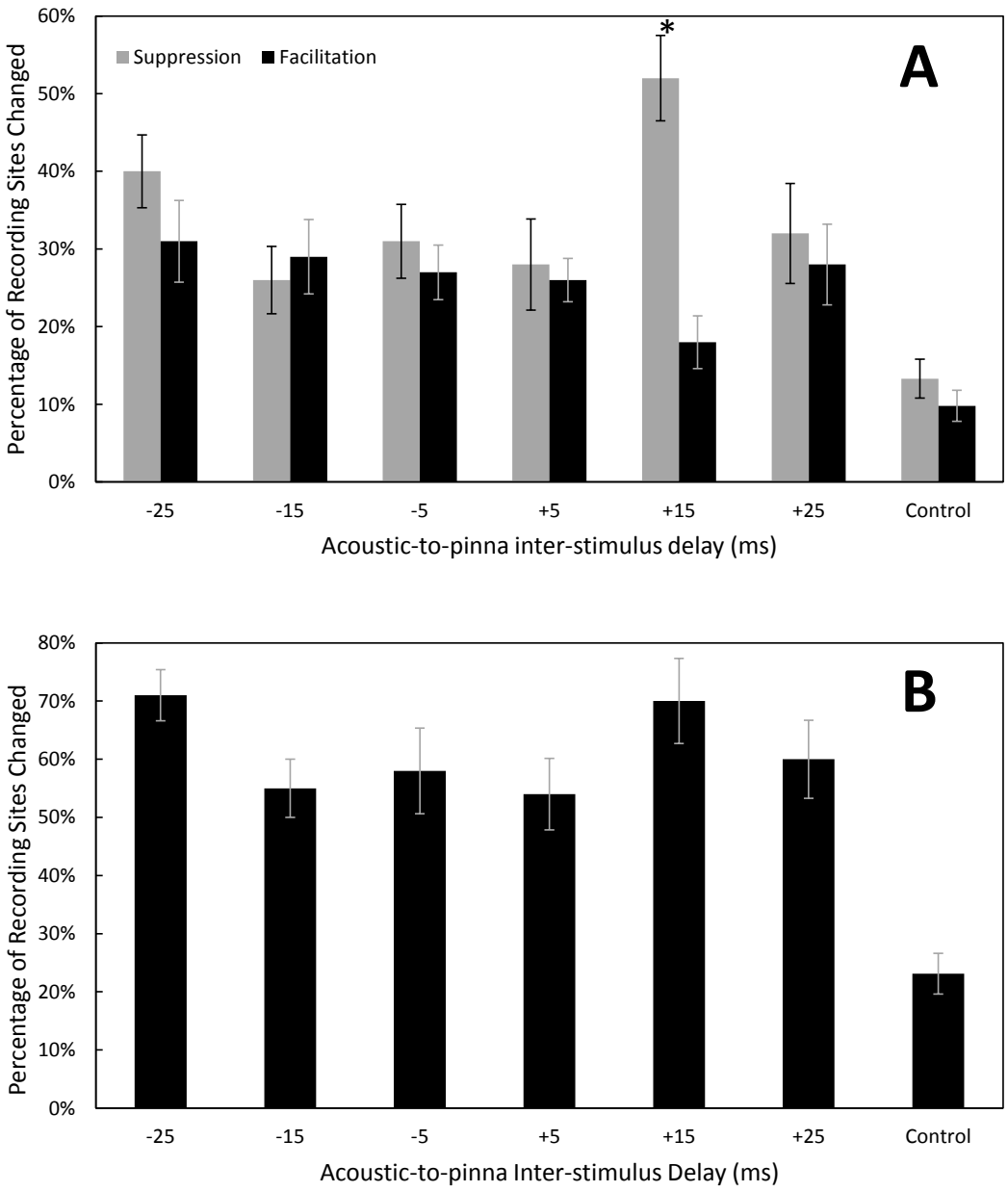


Figure 12: mSync-induced plasticity in anesthetized animals for different inter-stimulus delays.

The percentage of total recording sites in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) both immediately after and 30 minutes after

stimulation for different inter-stimulus delays is shown (**A**, n=247 recording sites for -25 ms, 250 for +15 ms, and 214 for all other delays). For classifying a site as suppressed or facilitated, significance was determined using a two-tailed, unequal variance, ranked t-test ($P<0.01$) when comparing post-stimulus activity to pre-stimulus activity. For three control animals (n=882 total recording sites), mSync was replaced with no stimulation in the same protocol. Error bars show standard error across animals, and an asterisk indicates a significant difference between the percentage of sites suppressed and facilitated ($P<0.05$). Only the inter-stimulus delay of +15 (acoustic before electrical stimulation) showed a significant difference between suppressed and facilitated sites. The total percentages of recording sites changed for each delay are shown in **B**. All delays elicited changes in more recording sites than control.

facilitated sites (Figure 12-A), and this trend was observed in all animals. No delay showed significantly more facilitation than suppression, and no delay induced significant long-term changes in all recording sites, with most delays changing activity in about 50-70% of all sites. All delays resulted in a higher percentage of sites changed than the control condition, for which mSync was replaced with no stimulation (Figure 12-B). This indicates that paired stimulation still had a plastic effect on spike activity for all inter-stimulus delays, even for those that elicited non-systematic facilitation/suppression.

Healing Touch Reduces Stress Levels

Awake guinea pigs can exhibit anxiety and stress during routine handling and mSync stimulation. To better control stress levels in these animals, we performed an

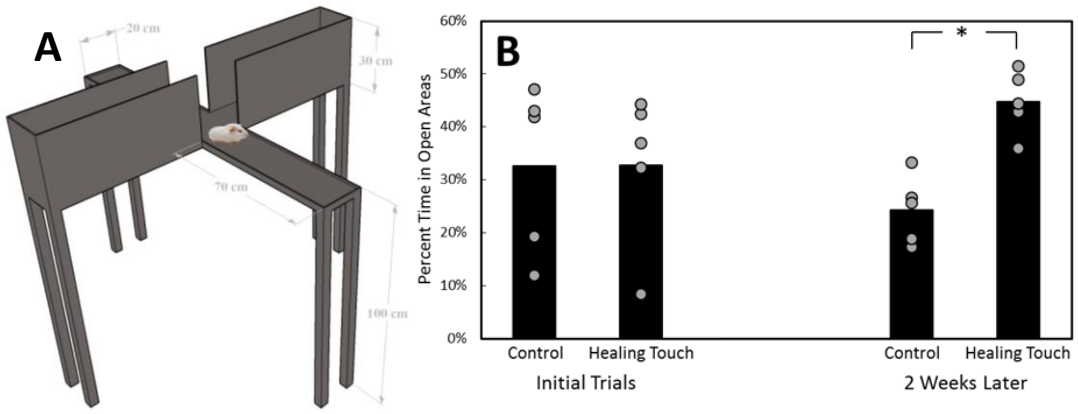


Figure 13: Elevated plus maze behavioral test for stress.

A schematic of the plus maze is shown (**A**). In an initial study in non-implanted animals, low-stress (Healing Touch) animals spent much more time in open areas of the maze compared to high-stress (Control) animals (**B**), and this difference is statistically significant (standard, two-tailed, unequal variance t-test, $P=8x10^{-4}$). The total percent time in open areas for each animal is shown in grey points. After treatment, every Healing Touch animal spent more time in the open areas of the maze than all five Control animals.

experiment with two groups of animals: one group receiving normal minimal handling and one group receiving stress relief treatment. For the treated group, Healing Touch for Animals (HTA) was performed on each animal by a certified HTA practitioner. Healing Touch is a popular form of clinical complimentary medicine (Hover-Kramer and Mentgen 2002; Wardell and Weymouth 2004) and is used to supplement clinical treatments for various health disorders (Eschiti 2007; Post-White et al. 2003; Wilkinson et al. 2002) and reduce stress in subjects (Maville et al. 2008; Shore 2004). Each animal in the HTA group received five sessions of HTA over two weeks, and each session lasted

15-45 minutes at the discretion of the practitioner. A schematic of our elevated plus maze is shown in Figure 13-A. Each animal was given the opportunity to roam around in the maze for three minutes, and the percentage of time the animal spent in the open areas of the maze was recorded and averaged across three separate days, in which higher values corresponded to lower stress levels.

Prior to the HTA sessions, the two groups of five animals exhibited similar results on the elevated plus maze, spending about 30% of their time in the open areas (Figure 13-B). After two weeks, the animals receiving HTA spent significantly more time in the open areas than the control animals ($P = 8 \times 10^{-4}$). In general, control animals became more stressed over time, while animals receiving HTA exhibited decreased stress levels, to the point that after two weeks, every HTA animal spent more time on the plus maze than all of the control animals. Overall, these findings confirm that HTA is an effective way to reduce stress in guinea pigs, and this allows us to assess the outcomes of mSync neuromodulation for chronically implanted awake animals with different stress levels.

Neuromodulatory Effects for Different Stress Levels in an Awake State

For chronic recording awake animals, we investigated plasticity effects in two groups of three animals, in which one group (Low-Stress Animals) received HTA and mSync sessions while another group (High-Stress Animals) received only mSync sessions, and in each group, we recorded from numerous sites (n=1728 for each of the high-stress and low-stress groups, with 288 sites for each delay). Control animals (n=1728 across 5 animals) were also implanted with d-drive electrode arrays and received

normal minimal handling; however, mSync stimulation was replaced with no stimulation within the protocol, and this was used to control for normal fluctuations in auditory cortical firing.

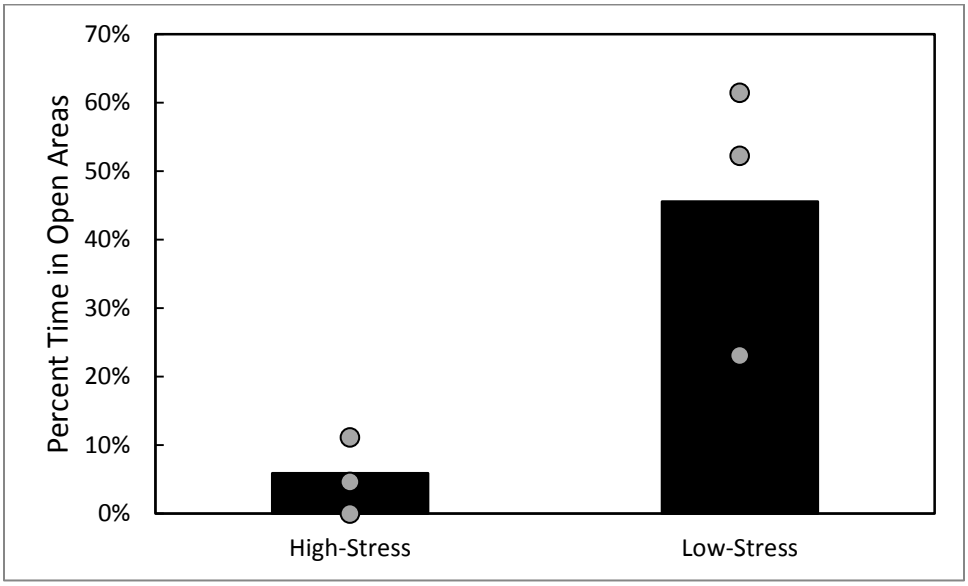
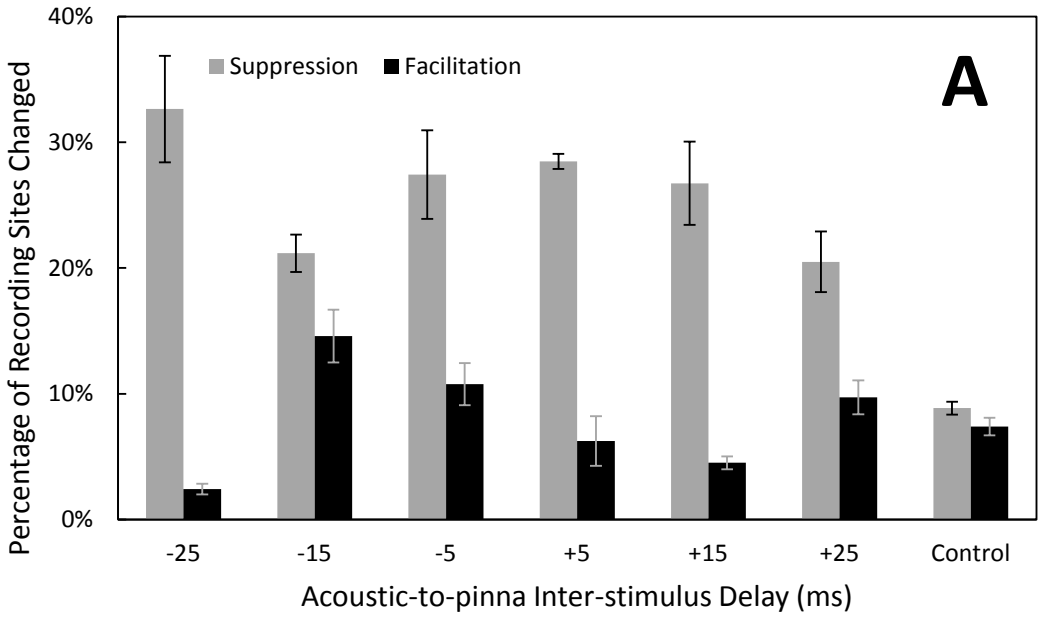


Figure 14: Stress levels high-stress and low-stress animals.

Low-stress animals spent much more time in open areas of the elevated plus maze compared to high-stress animals. The total percent time in open areas for each animal is shown with grey points.

Using the elevated plus maze test shown in Figure 13-A, we confirmed again that HTA sustained low stress levels in the low-stress group, as they spent more time in the open areas than the animals without HTA (Figure 14). Encouragingly, the open area times for the low-stress HTA animals were similar in two separate studies depicted in Figures 13-B and 14, in which the mean percentage was nearly 50%. This represents nearly free exploration, since the maze is 50% covered, indicating extremely low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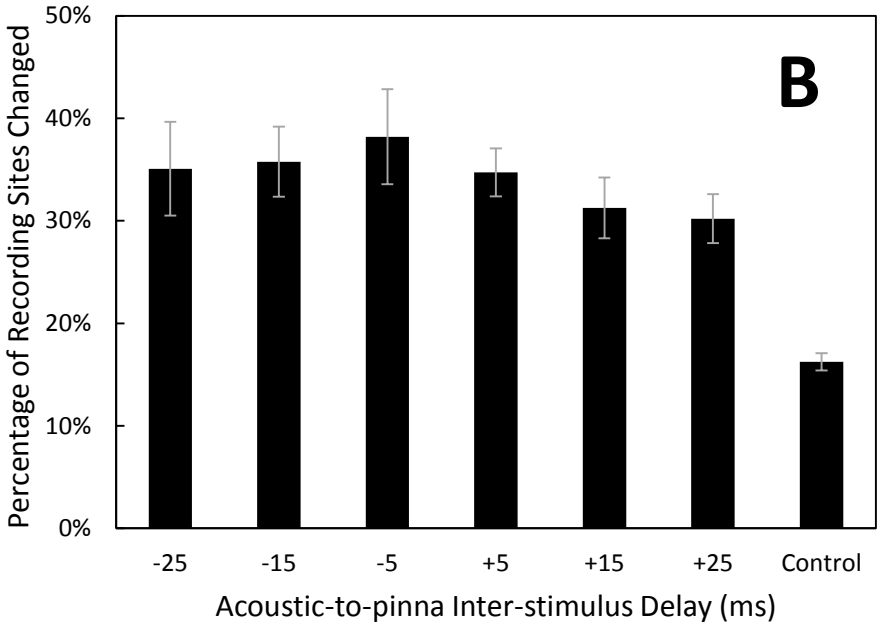
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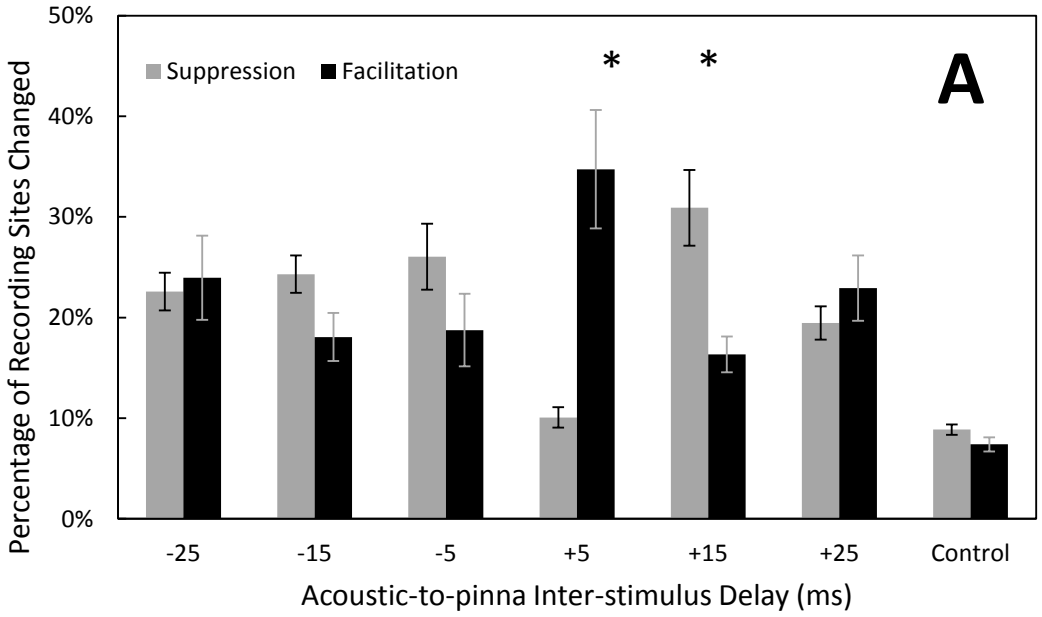
Control
Acoustic-to-pinna Inter-stimulus Delay (ms)

Figure 15: mSync-induced plasticity in awake high-stress animals.

The percentage of total recording sites (n=1728, with 288 recording sites per delay) in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) both immediately after and 30 minutes after stimulation is shown for high-stress animals (**A**). The same statistical methods as in Figure 12 were used. For the control group (n=1728 recording sites), mSync was replaced with no stimulation in the same protocol on animals that did not receive HTA treatment. Error bars show standard error across animals. Results were not consistent with anesthesia results, with fewer total sites changed and no clear systematic relationship between delays. **B** shows the total percentages of recording sites changed for each delay. In all cases, mSync induced changes in more recording sites than control.

stress/anxiety levels. The high-stress animals rarely spent any time in the open areas, and their open area times were much lower than the control animals in Figure 13-B, revealing that animals can become greatly stressed during additional manipulations such as mSync neuromodulation and procedures related to chronic probe implantation.

Similar mSync delays and analyses performed for the anesthetized animals were used for the high-stress (Figure 15) and low-stress (Figure 16) awake animals. We observed drastic differences in plasticity effects between these two groups. Only the low-stress group showed a systematic dependence on inter-stimulus timing, in which the +15 delay resulted in significantly more recording sites with suppressed spike activity than facilitated (similar to anesthesia results) and the +5 delay induced significantly more facilitation than suppression (Figure 16-A). These results were consistent across all three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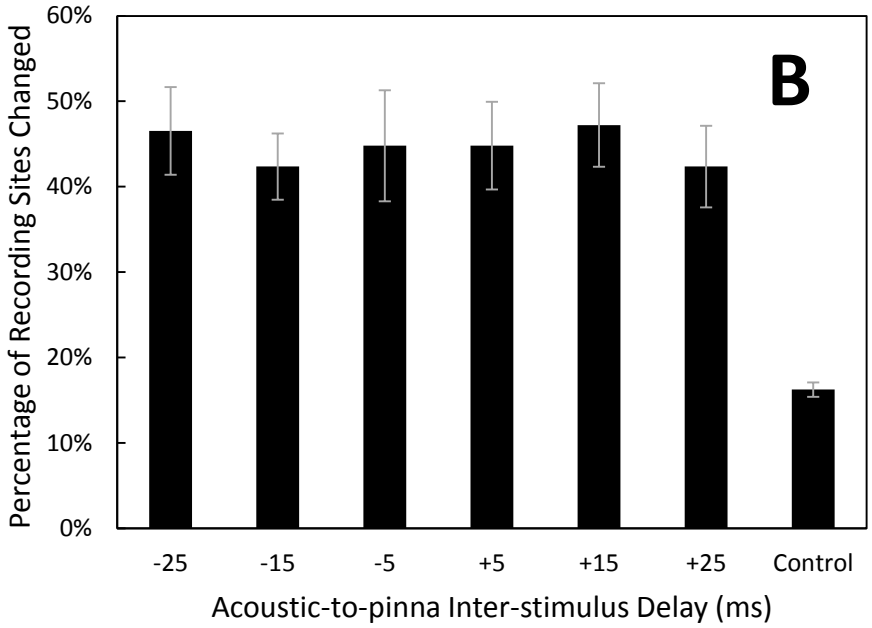
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Control
Acoustic-to-pinna Inter-stimulus Delay (ms)

Figure 16: mSync-induced plasticity in awake low-stress animals.

The percentage of total recording sites (n=1728, with 288 recording sites per delay) in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) both immediately after and 30 minutes after stimulation is shown for low-stress (HTA treated) animals (**A**). The same statistical methods as in Figure 12 were used. For the control group (n=1728 recording sites), mSync was replaced with no stimulation in the same protocol on animals that did not receive HTA treatment. The inter-stimulus delay of +15 showed significantly more suppressed than facilitated sites, and a delay of +5 showed significantly more facilitated than suppressed sites, indicated by asterisks. **B** shows the total percentages of recording sites changed for each delay. In all cases, mSync induced changes in more recording sites than control.

low-stress animals, and this reversal in suppressive versus facilitative effects as a function of inter-stimulus delay was not observed for the high-stress group, which generally exhibited inconsistent suppressive effects in neural firing in A1 with no time-dependence (Figure 15-A). Both groups still exhibited a higher percentage of total recording sites with significant changes in firing than the control animals for all delays (Figure 15-B and 16-B); however, across all delays, the low-stress group exhibited a much higher percentage of sites experiencing long-lasting plasticity than the high-stress group (Figure 17). Overall, these findings demonstrate that drastic differences in neuromodulatory effects can occur in animals with varying levels of stress, where more systematic and long-lasting plasticity effects are possible in animals with lower stress

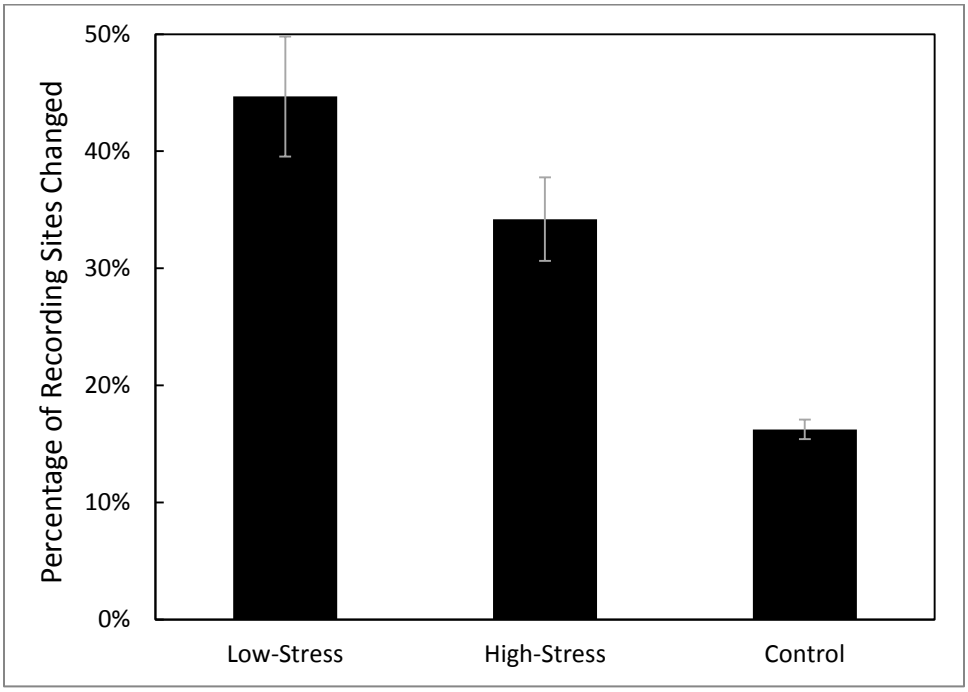


Figure 17: Total percentage of recording sites changed across groups

Low-stress animals exhibited changes in more total recording sites than high-stress animals, and both groups showed more changes than control. It may be easier to induce plasticity in low-stress animals overall.

levels, while results in high-stress animals are not systematic and do not match anesthesia results.

Discussion

The anesthesia experiments were performed to confirm which inter-stimulus delays of mSync would be relevant for timing-dependent plasticity in A1, such that an expectation could be established for planning and performing the awake chronic recording experiments. We became concerned about confounding effects in awake

animals, such as stress induced by the experimental protocol, chronic electrode implantation, and mSync stimulation, so we applied HTA to attempt to minimize stress levels in some animals.

From our series of experiments in anesthetized and awake animals, we discovered that timing-dependent mSync plasticity occurs in both anesthetized and awake low-stress animals. These findings have implications specifically for mSync treatment of tinnitus and also for the neuromodulation field in general. For the treatment of tinnitus, in which the phantom sound pain has been linked hyperactive or hyper-synchronous neurons in the auditory system (Eggermont and Roberts 2004; Henry et al. 2014; Kaltenbach 2011; Lanting et al. 2008; Lanting et al. 2009; Møller et al. 2010), the inter-stimulus delay between pinna and acoustic stimulation could be adjusted until a strong suppressive effect occurs to disrupt the pathogenic activity driving the phantom sound percept. For all neuromodulation approaches, especially those which induce plasticity in specific pathogenic brain regions, minimizing stress appears critical for enabling systematic and greater long-term plasticity outcomes. In our study, the animal subjects were naturally stressed, and one group received stress relief treatment. This is not all that different from clinical situations, where patients are often naturally stressed about a neuromodulation treatment, or about their disorder and its effects on their lives. Therefore, combining neuromodulation with some kind of stress relief treatment will likely improve the consistency of outcomes just as it did in the present study. Further studies in humans might be needed to confirm this inference in neuromodulation patients.

One interesting outcome in the awake animal experiments is the consistent facilitative nature of the +5 inter-stimulus delay in low-stress animals, which did not appear in the anesthesia results (where there was no significant difference in facilitation vs. suppression at the +5 delay). Previous studies have shown that invasive paired trigeminal nerve stimulation and acoustic stimulation with varied inter-stimulus delays can induce Hebbian-like effects in auditory cortex (Basura et al. 2015; Basura et al. 2012) and dorsal cochlear nucleus (Koehler and Shore 2013a; b), where one inter-stimulus delay is more suppressive of neural activity while a neighboring delay is more facilitative. In most of those previous studies, the facilitative and suppressive delays were about 8-15 ms apart (although few delays were tested sparsely in each of those experiments), which matches the 10 ms spacing between facilitative/suppressive delays in our data. It is encouraging that we have achieved these results consistently in our awake animals noninvasively, as the ability to modulate activity and induce timing-dependent plasticity using a noninvasive approach could eliminate the need for surgery, opening up treatment to a much larger population, which would be crucial for widespread disorders like tinnitus.

However, unlike our awake animal outcomes, the Hebbian-like effects were not observed in our anesthesia results. We propose two possible reasons for this. First, we used a ketamine-xylazine cocktail to anesthetize our animals, and ketamine has been shown to impair sensory perception (Oye et al. 1992), which is thought to occur in sensory cortical areas, and inhibit N-Methyl-D-Aspartate (NMDA) receptor activation

(Gonzales et al. 1995; Kim et al. 1996a; Silva et al. 1997), which is a glutamate receptor closely linked with plasticity induction. In fact, ketamine has been shown to inhibit plasticity induction in general (Forsythe and Westbrook 1988; Hu and Davies 1997; Kaltenbach et al. 2000), and most importantly, it has been shown to inhibit the excitation of spike activity in brain slices (Hu and Davies 1997) and in the auditory system in vivo (Kaltenbach et al. 2000). This may explain why we were able to achieve suppression at the +15 inter-stimulus delay but not significant facilitation at any other delays under anesthesia, and this same trend was found in an initial Hebbian plasticity study where the facilitative delay had much smaller effects than the suppressive delay (Tzounopoulos et al. 2007).

In addition to this likely ketamine effect, a second explanation could be the timing difference between the optimal facilitative and suppressive delays. In previous timing-dependent plasticity studies, the breadth of delays that would produce a specific facilitative/suppressive result could range up to 5 ms, while the time difference between the most optimal delays ranged between 8 and 12 ms, and the real range might be even larger considering that delays were often tested sparsely in these experiments. Based on this, if the initial suppressive delay in our anesthesia experiments (+15) happens to fall on the edge of the true range of suppressive delays (based on onsets of stimulation), it is possible that our two neighboring delays simply did not line up with the facilitative range. However, in order not to frighten or startle our awake animals, we moved the acoustic stimulation speaker about 2/3 meters away from the animals and adjusted the

volume accordingly. Since sound travels at approximately 340 m/s, this means that the auditory stimulus actually arrived at the awake animals' ears about 2 ms later than the anesthetized animals' ears (in which the speaker was coupled to an ear bar), and such a shift in onset delays could move the suppressive +15 delay into a different part of the true range of suppressive delays while also moving the +5 delay into the range of facilitative delays. Future studies that test many more delays in finer increments could give more insight into this phenomenon.

In conclusion, stress has adverse effects on plasticity outcomes with neuromodulation, as it obstructs the natural plasticity patterns that we expect to see. For naturally stressed animals, stress relief techniques can result in more consistent outcomes, and this concept might be applied to a variety of neuromodulation modalities to improve plasticity/modulatory results for the treatment of different types of neural disorders. Future studies could also attempt to pinpoint a precise mechanism for how stress affects plasticity induction by targeting specific neurotransmitters and/or receptors that have been shown to be affected by stress, which might open up possibilities of pharmacological compliments to improve neuromodulation outcomes in cases where stress cannot be easily reduced in patients. Given that patients are often heavily stressed about their neural disorders, this information could be extremely helpful in improving neuromodulation outcomes.

Chapter 5: Somatotopy, Thresholding, and Widespread Auditory Interactions within the Guinea Pig Somatosensory Cortex

Chapters 2 through 4 focused on mSync effects in the auditory cortex. However, the long-term goal is to incorporate other sensory inputs and modulate different sensory cortices for a variety of sensory disorders. In this chapter, we investigated the effects of multisensory stimulation in the somatosensory system, and we characterized auditory-somatosensory interactions is somatosensory cortex, which may have implications for the treatment of pain.

Summary

There has been growing interest in understanding multisensory integration in the cortex and in activating multiple sensory and motor pathways to treat various sensory disorders, such as pain and tinnitus. Specifically in the tinnitus field, a guinea pig model is often used for understanding pathological brain activity linked to tinnitus. Furthermore, a homunculus has been revealed in the somatosensory cortex of multiple species, including rats, cats, pigs, non-human primates, and humans; however, to our knowledge, a detailed mapping of body locations in the guinea pig is still lacking. In this study, we mapped the primary somatosensory cortex (**S1**) in response to electrical stimulation of different body locations and acoustic stimulation in anesthetized guinea pigs. The S1 topography of guinea pigs aligns similarly to that of the rat based on previous studies; however, there appears to be greater overlap in S1 body region responses in guinea pigs

than in rats. Interestingly, auditory broadband noise stimulation primarily excited S1 areas that typically respond to stimulation of lower body locations. Although there was only a subset of S1 locations excited by broadband noise stimulation, all S1 recording sites could be modulated by combined acoustic and somatosensory stimulation, and most of those changes were facilitative. These findings show that auditory inputs can excite or modulate firing across a widespread population of S1 neurons, which is relevant for sensory disorder treatments.

Introduction

Multisensory integration enables us to experience our surrounding environment as one complete perception instead of separate sensory events. It also helps us to integrate multiple sensory inputs originating from the same source for a more complete understanding of the environment around us. For example, the spatial maps of the visual and auditory scenes are overlapped and aligned in the superior colliculus (Drager and Hubel 1975), which can contribute to how we recognize when the visual perception of an object correlates with the sound that the object makes. Although we know this is made possible because our sensory systems are connected in the brain, there is still limited understanding of how such interactions are coded (Stein and Stanford 2008). Even less is known about how interactions are coded between the somatosensory and auditory systems. Therefore, this study investigates the spatial coding across the primary somatosensory cortex (**S1**) in response to somatosensory or auditory inputs in

anesthetized guinea pigs, as well as the modulatory effects of auditory stimuli on somatosensory coding in S1.

Before attempting to characterize and evaluate the coding interactions between somatosensory and auditory inputs in the guinea pig S1, we first mapped the guinea pig homunculus to better understand the organization of S1 neurons. Previous studies have shown a somatotopic representation in S1 in humans (Aminoff et al. 1985; Baumgartner et al. 1991; Hari et al. 1993; Itomi et al. 2000; Kakigi et al. 1995; Liguori et al. 1991; Mogilner et al. 1994; Nakamura et al. 1998; Narici et al. 1991; Nobre 2001; Penfield and Boldrey 1937; Woolsey et al. 1979; Yang et al. 1994b), rats (Cho et al. 2007; Godde et al. 2002; Petersen et al. 2001; Welker 1976), cats (Celesia 1963; Davenport et al. 2010; Dykes et al. 1980; Iwamura and Tanaka 1978; Shigenaga et al. 1989), pigs (Craner and Ray 1991), monkeys (Pons et al. 1985), and other mammals (Schott 1993); however, to our knowledge, no one has characterized somatotopy in the guinea pig S1. Additionally, past studies have investigated trends in somatosensory projections in the auditory system (Aitkin et al. 1981b), but there are no studies investigating topographic trends of auditory projections to S1. This study attempts to address both of these unknowns.

In addition to maps of body locations and sound stimuli, we also investigated the modulatory effects of their interactions within S1. Previous work has made great strides in understanding mechanisms of modulation and plasticity induction in S1 (Feldman and Brecht 2005; Foeller and Feldman 2004; Jones 2000; Schlaggar et al. 1993), including long-term potentiation and depression (Buonomano and Merzenich 1998; Feldman et al.

1999; Wolters et al. 2005), cortical reorganization due to amputation or restriction of usage (Borsook et al. 1998; Diamond et al. 1994; Jones and Pons 1998; Mogilner et al. 1993; Xerri et al. 1996; Yang et al. 1994a), alteration of GABA inhibitory circuits (Dykes 1997; Foeller and Feldman 2004), interhemispheric interactions (Clarey et al. 1996), NMDA receptor activation (Buonomano and Merzenich 1998; Garraghty and Muja 1996), and timing-dependent plasticity (Florence et al. 1997; Wolters et al. 2005). To expand upon these previous studies, we investigated how multisensory integration can play a role in the modulation of S1 neural firing. There have been several studies characterizing somatosensory and visual effects in the auditory system (Calvert et al. 1997; Foxe et al. 2002; Ghazanfar et al. 2005; Levine et al. 2003; Ma and Suga 2003; Murray et al. 2005) and lower level auditory or somatosensory areas (Aitkin et al. 1978; Foxe et al. 2000; Itaya and Van Hoesen 1982), as well as the superior colliculus (Drager and Hubel 1975; Groh et al. 2001; Meredith and Stein 1986), but little work has been done in S1. This study aims to explore how combined auditory and somatosensory stimuli can modulate firing rates in S1 neural populations, depending on which somatosensory location is stimulated. Clinically, the findings from this study can help better understand the mechanisms of action in multisensory integration and guide new neuromodulation approaches that are leveraging multimodal interactions to treat various sensory disorders. Cross-sensory or multimodal stimulation has been a recently investigated option for the treatment of tinnitus (Basura et al. 2015; Gloeckner et al. 2013; Levine and Oron 2014; Markovitz et al. 2015) and phantom limb pain (Chan et al. 2007; Ramachandran and Rogers-Ramachandran 1996), and we believe it can also be a

viable treatment option for neuropathic pain. Additionally, since guinea pigs are used in models for these disorders (Campbell et al. 1998; Cazals et al. 1998; Coomber et al. 2014; Fox et al. 2003; Koehler and Shore 2013a; Mulders et al. 2014; Norena et al. 2010; Vermeirsch et al. 2007), this study's characterization of somatosensory and auditory inputs to the guinea pig S1 may be useful to other studies as well.

Materials and Methods

Overview

Experiments were performed on nine young female Hartley guinea pigs (400–450 g; Elm Hill Breeding Labs, Chelmsford, MA) anesthetized with an initial intramuscular injection of ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL), with 0.1 mL supplements every 45–60 minutes to maintain an areflexive state. Neural recordings were performed inside an electrically-shielded and acoustic-attenuating room using hardware from Tucker-Davis Technology (Alachua, FL), and neural data was processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Surgery and Neural Recordings

A craniotomy was performed revealing the right somatosensory cortex of each animal. The animal's heart rate and blood oxygen content were continuously monitored using an H100 pulse oximeter from EdanUSA (San Diego, CA), and body temperature

was monitored using an Oakton Acorn series JKT thermocouple rectal probe (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN). The animal was held into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars. A 32-site recording electrode array (NeuroNexus Technologies, Ann Arbor, MI) was inserted into the right S1 of the guinea pig brain. This recording electrode array was comprised of four 5-mm long shanks separated by 500 μm with eight iridium sites linearly spaced at 200 μm along each shank (site area approximately 413 μm^2) and was placed at various locations across S1 in order to span the entire region. The array was inserted to a depth such that the main input Layer IV could be observed at approximately the middle of the eight recording sites on each shank (determined based on locating the initial sink using current-source density analysis similar to previous studies; (Lim and Anderson 2007a; Markovitz et al. 2013; Straka et al. 2014)), which usually resulted in the tip sites being inserted 1.1-1.3 mm below the surface of the cortex. The recording ground for the electrode array was inserted into the brain near the intersection of the lambda suture line and the midline. Recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine wave. Saline was routinely administered to the cortex after the probe was placed to limit the effects of drying.

Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of neural spike activity. A detection

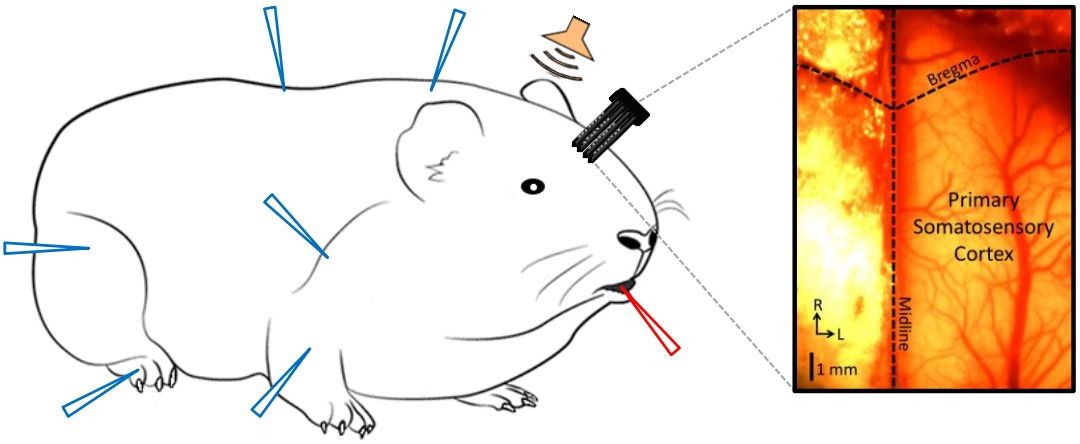


Figure 18: Neural recording and sensory stimulation

Subdermal needle electrodes were used to electrically stimulate the somatosensory system at locations that spanned the entire animal, including the tongue, neck, left and right shoulder, left and right arm, back, left and right hind leg, and left and right hind paw. Locations were stimulated independently during recordings. Contralateral (left side) somatosensory stimulation locations in the shoulder, arm, hind paw, and leg are not shown. Blue markers indicate subcutaneous electrodes, while the red marker on the tongue indicates that the electrode rests on the tongue's surface. The broadband noise auditory stimulus was presented to the contralateral (left) ear. Multiunit neural activity was recorded from the primary somatosensory cortex (S1) using a 32-site recording electrode array.

threshold of 3.5 times the standard deviation of the voltage noise floor was used to determine when spikes occurred. The timing of spikes relative to the beginning of a recording was used to construct Post-Stimulus Time Histograms (PSTHs) of the spike activity. Further details on these spike analysis methods are presented in previous studies

(Gloeckner et al. 2013; Lim and Anderson 2007a; 2006; Markovitz et al. 2015; Markovitz et al. 2013; Offutt et al. 2014; Straka et al. 2014).

Sensory Stimulation

Electrical stimulation (biphasic, charge-balanced, 205 μs per phase) of the skin was used to measure neural responses to somatosensory stimulation in S1. Subcutaneous needle electrodes (Rhythmlink International LLC, Columbia, SC) were placed within the left and right shoulders, neck, left and right arms, back, left and right hind legs, left and right hind paws, and onto the tongue of the animal (Figure 18). The tongue electrode was placed on top of the tongue, taking care not to puncture it. The neck electrode was inserted centrally halfway between the ears and the shoulder joints. The shoulder electrodes were inserted dorsal of the shoulder joints, and the arm electrodes were inserted on the lateral side of the arm, halfway between the shoulder joint and elbow joint. The back electrode was inserted along the spine halfway between the neck electrode and the end of the spine. The leg electrodes were placed laterally halfway between the hip joint and the knee joint, and the hind paw electrodes were placed in the center of the bottom of the paw. For the tongue, neck, arm, hind paw, and back stimuli, the stimulation ground was distributed between the four shoulder and leg electrodes, and for the shoulder and leg stimuli, the stimulation ground was distributed between the four arm and hind paw electrodes. By spreading the ground across four distinct body regions, unintended activation of ground areas was eliminated, and control experiments were conducted to ensure that no ground areas caused activation in S1 even at the highest

stimulation current levels. We used needle electrodes instead of surface electrodes because they were easier to consistently position across animals and reduced variability due to each animal's skin and fur (Gloeckner et al. 2013; Markovitz et al. 2015).

Acoustic broadband noise auditory stimuli (50 ms duration, 0.5 ms rise/fall time, 70 dB SPL, equal energy between 625 Hz and 40 kHz) were presented to the animal's left ear using a speaker (Tucker-Davis Technology, Alachua, FL) coupled to the left ear bar. The speaker-ear bar system was calibrated using a 0.25 in. condenser microphone (ACO Pacific, Belmont, CA).

Somatosensory and Auditory Mapping in S1

We mapped somatosensory and auditory activation in S1 in five animals. At each recording location in S1, 100 trials (2 per second) of spontaneous spike activity were recorded. Afterward, each somatosensory stimulation location was independently electrically stimulated at amplitudes ranging 0.11 – 2.82 mA in 2 dB steps (relative to 1 μA) for a total of 15 levels, and neural spike responses were recorded. For each current level, 100 trials were performed, starting with the lowest level and increasing thereafter with a 30-second delay in between each level. After all levels of stimulation were performed, a different somatosensory stimulation location was used until all body locations were stimulated, and the order of somatosensory stimulation locations was randomized across experiments to mitigate cumulative effects. After all somatosensory stimulation locations were tested, an acoustic stimulus was presented for 100 trials.

For each recording location, neural signals were windowed between 5 and 55 ms following the electrical somatosensory stimulus, and spike counts within that window were determined for each of the 100 trials for each stimulation level (the same length of window was used for spontaneous activity, even though no stimulus was presented). The spike counts for each stimulation paradigm were compared to spontaneous spike counts using Signal Detection Theory (d'=1) to determine if stimulus responses were statistically significant, similar to previous studies (Celebrini and Newsome 1994; Green and Swets 1966; Lim and Anderson 2007a; Markovitz et al. 2015), and these results were checked visually on PSTHs. Stimulation thresholds of activation were determined to be the lowest current level with a statistically significant response for each somatosensory stimulation location. For auditory stimulation, neural signals were windowed between 5 and 80 ms, and Signal Detection Theory (d'=1) was used to determine if stimulus responses were statistically significant for a stimulation level of 70 dB SPL.

Paired Somatosensory and Auditory Stimulation

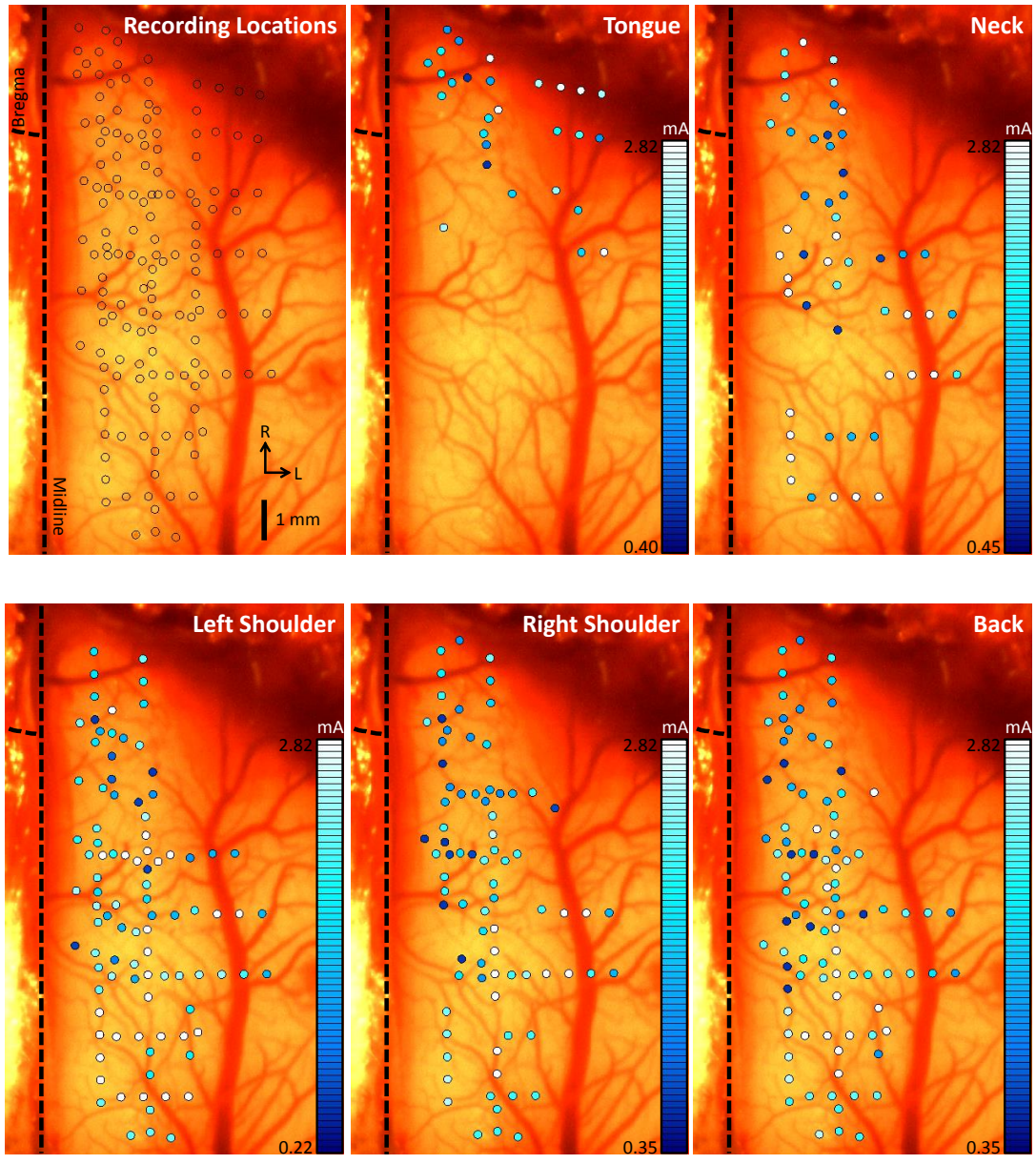
We investigated the modulatory effects of paired stimulation on S1 activity in nine animals (five mapping animals plus four additional control animals). At each S1 recording location, 100 trials of paired simultaneous broadband noise acoustic stimulation (70 dB SPL) and electrical somatosensory stimulation (1.8 mA) of one body location were performed, and neural spike responses were recorded. This was repeated for all body stimulation sites, and the order of locations was randomized across experiments to mitigate cumulative effects. For locations that did not exhibit a response

to the auditory stimulus in the mapping study, spike counts across trials for responses to paired stimulation were compared to those of only somatosensory stimulation using a two-tailed, unequal variance, ranked t-test (Ruxton 2006) to determine if paired stimulation responses were significantly different than somatosensory responses alone ($P<0.01$). S1 locations that exhibited auditory responses were not considered, due to the difficultly in comparing paired responses to the combination of separate somatosensory and auditory responses. In the four control animals, the same protocol was used, except that paired stimulation was replaced with somatosensory stimulation only.

S1 Reconstructions

For each S1 recording electrode placement location, a photograph of the placement was taken perpendicular to the cortical surface using a Moticam 2300 3-megapixel USB microscope camera (Motic, Kowloon, Hong Kong) through a Carl Zeiss surgical microscope (Oberkochen, Germany) at 25x magnification. These photographs showed the individual electrode shanks after insertion, the midline, the Bregma suture line, and a 2-D scale bar measuring 4 mm by 4 mm with markings every 1 mm. Using Rhinoceros software (Seattle, WA), images for each animal were scaled in the horizontal and vertical directions such that scale bars on all images were identical. The images were then stacked together and aligned at the intersection of the Bregma suture line and the midline, such that the midlines and intersections of all photos were aligned. These steps ensured that the electrode placement locations were superimposed onto the same plane such that a full map of S1 could be visualized. An individual threshold map for each body

stimulation location was generated, where a colored circle was placed at each recording location and the color representing the stimulation threshold of activation.



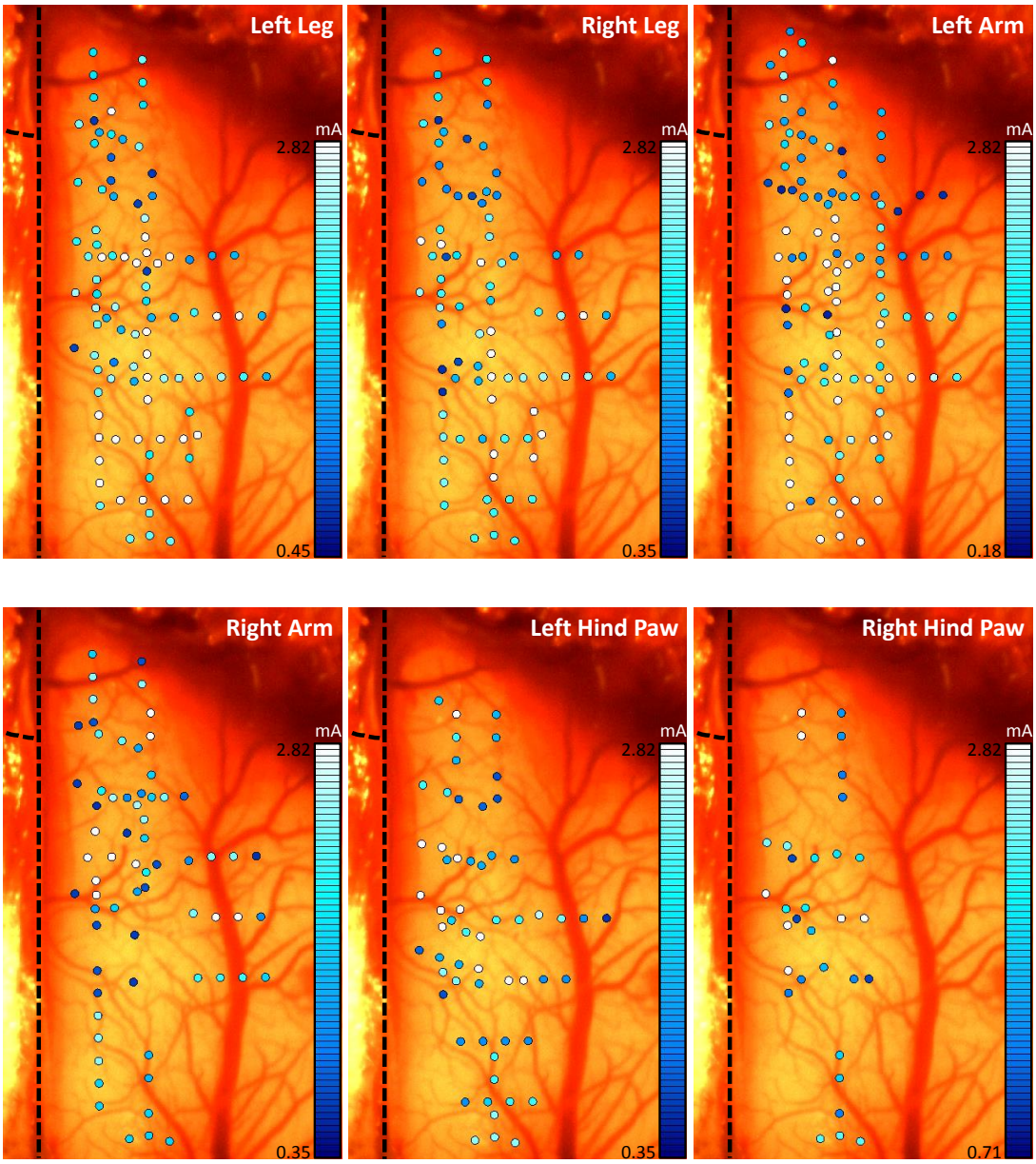


Figure 19: S1 responses to somatosensory stimulation of individual body locations

The Recording Locations map shows all recording locations that responded to at least one somatosensory stimulus location (recording locations that did not respond are omitted). All other maps show the thresholds of activation (indicated by color) for all responding S1

recording locations for a given somatosensory stimulation location. Each stimulation location has a unique range of thresholds, so each map has its own color scale.

Results

Somatosensory and Auditory Mapping in S1

Thresholds of activation for each somatosensory stimulation location at each cortical recording location are shown in Figure 19. For simplicity, we did not show any recording locations that elicited no somatosensory responses, but many non-responding perimeter locations were tested to ensure that we spanned the entire functional S1. Tongue stimulation primarily activated rostral areas of S1 near the Bregma suture line, and it also had the greatest rostral-lateral reach of all somatosensory stimulation locations tested. The remaining somatosensory stimulation locations generally activated a large area of S1 caudal of the tongue area, in which each stimulation location had its lowest thresholds in different areas. As the stimulation location was moved from the head toward the rear of the animal, the cortical locations of lowest threshold roughly trended from the rostral portion to the caudal portion of S1. This trend can be seen more clearly in Figure 20-A, which shows a normalized homunculus map of all stimulation locations. Here, at each recording location, the stimulation location with the lowest normalized threshold is indicated by the color of the circle. For each body stimulation location, we normalized the thresholds on a 0 to 1 scale for all S1 recording sites, in which 0 represented the lowest threshold observed across all S1 recording sites in response to

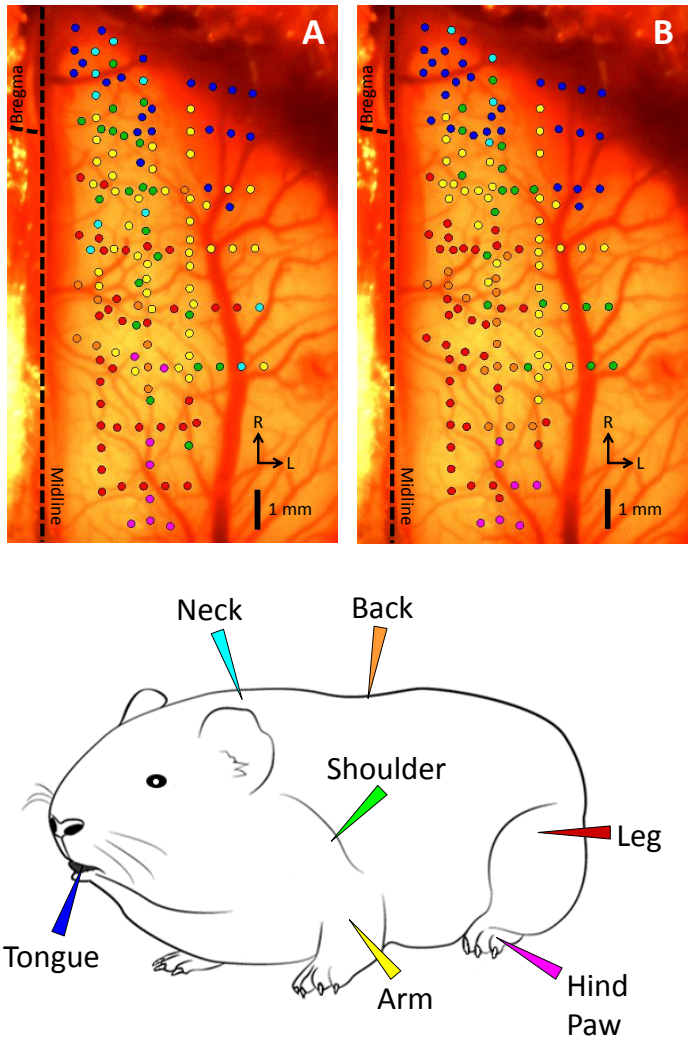


Figure 20: Normalized S1 response map

A: Thresholds for all stimulation locations were normalized based on their lowest threshold of activation across S1, and the stimulation location with the lowest normalized threshold at each recording location is indicated by a color corresponding to the colored arrows on the guinea pig drawing. **B:** The normalized threshold map is smoothed by averaging the normalized thresholds of all recording sites within 0.5 mm of a given S1 location.

stimulation of that somatosensory stimulation location and 1 represented the highest stimulation level possible. This normalization was performed because some somatosensory stimulation locations may be more easily activated by electrical stimulation than others, which can partly depend on the stimulation electrode interface and the conductive properties of the body site. In the case that a body site had both left and right stimuli (e.g. left and right shoulder), only the contralateral location was used in the normalized threshold maps to avoid overrepresentation of a particular dermatome.

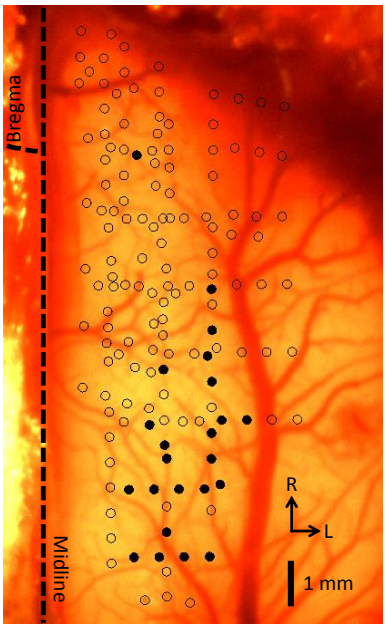


Figure 21: S1 responses to auditory stimulation

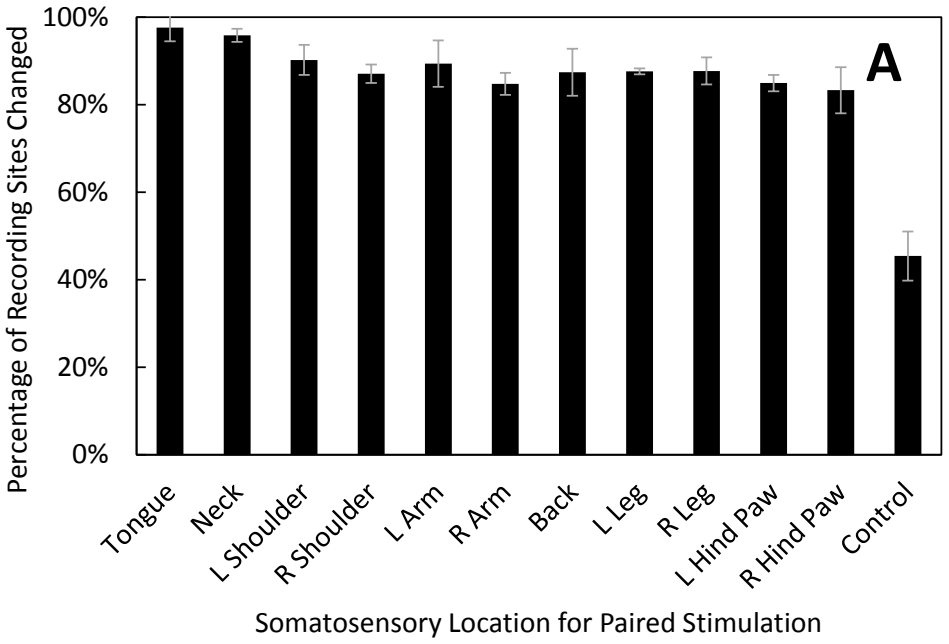
Recording locations that responded to auditory simulation are shown in black, while recording locations that did not respond to auditory stimulation are indicated with an empty circle. Only locations that responded to at least one somatosensory stimulation location are included. S1 responses to auditory stimulation are primarily located in lower body (caudal) areas.

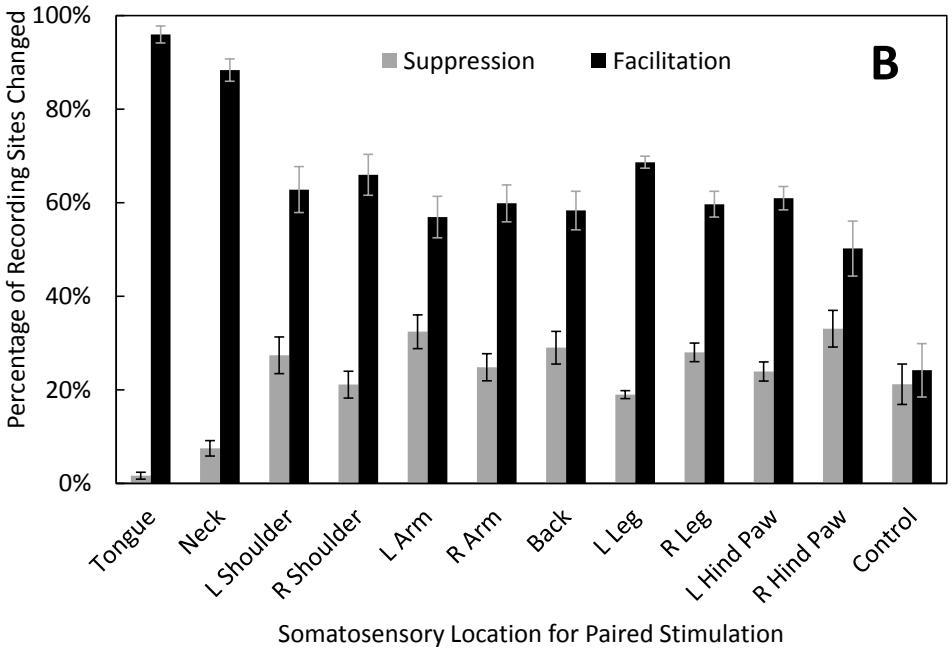
The data is spatially smoothed in Figure 20-B by averaging threshold values of a recording location with those of all other recording locations within 0.5 mm, and this more clearly depicts the somatotopic organization within S1.

Similarly, a map of S1 responses to contralateral acoustic stimulation is shown in Figure 21. Interestingly, recording locations that responded to auditory stimulation were localized primarily in the caudal half of S1. Of the recording locations that responded to tongue stimulation (through cranial nerves instead of the spinal cord), none of them responded to the acoustic stimulus. Only 4% of all recording sites sensitive to somatosensory stimulation were also excitable by auditory stimulation (46 out of 1176 sites), and even at S1 locations where neurons responded to acoustic stimulation, only 25% of the electrode recording sites at these locations (46 out of 184) showed an acoustic-driven response (note that there are multiple recording sites along each electrode shank for a given S1 recording location). This indicates that acoustic stimulation only excites a small subset of neurons in S1.

Paired Somatosensory and Auditory Stimulation

In addition to solely excitatory effects of acoustic stimulation alone, we investigated suppressive and facilitative effects by analyzing S1 responses to paired somatosensory and auditory stimulation. We compared S1 responses to paired simultaneous broadband noise and somatosensory stimulation of a particular body location with responses to somatosensory stimulation alone (Figure 22-A). For each individual somatosensory-auditory combination, at least 80% of all analyzed S1





P-Values comparing facilitation to control

Tongue	Neck	L Shoulder	R Shoulder	L Arm	R Arm	Back	L Leg	R Leg	L Hind Paw	R Hind Paw
2×10^{-4}	3×10^{-4}	2×10^{-3}	1×10^{-3}	3×10^{-3}	2×10^{-3}	2×10^{-3}	1×10^{-3}	3×10^{-3}	2×10^{-3}	8×10^{-3}

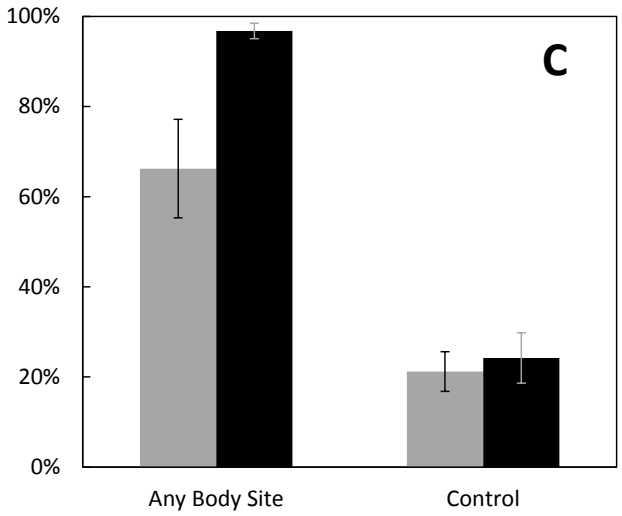


Figure 22: Spike activity is modulated with paired simultaneous somatosensory and auditory stimulation

Recordings of spike activity in S1 in response to paired stimulation (70 dB SPL broadband noise acoustic stimulus presented to the contralateral ear paired with a 1.8 mA electrical somatosensory stimulus) were compared to electrical stimulation alone. The x-axis indicates the body location that was used for somatosensory stimulation, and the control compares responses to two identical somatosensory stimuli (no paired stimulation) to indicate the natural variability of S1 stimulus-driven spike rates. A two-tailed, unequal variance, ranked t-test ($P<0.01$) was used to determine if neural firing at a given recording site was significantly increased (black) or decreased (grey). Error bars show standard error across animals. For all paired stimulation combinations, over 80 % of all recording sites exhibited changes in activity (**A**), and all S1 recording sites were modulated by at least one combination, indicating that paired acoustic and somatosensory stimulation can modulate activity across all of S1. Paired stimulation with upper body locations induced suppression more often than lower body locations, and all paired stimulation combinations induced

facilitation in significantly more recording sites than suppression (**B**, P-values shown below chart). 97% of S1 recording sites were facilitated by at least one paired stimulation combination, while 66% were suppressed at least once (**C**), indicating that different body regions can have differential effects on the same neuron since most recording sites exhibited both suppression and facilitation at least once.

recording sites exhibited a modulatory effect to paired stimulation, with some combinations resulting in over 90% of sites changed. We also observed that 100% of the recording sites in S1 were modulated by at least one paired stimulation combination. This finding demonstrates that even though only some S1 neurons may be significantly excited by a broadband stimulus alone (as shown in Figure 21), we can modulate neurons fully spanning S1 with paired acoustic and somatosensory stimulation. Note that only sites that did not respond to auditory stimulation are included in this analysis, since the comparison of the summation of activity of two separate stimuli and responses to their combination is not possible unless we assume linear spike activity summation, which may be incorrect, although all 46 sites responding to acoustic stimulation were significantly facilitated with paired stimulation.

Figure 22-B shows the percentage of analyzed recording sites that were significantly suppressed or facilitated for paired stimulation with each somatosensory stimulation location. For all somatosensory stimulation locations, there were significantly more recording sites that exhibited facilitation than the control condition, which illustrates the intrinsic variability that occurs with somatosensory stimulation alone

($p<0.05$, p-values are shown below the chart). There were fewer recording sites that exhibited suppressive effects. Interestingly, a trend can be observed in which the percentage of recording sites with facilitated activity decreases (and that of suppressed activity increases) as the somatosensory stimulation location is moved from the head toward the rear of the animal. Additionally, 97% of all analyzed recording sites could be facilitated by at least one somatosensory stimulation location, while 66% could be suppressed (Figure 22-C). This result demonstrates the ability to induce differential modulatory effects in a given recording location depending on somatosensory stimulation location selection, especially since more than half of the recording sites could be suppressed or facilitated by at least one paired stimulation combination.

Discussion

Guinea Pig Homunculus

To our knowledge, this is the first study to characterize the somatotopic map of the guinea pig homunculus in S1. Compared to the original rat homunculus (Welker 1976), the overall somatosensory trends and orientation are consistent. In rats, the somatotopic map exhibits a head-to-tail organization in the rostral-to-caudal direction, just as we have shown in our results. However, there are two main differences between the two datasets. First, Welker showed a much smaller back area than paw area in the rat homunculus, while we show a greater area of responses to back stimulation than rear paw stimulation in our guinea pig homunculus. Second, while Welker showed specific recording locations that responded only to a small area of somatosensory stimulation, we

show a relatively large amount of overlap between stimulation locations in our S1 map. It should be noted that Welker showed S1 locations that responded to tactile stimulation and did not show electrical stimulation threshold maps, which makes a direct comparison difficult.

One possible explanation for these discrepancies, beyond species differences, is that we used electrical stimulation, which may activate somatosensory receptors and/or axons within somatosensory nerves differently than mechanical stimulation. If we are activating receptors more strongly with electrical stimulation, the response may recruit more neurons in S1, which matches our observation that larger areas of S1 are only activated with higher amplitudes of electrical somatosensory stimulation based on the threshold maps in Figure 19 (see Appendix A for a comparison of S1 responses to tactile and electrical stimulation). This result could imply that the area of an S1 response is positively proportional to the intensity of somatosensory activation, as a more intense stimulus would activate a greater area of S1 for a stronger perception of the stimulus. Perhaps tactile stimulation, which may not activate all types of receptors simultaneously, is not capable of eliciting as large of response areas as electrical stimulation. Another possible explanation is that higher levels of electrical stimulation may lead to greater current spread, causing a larger area of the animal's body area to be activated. In a future experiment, this possibility could be further mitigated by placing a stimulation ground fairly close to the stimulation lead for each somatosensory stimulation location. However,

this may limit the ability to activate many receptors simultaneously if current spread is minimized to a small, narrow area between needles.

Auditory Activation and Modulation in S1

Sparse excitatory S1 responses to acoustic stimulation were observed in the guinea pig. Previous studies have also shown sparse responses in one sensory system caused by a different sensory input. For example, in the inferior colliculus (a multimodal center in the central auditory pathway), only sparse responses to somatosensory stimulation were found (Aitkin et al. 1978; Aitkin et al. 1981b), and excitatory visual responses were limited to a small percentage of neurons (Mascetti and Strozzi 1988). Similarly, auditory responses were observed on only a limited number of visual cortex neurons (Fishman and Michael 1973), mainly in secondary visual cortex (Barth et al. 1995). Because multisensory excitatory activity is not widespread in other sensory systems, our sparse auditory map in S1 is not surprising.

In contrast, there appears to be greater multimodal interactions observed in sensory centers when assessing modulation effects. For example, modulatory auditory-somatosensory interactions have been shown in auditory cortex (Kanold et al. 2011; Kayser et al. 2005; Ma and Suga 2003), the superior colliculus (Meredith and Stein 1986), visual cortex (Watkins et al. 2006), the inferior colliculus (Gloeckner et al. 2013), and occipital temporal cortex (Amedi et al. 2001; Beauchamp 2005). Our findings indicate that these multimodal interactions can occur much more extensively in a sensory cortical region than what was previously estimated. We observed that over 80% of S1

recording sites could be modulated by paired somatosensory and acoustic stimulation for each paired stimulation combination, and every recording site was modulated by at least one combination, revealing that multisensory interactions are capable of modulating neurons fully spanning S1. These extensive interactions between sensory systems could have implications on treatments for sensory disorders, especially those related to abnormal cortical firing patterns like tinnitus and pain.

An interesting observation from our mapping study is that an auditory stimulus alone yields excitation primarily in S1 lower body areas, despite the fact that paired stimulation is more facilitative for upper body regions than lower body regions. One explanation for this is that animals receive significant auditory and lower body somatosensory stimuli when they move on cage bedding, which makes loud noises as the animals move around. Based on previous studies showing that the environment can induce plasticity in the S1 homunculus (Bourgeon et al. 2004; Coq and Xerri 1998; Feldman and Brecht 2005; Xerri et al. 1996), it's possible that these natural auditory-somatosensory stimulus combinations could lead to different plasticity results for lower body areas compared to upper body areas. It would make sense that auditory stimulation alone would induce excitatory S1 activity if lower body areas are always activated during noises (when the animal is moving) in its natural environment, since the brain would already naturally be connecting the two excitatory stimuli.

Additionally, it has been shown that the timing of firing between neurons can code for somatosensory location in S1 (Panzeri et al. 2001; Petersen et al. 2001), so

perhaps different delays between auditory and somatosensory stimuli might produce different plasticity results with paired stimulation. We only used simultaneous somatosensory and acoustic stimulation, and our selected timing may have led to predominantly facilitative versus suppressive effects. Greater suppressive modulatory effects, or even differential effects, may be possible for different somatosensory stimulation locations if other inter-stimulus delays are used.

One important note to consider is that all of the somatosensory responses in this study were generated through electrical stimulation of the skin. It is unclear precisely how electrical stimulation activates the somatosensory system, possibly through a combination of receptor activation and/or axonal activation. The stimulation of specific mechanoreceptor groups could have different effects in different areas of somatosensory cortex, and this needs to be considered in future studies when comparing electrical stimulation findings to those of tactile stimulation. Appendix A of this dissertation briefly investigates the S1 responses of different forms of somatosensory stimulation.

Future Studies

Further studies that further investigate auditory interactions in S1 and somatosensory interactions in auditory cortex are needed to better understand how these different sensory systems modulate each other, especially in awake and behaving animals. Tracing studies can give us clues for how multisensory projections reach each cortical area. In terms of clinical applications, tinnitus and some forms of pain are neurological disorders affecting millions of people worldwide (Diatchenko et al. 2005;

Møller et al. 2010; Smith et al. 1999), and have been linked to hyperactivity or alterations in coding in auditory and somatosensory cortex, respectively (Eggermont and Roberts 2004; Flor et al. 1997; Kaltenbach 2011; Millan et al. 1987; Zhao et al. 2006). The ability to systematically alter firing properties across neurons in these sensory cortices by using paired sensory stimulation with varying delays may prove to be an effective approach to interfere with the abnormal firing patterns driving the debilitating symptoms, but it is unclear if our animal results will translate to humans. Since paired multisensory stimulation is easy and noninvasive, human trials should be conducted in the future to test the efficacy of paired stimulation for tinnitus and pain in human subjects.

Chapter 6: Inducing Plasticity Across Five Sensory Cortices Using Multisensory Neuromodulation

Building upon our findings in Chapter 5, we investigated the effects of paired multisensory stimulation across five major sensory cortices to determine if plasticity and modulatory effects can be induced beyond the somatosensory and auditory systems. We also incorporated stimulation of these five sensory systems for a more diverse mSync implementation.

Summary

Plasticity effects of paired noninvasive multisensory stimulation were investigated in olfactory piriform cortex (**OC)**, gustatory cortex (**GC**), primary somatosensory cortex (**S1**), primary auditory cortex (**A1**), and primary visual cortex (**V1**) of ketamine-anesthetized guinea pigs. Significant changes in cortical firing ($P<0.01$) were induced with paired sensory stimulation that depended on sensory input and recording location. Pairing somatosensory and auditory stimulation induced differential effects in S1 and A1 depending on somatosensory stimulation location. Paired gustatory and somatosensory stimulation suppressed or facilitated GC depending on contralateral/ipsilateral somatosensory stimulation location. OC was facilitated by paired olfactory and somatosensory stimulation, but was suppressed with paired olfactory and acoustic stimulation. V1 was facilitated by paired visual and acoustic, gustatory, or contralateral somatosensory stimulation, but was suppressed by paired visual and olfactory or ipsilateral somatosensory stimulation. Overall, we can induce differential effects in neural

firing in sensory cortices using multisensory neuromodulation, and we can control the type, location, and amount of cortical plasticity through the strategic selection of parameters, which may potentially treat neural sensory disorders noninvasively by altering abnormal spike patterns that may drive symptoms.

Introduction

Multisensory integration allows humans to comprehend their surrounding environment as one complete perception instead of separate sensory viewpoints. It also helps us make sense of multiple sensory observations from the same source by combining them in a way that is easy to understand. For example, the spatial maps of the visual and auditory perceptions are integrated and aligned in the superior colliculus (Drager and Hubel 1975; King and Palmer 1985; Meredith and Stein 1986; Wallace et al. 1998), such that we can understand how the visual perception of an object correlates with the sound it makes. In primary auditory cortex (**A1**), there is evidence that neurons respond differently to a voice when the visual perception of a face making speech motions is also seen (Ghazanfar et al. 2005), and auditory cortex is activated during silent lip-reading (Calvert et al. 1997). We know that such phenomena are possible because all of our sensory systems are connected through sensory projections in the brain (Ghazanfar and Schroeder 2006; Murray and Wallace 2011), but it is unclear precisely how these relationships are coded in cortical areas (Stein and Stanford 2008). Even when we can map multisensory connections in subcortical brain regions, we have a limited

understanding of how one sensory system modulates neural firing in another, or how multisensory integration can lead to plasticity.

Many previous studies have shown examples of how each sensory system is interconnected with every other sensory system. A1 plasticity has been induced using electrical stimulation of somatosensory receptors (Gloeckner et al. 2013; Kayser et al. 2005; Markovitz et al. 2015) or direct electrical stimulation of somatosensory cortex (Ma and Suga 2003). Excitatory activity has also been induced by somatosensory stimulation in A1 (Foxe et al. 2002; Murray et al. 2005) and in subcortical auditory areas such as the cochlear nucleus (Kanold et al. 2011) and inferior colliculus (Aitkin et al. 1978; Aitkin et al. 1981b). Additionally, olfactory stimulation has been shown to induce plasticity through reorganization in auditory cortex (Cohen et al. 2011), and visual-auditory interactions in A1 have been well-documented as well (Calvert et al. 1997; Ghazanfar et al. 2005). Modulation of activity in primary somatosensory cortex (**S1**) with auditory stimuli has been shown (Foxe et al. 2000), and primary visual cortex (**V1**) has been modulated by natural auditory stimuli (Watkins et al. 2006). Visual cortex receives direct projections to and from the auditory and somatosensory systems (Dehay et al. 1988; Innocenti et al. 1988), especially cortical areas (Miller and Vogt 1984), and these often result in excitatory activity (Giraud et al. 2001; Yaka et al. 1999). In fact, in blind patients, somatosensory inputs from brail reading activates V1 reliably (Sadato et al. 1996). Evidence of multisensory integration also exists in gustatory cortex (**GC**), which has interactions with the auditory (Yan and Dando 2015), visual (Rolls and Baylis 1994;

Spence 2013; Zampini et al. 2007), somatosensory (de Araujo and Simon 2009; Simon et al. 2006; Simon et al. 2008), and olfactory (de Araujo and Simon 2009; Rolls and Baylis 1994) systems, and a review paper describes how all five sensory systems are directly involved in gustation and neural flavor perception (Auvray and Spence 2008). Piriform olfactory cortex (**OC**), which already functions much like associative cortex with a high density of neighboring axonal outputs and a lack of columnar organization (Johnson et al. 2000), is directly modulated by the auditory (Seo and Hummel 2011; Wesson and Wilson 2010), somatosensory (Demattè et al. 2006; Fiore 1993), and visual (Gottfried and Dolan 2003; Leonard and Masek 2014) systems, and it has a more direct relationship with gustatory cortex, with many direct network projections (Maier et al. 2015) and modulatory features (Rolls and Baylis 1994). The olfactory system also has direct connections with behavior, as olfactory bulb removal can eliminate maternal behavior in mice (Gandelman et al. 1971), making it even more of a multimodal center.

In addition to all of these direct sensory interactions, there are also other areas of the brain that have been classified as multisensory. The superior and inferior colliculi have already been mentioned, and the occipital temporal cortex receives projections from the visual, somatosensory, and auditory systems (Amedi et al. 2001; Beauchamp 2005). There is even a provocative hypothesis that the entire neocortex is multisensory, since there are so many projections from other sensory systems to traditional sensory cortical areas (Ghazanfar and Schroeder 2006), although there is clear evidence that primary

sensory cortical areas do have a primary sensory system input that appears to be far more active than all other sensory inputs.

From all of these previous studies and reviews, it is clear that multisensory integration plays an important role in the processing of sensory inputs. However, the potential for such connectivity and plasticity induction to be used to treat neural disorders has been sparsely investigated. Many neural sensory disorders are characterized by abnormal firing patterns, including tinnitus (Eggermont and Roberts 2004; Kaltenbach 2011; Lanting et al. 2008; Lanting et al. 2009), phantom limb pain (Smith et al. 1999), and some forms of chronic pain induced by alterations in neural opioid systems (Millan et al. 1987), increases in neurotransmitter release (Zhao et al. 2006), S1 reorganization (Flor et al. 1997), and increased gain in pain-related neural activity (Woolf and Salter 2000). In theory, all of these abnormal firing patterns could be corrected or improved with careful strategic plasticity induction through neuromodulation treatments that specifically address patient-specific problems in neural firing. Neuromodulation is a quickly growing field of neural stimulation for the modulation of activity to treat various neural disorders (Johnson et al. 2013), and some neuromodulation techniques target plasticity in specific brain regions to induce permanent solutions to symptoms (Engineer et al. 2011). Such techniques can be applied to sensory disorders. Noninvasive neuromodulation has already been used to treat phantom limb pain through mirror therapy, which uses visual stimuli to trick the brain into thinking that an amputated limb still exists (Chan et al. 2007). Chronic pain neuromodulation treatments include peripheral stimulation (Gildenberg 2006),

spinal cord stimulation (Alo and Holsheimer 2002), and brain stimulation (Charleston et al. 2010). Tinnitus is also treated with both noninvasive and invasive techniques, including transcranial magnetic stimulation (De Ridder et al. 2011b), deep brain stimulation (Cheung and Larson 2010), and various other stimulation treatments (Vanneste and De Ridder 2012).

Sensory system stimulation for neuromodulation has not been routinely attempted for neural disorder treatment, but there are a few cases of sensory stimulation for treating brain disorders. For example, trigeminal nerve stimulation, which is essentially an invasive way of stimulating the somatosensory and motor systems of the face, has been used to treat epilepsy (DeGiorgio et al. 2013) and depression (Cook et al. 2013), and multisensory stimulation has been attempted for tinnitus treatment (Dehmel et al. 2008b; Levine et al. 2003). We have recently attempted noninvasive paired somatosensory and sound stimulation to induce controlled plasticity in the auditory system for the treatment of tinnitus (Gloeckner et al. 2013; Markovitz et al. 2015), but we have not investigated the effects of paired multisensory stimulation on other sensory systems, which might be useful for treating a variety of neural sensory disorders based on how interconnected the senses are in the brain. In this study, we investigated the plasticity effects and immediate modulatory effects of a variety of paired multisensory stimulation combinations on all five major sensory cortices. We also briefly looked into the potential of inducing differential effects, and the ability to control the type, amount, and location of plasticity,

which might be useful in treating disorders with a variety of symptoms and/or pathogeneses across patients.

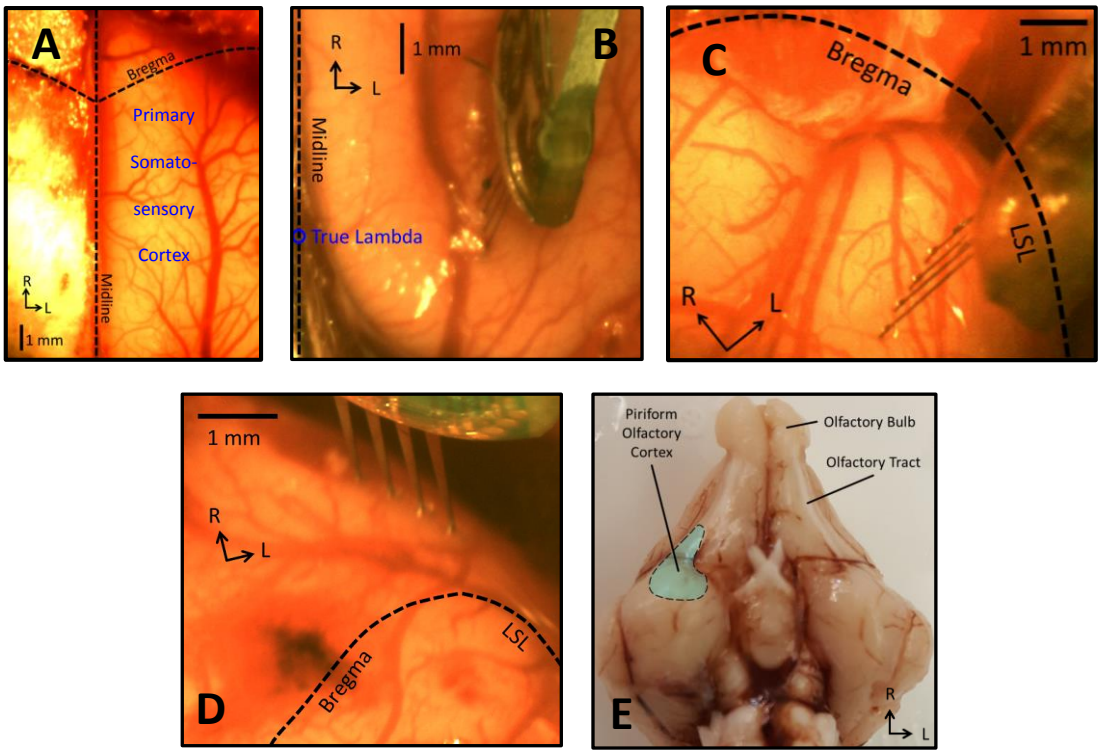


Figure 23: Recording locations

Primary sensory cortices were located using previous mapping studies and functional responses to sensory stimuli. Primary somatosensory cortex is located adjacent to the midline and spans from just rostral of the Bregma suture line to about halfway between the Bregma suture line and the Lambda suture line (**A**). Primary visual cortex is located adjacent to the midline near the Lambda suture line, caudal of somatosensory cortex (**B**). Primary auditory cortex is located between the pseudosylvian sulcus and the lateral suture line (LSL), caudal of the Bregma suture line (**C**). Gustatory cortex is located on the lateral edge of the brain near the Bregma suture line, rostral of auditory cortex (**D**, the electrode placement in the

picture shows how one would typically reach gustatory cortex by inserting the electrode dorsal of the target at the very lateral edge of the brain, and pushing it deeper than normal to reach the target). Piriform olfactory cortex is located on the underside of the brain as shown in the figure (**E**, this picture shows the ventral side of the brain; in order to reach olfactory cortex in a live animal, one must insert a probe in to the dorsal cortex and penetrate all the way through the brain to the ventral side).

Materials and Methods

Overview

Plasticity experiments were performed on 40 young female Hartley guinea pigs (400–450 g; Elm Hill Breeding Labs, Chelmsford, MA) anesthetized with an initial intramuscular injection of a ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL) cocktail, with varying supplements every 45–60 minutes to maintain an areflexive state. Electrophysiology neural recordings were performed inside an electrically-shielded and acoustic-attenuating room using hardware from Tucker-Davis Technology (Alachua, FL), and resulting data was processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Surgery and Neural Recordings

A craniotomy was performed revealing the target sensory cortical area on the right side of the guinea pig brain, and a 32-site Michigan-style recording electrode array (NeuroNexus Technologies, Ann Arbor, MI) was inserted into the target area. For S1 recordings, the skull and dura were removed spanning from the midline to the lateral suture line and from the Lambda suture line to about 0.5-1.0 cm rostral of the Bregma suture line (Figure 23-A, the Lambda suture line would be located caudal of the bottom edge of the picture). The recording electrode was inserted into S1 within 5 mm of the midline at a location with strong responses to stimulation of the somatosensory location being used, based on our own S1 mapping studies and previous somatosensory cortical mapping studies (Campos and Welker 1976; Cho et al. 2007; Godde et al. 2002; Welker 1976). For V1 recordings, the skull and dura were removed spanning from midline to the lateral suture line and from the cerebral transverse fissure to about 1 cm rostral of True Lambda, which is the intersection of the Lambda suture line and the midline. The recording electrode was inserted into V1 within 5 mm of the midline (Figure 23-B, the cerebral transverse fissure would be located just caudal of the bottom edge of the picture) at a location that responded well to visual stimulation, based on previous visual cortical studies (Choudhury 1978; Coogan and Burkhalter 1993; Orbach et al. 1985; Wang and Burkhalter 2007). For A1 recordings, the skull and dura were removed spanning from the midline to the lateral suture line and from the Bregma suture line to about 1 cm caudal of Bregma (Figure 23-C). The recording electrode was inserted into A1 between the

pseudosylvian sulcus and the lateral suture line at a location with strong responses to acoustic stimulation frequencies of 1-20 kHz, based on previous studies mapping the locations of auditory cortical areas (Markovitz et al. 2013; Redies et al. 1989; Straka et al. 2014; Wallace et al. 2000). For GC recordings, the skull and dura were removed spanning from the midline past the lateral edge of the brain and from the olfactory bulb to about 1 cm caudal of the Bregma suture line (such a large area was removed to make it easier to remove the lateral side of the skull without damaging the cortex). The recording electrode was inserted into GC near the Bregma suture line at the very lateral edge of the cortex at a location that responded well to gustatory stimulation (Figure 23-D), based on previous gustatory cortical studies (Accolla et al. 2007; Accolla and Carleton 2008; Katz et al. 2002; Kosar et al. 1986a; b). For OC recordings, the skull and dura were removed spanning from the midline past the lateral edge of the brain and between the olfactory bulb and the Lambda suture line (such a large area was removed to make it easier to angle the recording probe appropriately for insertion through the brain). The recording electrode was inserted into the dorsal cortex and penetrated through the entire brain to reach the ventral cortex, resting in OC at a location that responded well to olfactory stimulation (Figure 23-E shows the ventral side of the brain where OC resides), based on previous olfactory cortical studies (Biella and De Curtis 2000; Heimer 1978; Johnson et al. 2000; Schoenbaum and Eichenbaum 1995). In all cases, the recording electrode ground was either inserted into the neck of the animal or another part of the brain unrelated to sensory systems, depending on background noise levels.

Heart rate and blood oxygen content were continuously monitored using an H100 pulse oximeter from EdanUSA (San Diego, CA), and body temperature was monitored using an Oakton Acorn series JKT thermocouple rectal probe (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN). The animal's head was fixed into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars.

Recording electrode arrays for A1, S1, V1, and GC experiments contained four 5-mm long shanks separated by 500 μm with eight iridium sites linearly spaced at 200 μm along each shank (site area approximately 413 μm^2), and were inserted to a depth such that the main input Layer IV could be observed at approximately the middle of the eight recording sites on each shank (determined based on locating the initial sink using current-source density) (Lim and Anderson 2007a; Markovitz et al. 2013; Straka et al. 2014), which generally resulted in the tip sites being inserted 1.0-1.5 mm below the surface of the cortex (for GC, Layer IV was initially found by inserting the probe at an angle such that it spanned multiple layers, and then the probe was reinserted parallel to Layer IV such that most sites showed Layer IV activity). Recording electrode arrays for OC experiments contained two 10-mm long shanks (site area approximately 413 μm^2) with 16 recording sites per shank at a spacing of 100 μm, and were inserted completely through the brain from the dorsal cortex to the ventral cortex. In all cases, recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine

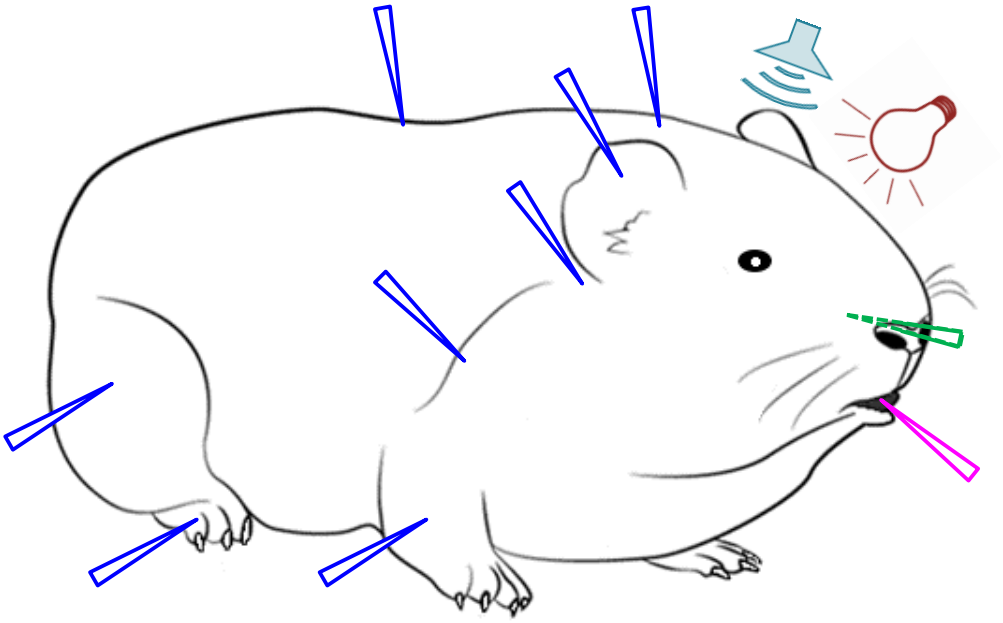


Figure 24: Stimulation methods

Subdermal needles were used to electrically stimulate the somatosensory system (blue triangles) at locations that spanned the entire animal, and these locations were stimulated independently during recordings. Contralateral (left side) somatosensory stimulation locations in the shoulder, mastoid, foot, leg, and pinna are not shown. A surface electrode was used to stimulate the gustatory system (magenta triangle). A dull needle electrode was placed in the olfactory cavity for olfactory stimulation (green triangle), and the needle was moved around until excitatory activity could be observed in olfactory cortex in response to electrical stimulation of the cavity. Broadband noise was played into the left ear for auditory stimulation, and flashes of white light using an LED light were used for visual stimulation.

wave. Saline was routinely administered to the cortex after the probe was placed to limit the effects of drying. Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of neural spike activity. A detection threshold of 3.5 times the standard deviation of the voltage noise floor was used to determine when spikes occurred for A1, S1, V1, and GC experiments (3.0 for OC experiments due to a higher noise floor), and spike voltage waveforms were visually inspected to ensure that no noise was falsely detected.

Sensory Stimulation

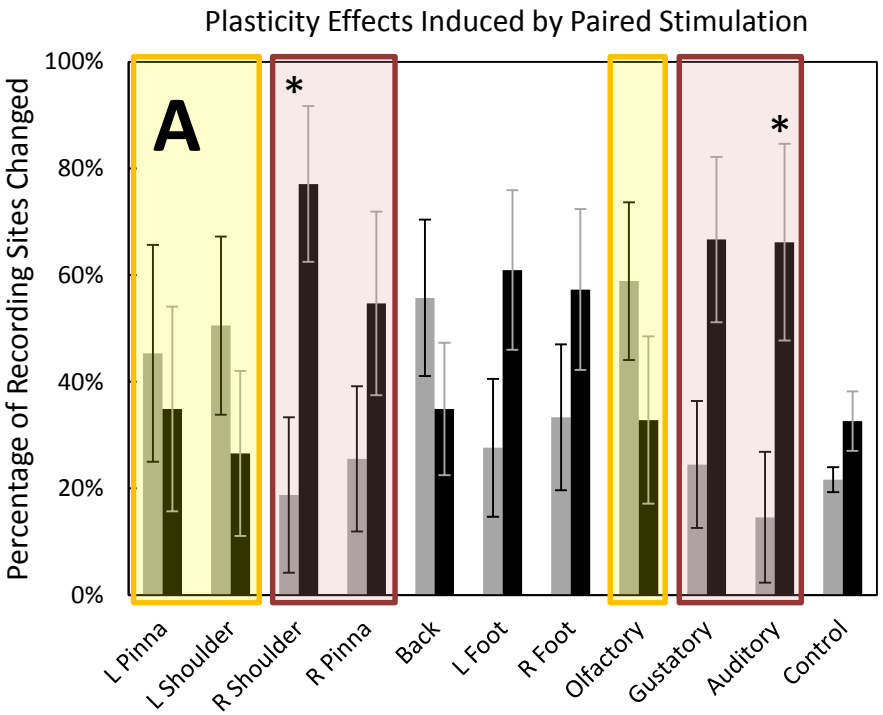
A diagram of all sensory stimuli is shown in Figure 24. Electrical somatosensory stimulation (biphasic, 205 μs per phase, 0.63 mA for A1 experiments, 1.8 mA for all others) of the skin was performed using subcutaneous needle electrodes (Rhythmlink International LLC, Columbia, SC) placed within the left and right pinnas, left and right mastoids, left and right shoulders, neck, left and right arms, back, left and right hind legs, and left and right hind paws of the animal. The pinna electrodes were placed on the surface of the center of each pinna, and the left and right mastoid electrodes were placed over the mastoid portions of the temporal bones just beneath the skin in the ventral direction. The shoulder electrodes were inserted dorsal of the shoulder joints, and the arm electrodes were inserted on the lateral side of the arm, halfway between the shoulder joint and elbow joint. The neck electrode was inserted centrally halfway between the ears and the shoulder joints. The back electrode was inserted along the spine halfway between the

neck electrode and the end of the spine. The leg electrodes were placed laterally halfway between the hip joint and the knee joint, and the hind paw electrodes were placed in the center of the bottom of the paw. For neck, arm, foot, and back stimuli, the stimulation ground was distributed between the four shoulder and leg electrodes, and for the shoulder, leg, pinna, and mastoid stimuli, the stimulation ground was distributed between the four arm and foot electrodes. Spreading the ground across four distinct body locations helped to mitigate unintended activation of ground areas, and control experiments were performed to confirm that no ground areas elicited activation in S1 even at the highest stimulation current levels. Electrical gustatory stimulation (1.8 mA) was performed by placing a needle electrode on the surface of the tongue, and care was taken to ensure that the tongue was not punctured. Electrical olfactory stimulation (1.8 mA) was performed by carefully inserting a needle electrode into the olfactory cavity through the nostril, and the electrode was tactfully moved around until stimulation resulted in olfactory cortical activity. Acoustic broadband noise stimuli (50 ms duration, 0.5 ms rise/fall time, 70 dB SPL, equal energy between 625 Hz and 40 kHz) were presented to the animal's left ear for auditory stimulation using a speaker (Tucker-Davis Technology, Alachua, FL) coupled to the left ear bar. The speaker-ear bar system was calibrated using a 0.25 in. condenser microphone (ACO Pacific, Belmont, CA). Visual stimulation was performed using a white LED light placed in front of the left eye and powered by a 1 ms electrical pulse. The animal was allowed to rest in the dark for at least five minutes before each visual stimulation session.

In all cases, multisensory stimulation consisted of paired stimulation of the sensory system being recorded from (e.g. auditory for A1) with the stimulation of a different sensory system. For S1, V1, GC, and OC recordings, the two sensory stimuli were always simultaneous, and for A1 recordings, auditory stimulation occurred 5 ms later than the other stimulus to match previous studies.

General Protocol

For each cortical recording, we recorded 100 trials (2 per second) of responses to a normal sensory stimulus corresponding with the cortical area being recorded from (e.g. auditory stimulation was used for A1 recordings). Next, either 1000 trials (A1, V1, GC, OC) or 500 trials (S1) of paired sensory stimulation (2 per second) were performed. This was followed by another 100 trials of normal sensory stimulus. To test for plasticity, responses before paired stimulation were compared to those after to determine if paired stimulation had induced significant changes in spike activity (two-tailed, unequal variance, ranked t-test, $P<0.01$). To test for changes during paired stimulation, responses before paired stimulation were compared to responses during the first 100 trials of paired stimulation using the same statistical method. After all of this was completed, the next experiment with a different set of parameters was performed depending on the experiment, and the order of parameter combinations was randomized to mitigate cumulative effects. Additionally, control experiments were performed for each sensory cortex in which paired stimulation was replaced with no stimulation.


Plasticity Effects Induced by Paired Stimulation
Percentage of Recording Sites Changed
100%
80%
60%
40%
20%
0%
A
*
*
L Pinna
L Shoulder
R Shoulder
R Pinna
Back
L Foot
R Foot
Olfactory
Gustatory
Auditory
Control

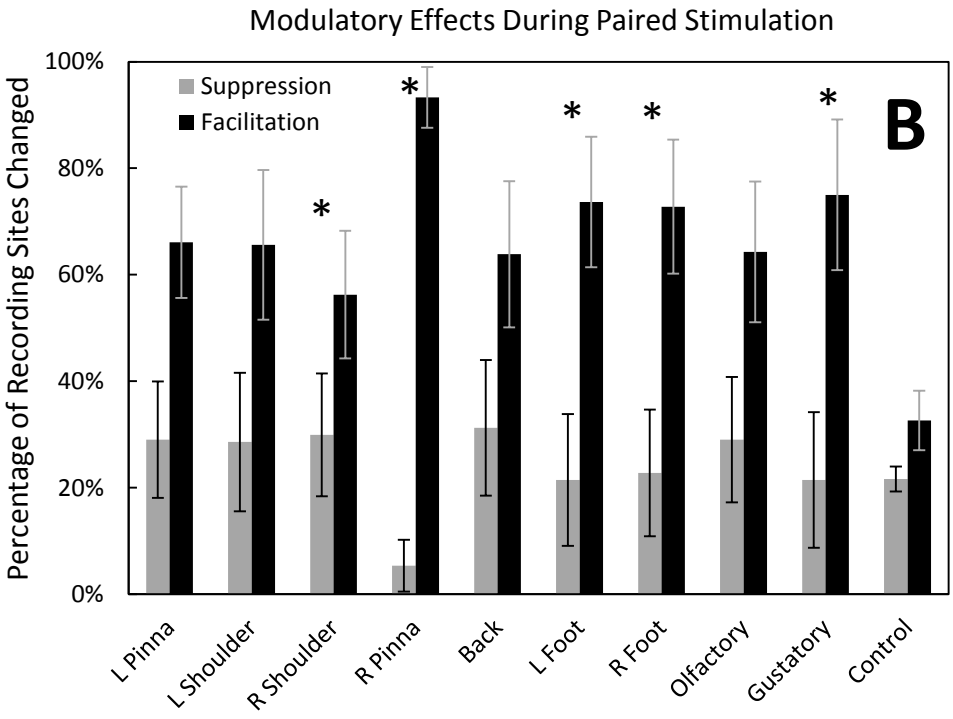

Modulatory Effects During Paired Stimulation
Percentage of Recording Sites Changed
100%
80%
60%
40%
20%
0%
Suppression
Facilitation
B
*
*
*
*
*
L Pinna
L Shoulder
R Shoulder
R Pinna
Back
L Foot
R Foot
Olfactory
Gustatory
Control

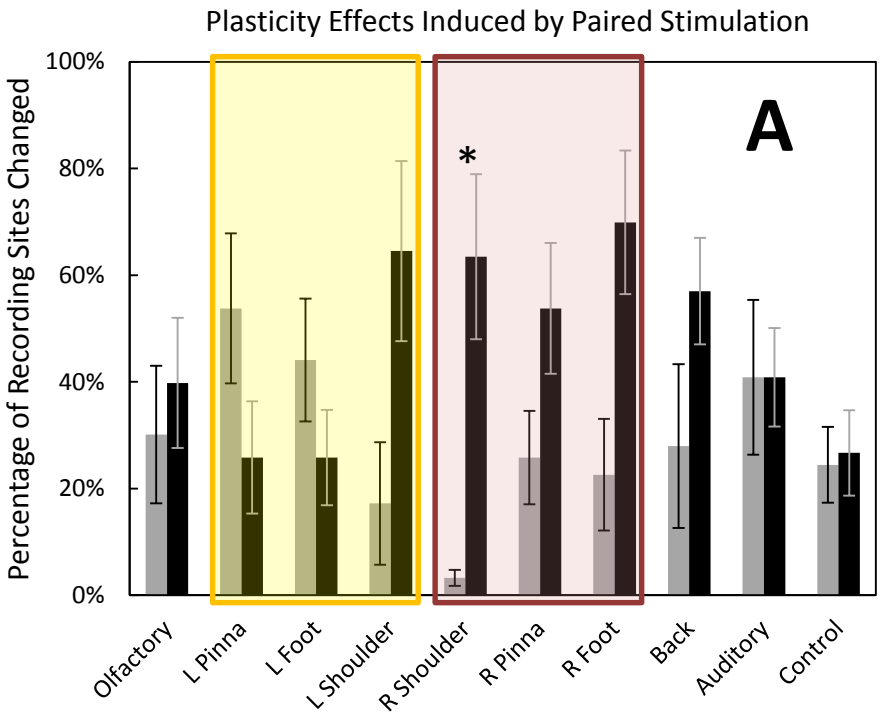
Figure 25: Plasticity and modulation of activity in visual cortex

The percentage of total V1 recording sites in which visual-driven spike activity was suppressed (grey) or facilitated (black) immediately after stimulation is shown for visual stimulation paired with simultaneous stimulation of another sensory system in four animals (**A**). Tested somatosensory locations included the left and right pinnas, left and right shoulders, back, and left and right foot, in addition to auditory stimulation (70 dB broadband noise), gustatory stimulation, and olfactory stimulation (n=224 for each of the paired stimulation combinations). For control trials, paired stimulation was replaced with no stimulation in the same four animals (n=1760) before paired stimulation was used. Error bars show standard error across animals. For contralateral upper body somatosensory stimulation locations, paired stimulation induced more suppressed recording sites than facilitated sites (left yellow box), while ipsilateral upper body locations induced more facilitated sites than suppressed sites (left maroon box). Similarly, olfactory stimulation was more suppressive (right yellow box) while gustatory and auditory stimulation were more facilitative (right maroon box). Right shoulder and auditory stimuli both induced significantly more facilitated recording sites than suppressed sites ($P<0.05$, marked with asterisks). In all cases, paired stimulation induced changes in more recording sites than control. When comparing visual-driven activity before stimulation to activity during paired stimulation, activity was generally facilitated, although different combinations yielded different ratios of facilitated sites vs. suppressed sites (**B**, parameters with significantly more facilitated sites than suppressed sites are marked with asterisks, $P<0.05$).

Results

Primary Visual Cortex

For V1 recordings, visual stimulation was paired with gustatory, olfactory, auditory, or somatosensory stimulation of different body locations. Plasticity results are shown in Figure 25-A, where paired stimulation induced a higher percentage of recording sites with significantly changed spike activity than control (no stimulation) in all cases. Interestingly, contralateral (left) somatosensory stimulation locations tended to be more suppressive (left yellow box), while ipsilateral (right) somatosensory locations tended to be more facilitative (left maroon box) when comparing pinna and shoulder locations. Additionally, olfactory stimulation tended to be more suppressive (right yellow box), while gustatory and auditory stimulation tended to be more facilitative (right maroon box). In some cases, such as right shoulder stimulation, the percentage of recording sites that was facilitated was significantly higher than that of those suppressed (standard t-test, $P<0.05$), and those cases are marked with asterisks on the figure. When looking at modulatory effects during paired stimulation, activity was generally facilitated more often than suppressed when compared to activity before paired stimulation, and more total changes were induced than control in all cases (Figure 25-B). This data indicates that multisensory inputs to V1 are facilitative by nature, causing neurons to fire more often when already responding to a visual stimulus; however, the plasticity effects of paired stimulation are dependent on stimulation parameters, as different paired combinations induce different changes in firing rates following stimulation.


Plasticity Effects Induced by Paired Stimulation
Percentage of Recording Sites Changed
100%
80%
60%
40%
20%
0%
A
*
Olfactory
L Pinna
L Foot
L Shoulder
R Shoulder
R Pinna
R Foot
Back
Auditory
Control

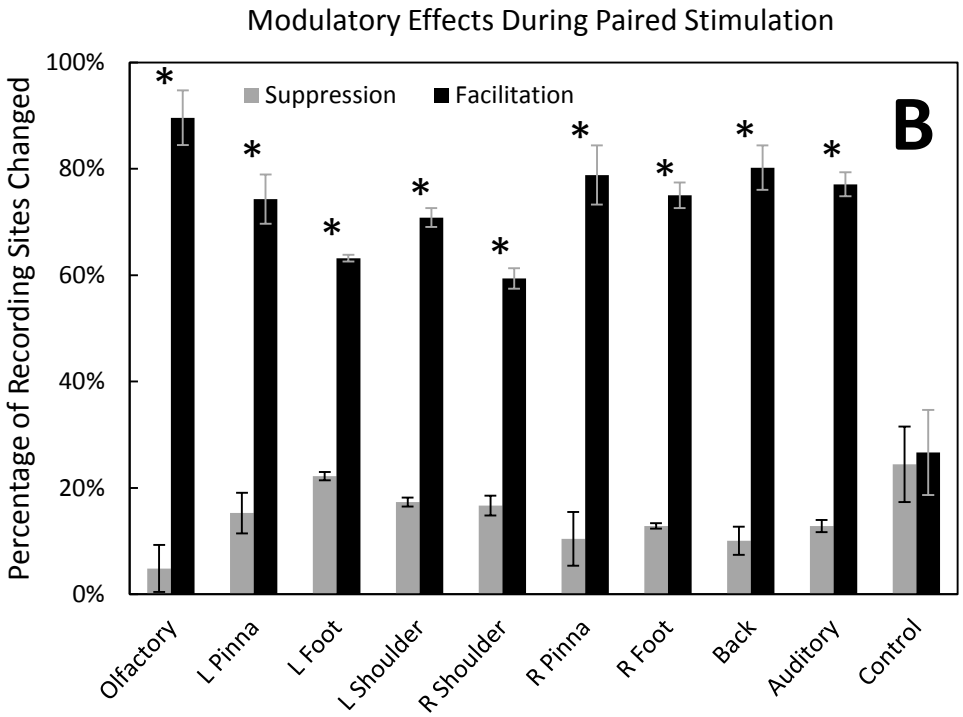

Modulatory Effects During Paired Stimulation
Percentage of Recording Sites Changed
100%
80%
60%
40%
20%
0%
Suppression
Facilitation
B
*
Olfactory
L Pinna
L Foot
L Shoulder
R Shoulder
R Pinna
R Foot
Back
Auditory
Control

Figure 26: Plasticity and modulation of activity in gustatory cortex

The percentage of total GC recording sites in which gustatory-driven spike activity was suppressed (grey) or facilitated (black) immediately after stimulation is shown for gustatory stimulation paired with simultaneous stimulation of another sensory system in four animals (**A**). Tested somatosensory locations included the left and right pinnas, left and right shoulders, back, and left and right foot, in addition to auditory stimulation (70 dB broadband noise) and olfactory stimulation (n=93 for each of the paired stimulation combinations). For control trials, paired stimulation was replaced with no stimulation in two animals (n=297). Error bars show standard error across animals. For contralateral somatosensory stimulation locations, paired stimulation induced more suppressed recording sites than facilitated sites (yellow box), while ipsilateral body locations induced more facilitated sites than suppressed sites (maroon box). Only paired stimulation with the right shoulder had significantly more facilitated recording sites than suppressed sites ($P<0.05$, marked with an asterisk), and no combination had significantly more suppressed sites than facilitated sites. When comparing gustatory-driven activity before stimulation to activity during paired stimulation, all combinations had significantly more facilitated than suppressed sites ($P<0.05$, marked with asterisks), although different combinations yielded different ratios of facilitated vs. suppressed sites (**B**).

Gustatory Cortex

For GC recordings, gustatory stimulation was paired with either olfactory or auditory stimulation, or with somatosensory stimulation of different body locations. Plasticity results are shown in Figure 26-A, where paired stimulation induced a higher

percentage of recording sites with significantly changed spike activity than control in all cases. Contralateral (left) somatosensory stimulation locations tended to be more suppressive (yellow box), while ipsilateral (right) somatosensory locations tended to be more facilitative (maroon box). Even with shoulder stimulation, the trend holds true, as although the left shoulder was more facilitative than suppressive, its facilitation/suppression ratio is still much smaller than that of its right counterpart. This contralateral/ipsilateral trend is similar to that of visual cortex. During paired stimulation, modulatory effects were generally facilitative more often than suppressive when compared to activity before paired stimulation, although different stimulation modalities elicited different ratios of facilitation/suppression (Figure 26-B). Once again, cases where there were significantly more facilitated sites than suppressed sites are marked with asterisks ($P<0.05$). Just as in V1, multisensory inputs to GC appear to be primarily excitatory during paired stimulation, but the plasticity effects are dependent on paired combinations. Based on this, it may be possible to control facilitative versus suppressive plasticity effects with the appropriate stimulation combinations.

Piriform Olfactory Cortex

For OC recordings, olfactory stimulation was paired with gustatory, auditory, or somatosensory stimulation of different body locations, and plasticity results are presented in Figure 27-A, where paired stimulation induced a higher percentage of recording sites with significantly changed spike activity than control in all cases. Unlike V1 and GC, there is not a clear contralateral/ipsilateral trend for somatosensory stimulation locations

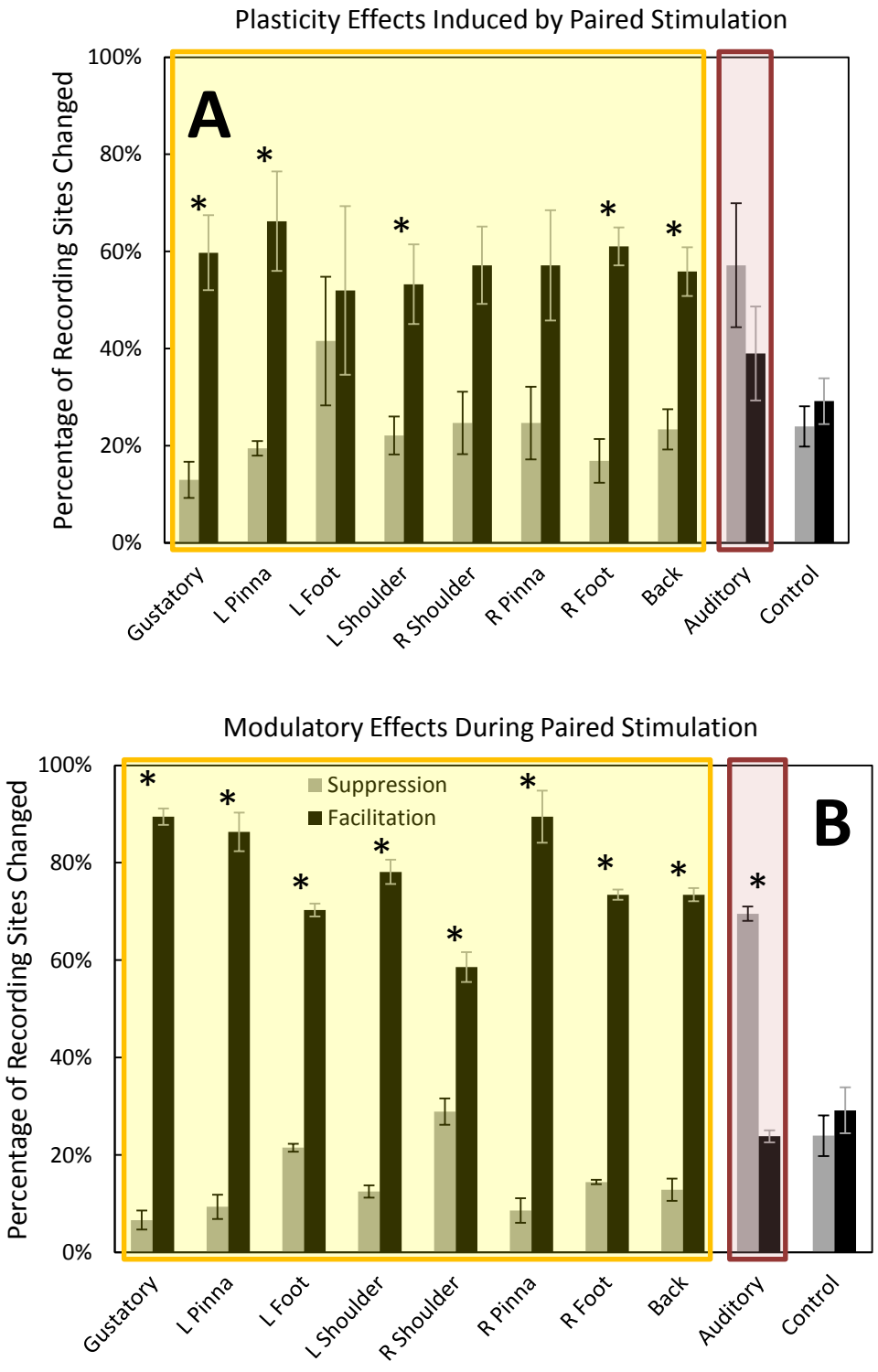

Plasticity Effects Induced by Paired Stimulation
Percentage of Recording Sites Changed
100%
80%
60%
40%
20%
0%
A
*
Gustatory
L Pinna
L Foot
L Shoulder
R Shoulder
R Pinna
R Foot
Back
Auditory
Control
Modulatory Effects During Paired Stimulation
Suppression
Facilitation
B

Figure 27: Plasticity and modulation of activity in olfactory cortex

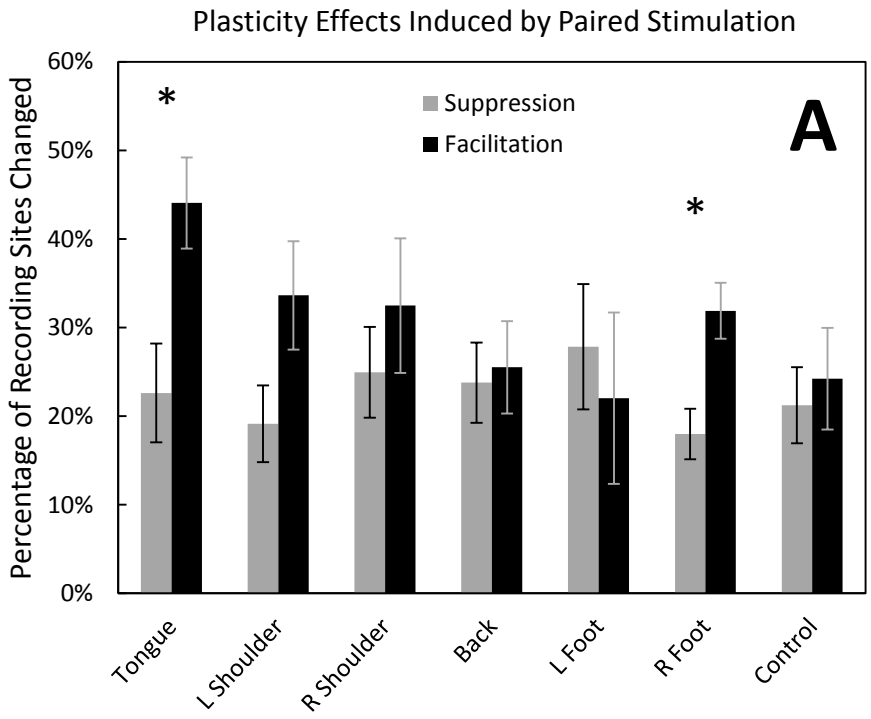
The percentage of total OC recording sites in which olfactory-driven spike activity was suppressed (grey) or facilitated (black) immediately after stimulation is shown for olfactory stimulation paired with simultaneous stimulation of another sensory system in four animals (**A**). Tested somatosensory locations included the left and right pinnas, left and right shoulders, back, and left and right foot, in addition to auditory stimulation (70 dB broadband noise) and gustatory stimulation (n=77 for each of the paired stimulation combinations). For control trials, paired stimulation was replaced with no stimulation in four animals (n=396). Error bars show standard error across animals. In general, paired stimulation with somatosensory or gustatory stimulation induced more facilitated recording sites than suppressed sites (yellow box), while paired stimulation with auditory stimulation induced more suppressed sites than facilitated sites (maroon box). Gustatory stimulation and somatosensory stimulation of the left pinna, left shoulder, right foot, and back all induced significantly more facilitated recording sites than suppressed sites ($P<0.05$, marked with asterisks). Similar trends were observed when comparing olfactory-driven activity before stimulation to activity during paired stimulation (**B**), as paired stimulation with somatosensory or gustatory stimulation was more facilitative and paired stimulation with auditory stimulation induced more suppression.

in OC. Instead, gustatory and somatosensory stimulation tended to be more facilitative regardless of location (yellow box) although different locations yielded different facilitation/suppression ratios. Meanwhile, auditory stimulation tended to be more suppressive (maroon box). Many stimulation combinations had significantly more

facilitated recording sites than suppressed sites, and these are marked with asterisks ($P<0.05$). The same trends can be seen in modulatory effects when comparing activity before paired stimulation to that during paired stimulation (Figure 27-B), where auditory stimulation resulted in significantly more suppressed than facilitated sites, while all other combinations were significantly facilitative. Compared to trends in GC and V1, olfactory cortex has unique relationships, which might signify the ability to induce differential effects across sensory systems.

Primary Somatosensory Cortex

For S1 recordings, somatosensory stimulation of different body locations was always paired with auditory stimulation, and recordings were performed at S1 locations where responses from stimulation of a given body location alone were observed. Plasticity results are shown in Figure 28-A, and while there are no clear trends across somatosensory stimulation locations, each location does induce a unique effect, as some are more facilitative than others. Tongue and right foot stimulation both induced significant differences between suppressed sites and facilitated sites ($P<0.05$). Meanwhile, modulatory effects during paired stimulation were always facilitative, but upper body stimulation locations tended to have higher facilitation/suppression ratios than lower body locations (Figure 28-B). In all cases, more recording sites were facilitated during paired stimulation than control.


Plasticity Effects Induced by Paired Stimulation
Percentage of Recording Sites Changed
60%
50%
40%
30%
20%
10%
0%
Suppression
Facilitation
A
*
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Tongue
L Shoulder
R Shoulder
Back
L Foot
R Foot
Control

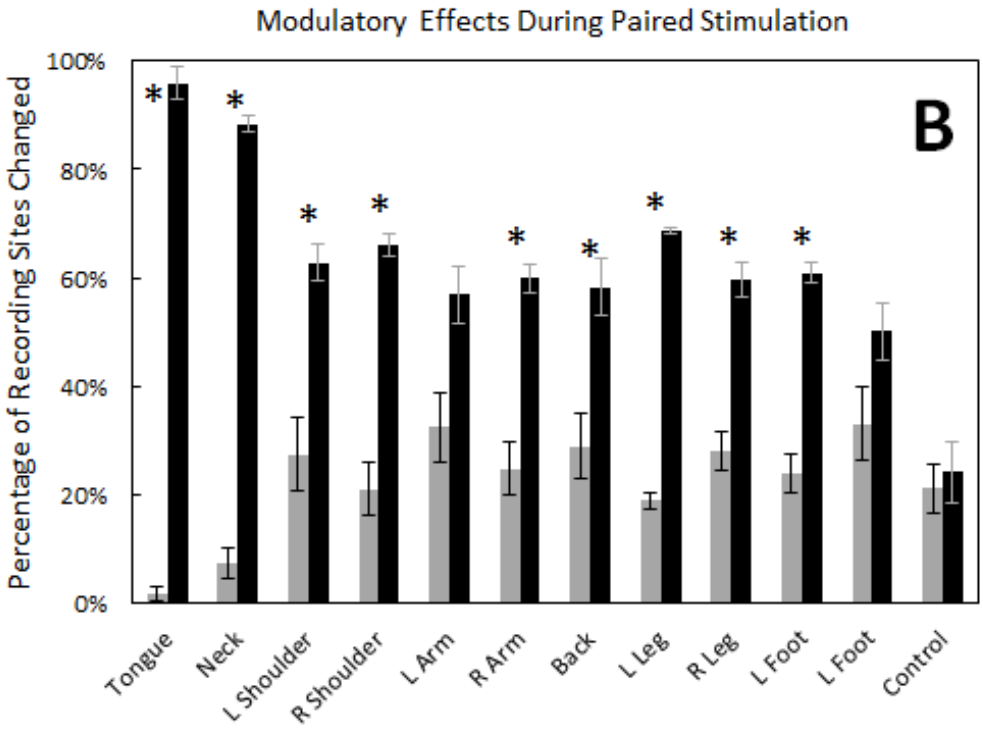
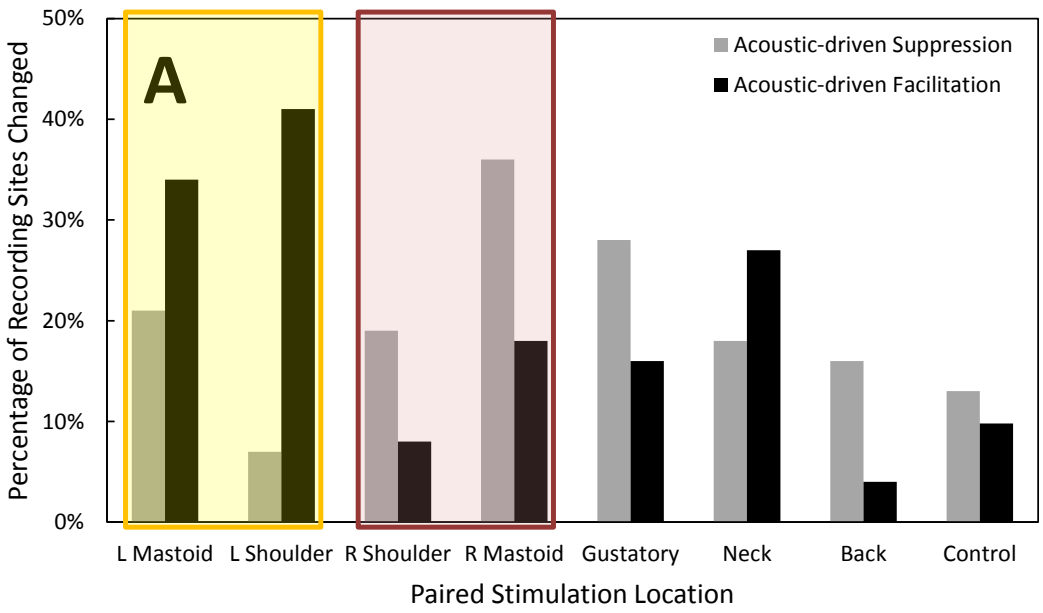

Modulatory Effects During Paired Stimulation
Percentage of Recording Sites Changed
100%
80%
60%
40%
20%
0%
B
Tongue
Neck
L Shoulder
R Shoulder
L Arm
R Arm
Back
L Leg
R Leg
L Foot
L Foot
Control

Figure 28: Plasticity and modulation of activity in somatosensory cortex

The percentage of total S1 recording sites in which somatosensory-driven spike activity was suppressed (grey) or facilitated (black) immediately after stimulation is shown for paired simultaneous acoustic (70 dB broadband noise) and somatosensory stimulation with different somatosensory stimulation locations in four animals (**A**). Tested somatosensory locations included the tongue (n=224), left shoulder (n=608), right shoulder (n=576), back (n=576), left foot (n=544), and right foot (n=320). For two control animals, no stimulation was used (n=800). Error bars show standard error across animals. While there are no significant trends, different somatosensory stimulation locations yielded different results, indicating the ability to induce differential effects with different parameters of stimulation. Paired stimulation with the tongue and right foot induced significantly more facilitated recording sites than suppressed sites ($P<0.05$, marked with asterisks). When comparing somatosensory-driven activity before stimulation to activity during paired stimulation, activity was primarily facilitated regardless of somatosensory stimulation location, but lower body locations had a smaller ratio of facilitated sites to suppressed sites than upper body locations (**B**). In addition to those in A, tested somatosensory locations in B included the neck (n=448), left arm (n=704), right arm (n=448), left leg (n=640), and right leg (n=608). Most stimulation locations resulted in significantly more facilitation than suppression ($P<0.05$, marked with asterisks).

Primary Auditory Cortex

For A1 recordings, auditory stimulation was always paired with either gustatory stimulation or somatosensory stimulation of different body locations. Plasticity results are


A
Acoustic-driven Suppression
Acoustic-driven Facilitation
Percentage of Recording Sites Changed
50%
40%
30%
20%
10%
0%
L Mastoid
L Shoulder
R Shoulder
R Mastoid
Gustatory
Neck
Back
Control
Paired Stimulation Location

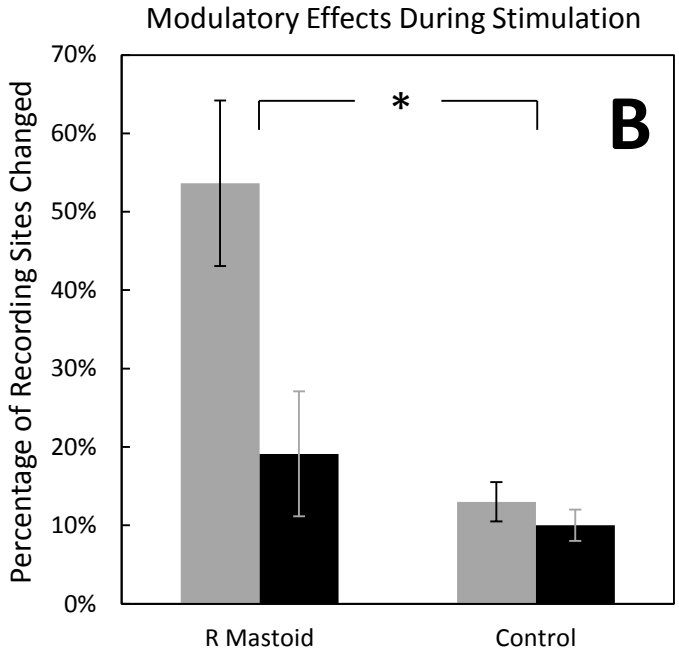

Modulatory Effects During Stimulation
B
*
Percentage of Recording Sites Changed
70%
60%
50%
40%
30%
20%
10%
0%
R Mastoid
Control

Figure 29: Plasticity and modulation of activity in auditory cortex.

The percentage of total A1 recording sites in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) immediately after stimulation is shown for paired acoustic stimulation and gustatory somatosensory stimulation with different somatosensory locations (**A**). In all cases, somatosensory/gustatory stimulation preceded acoustic stimulation by 5 ms. Tested somatosensory locations included the left mastoid (n=90 across 4 animals), right mastoid (n=486 across 6 animals), neck (n=79 across 5 animals), left shoulder (n=58 across 3 animals), right shoulder (n=86 across 3 animals), and back (n=57 across 2 animals). Gustatory stimulation (n=108 across 5 animals) was also tested. For three control animals (n=882), no stimulation was used. In general, contralateral (left) somatosensory locations induced more facilitated recording sites than suppressed (yellow box), while ipsilateral (right) locations induced more suppressed than facilitated sites (maroon box). When comparing acoustic-driven activity before stimulation to activity during paired stimulation, activity was primarily suppressed with right mastoid stimulation, and more total recording sites (facilitated and suppressed) were significantly changed when compared to control (**B**, $P=1x10^{-4}$, error bars show standard error across animals).

shown in Figure 29-A. Contralateral (left) somatosensory stimulation locations tended to be more facilitative (shown in the yellow box), while ipsilateral (right) somatosensory locations tended to be more suppressive (maroon box). Interestingly, this is opposite of the effects seen in V1 and GC, where contralateral locations induced more suppressive plasticity. Gustatory stimulation was also suppressive. Modulatory effects of paired acoustic and somatosensory stimulation of the right mastoid were generally suppressive

when compared to activity before stimulation, matching right mastoid plasticity data (Figure 29-B), and significantly more total changes were induced than control (Figure 29-B, $P<0.05$). Unfortunately, in our preliminary A1 experiments, we were unable to analyze data during paired stimulation due to problems with our recording equipment, which is why only data for right mastoid stimulation is available in this dissertation. We have since remedied these problems, and will perform experiments in the future to obtain a more complete dataset.

Discussion

In general, similarities in outcomes exist across cortical locations, but there are also many differences. For example, for paired stimulation that included a somatosensory component, contralateral somatosensory stimulation locations were suppressive (and ipsilateral facilitative) for gustatory and visual cortices, but the opposite was true for auditory cortex, and there was no real ipsilateral/contralateral relationship for olfactory cortex. Additionally, combining different sensory systems had different effects for most cortical areas, but the relationships varied depending on where neural activity was recorded. For example, in V1, paired stimulation with a gustatory or auditory component was facilitative, but in OC, paired stimulation with a gustatory component was facilitative while auditory was suppressive, and auditory stimulation was equally suppressive and facilitative in GC. This indicates that the relationships between different sensory systems are not consistent, and each relationship likely has a unique role in multisensory perception.

Plasticity results did not always match immediate modulatory results during stimulation. For example, paired stimulation in V1 was always facilitative during stimulation regardless of what paired combination was used, but some combinations induced suppressive plasticity while others induced facilitative plasticity when comparing activity before and after. Conversely, plasticity results in OC were similar to its immediate modulatory effects during paired stimulation. Differences may be mechanism-related, and more studies need to be performed to understand why these differences exist. Additionally, inconsistencies could be related to anesthesia. Ketamine has been shown to inhibit N-Methyl-D-Aspartate (NMDA) receptors (Gonzales et al. 1995; Hu and Davies 1997; Kim et al. 1996a; Silva et al. 1997), which have implications on the ability to induce plasticity, and studies have shown that ketamine can actually inhibit plasticity induction (Forsythe and Westbrook 1988) and reduce excitation (Hu and Davies 1997; Kaltenbach et al. 2000). These anesthesia effects could also explain why even the most suppressive or facilitative stimulation combinations still induce opposite effects in a smaller percentage of recording sites, and awake studies could confirm whether or not cleaner results are attainable.

One interesting observation in our data is that changes in the control were higher for somatosensory, visual, gustatory, and olfactory cortices than for auditory cortex. One reason for this could be that auditory cortex is less plastic to repeated, single stimuli than other cortical sensory areas (since out controls include single-sensory stimulation for firing rate measurements). Another possible explanation is that we used acoustic stimuli

to measure cortical activity for auditory cortex, which is a much more natural stimulus than the electrical stimulation used for gustatory, olfactory, and somatosensory cortex. Perhaps electrical stimulation alone induces more plasticity for these cortices than a natural stimulus in auditory cortex, since electrical stimulation may be activating more fibers/receptors simultaneously (see Appendix A for details on S1 responses to electrical stimulation versus tactile stimulation). Visual cortex may exhibit greater changes because the animal is in a dark room receiving a bright light stimulus, which may be more disruptive than 70 dB SPL broadband noise due to differences in contrast between stimulation and the quiet environment preceding stimulation. It would be interesting to compare electrical stimulation controls in this study to controls using electrical stimulation of the cochlea in auditory cortex for comparison. Electrical stimulation could also be replaced with a more natural stimulus for gustatory, olfactory, and somatosensory cortex recordings, but it would be difficult to implement a short, precise taste or odor stimulus, making a direct comparison fairly difficult.

We have observed that combining stimulation of different sensory systems induces a wide range of significant cortical plasticity effects and can elicit immediate modulatory effects on cortical responses to normal stimuli alone. This happens in all five of the major sensory cortices, indicating widespread multisensory integration between them and revealing a strong ability of multisensory pathways to change or modulate neural activity. This is an interesting finding from a neuroscience perspective, but the ability to induce random plasticity alone is not as useful from a clinical perspective. Of

high importance is the consistent and unique plasticity and modulatory results of each paired stimulation combination. Because some paired stimulation paradigms induce more suppression than facilitation while others induce more facilitation than suppression, and because ratios of facilitation/suppression vary across parameter sets in all five sensory cortices, we can strategically select parameters to attempt to achieve a specific neuromodulatory outcome. It is also likely that different combinations of paired stimulation will target different groups of neurons in the same cortical area, which will allow us to control the location of plasticity in addition to the type, amount, and timing thereof.

These findings are potentially promising for the neuromodulation treatment of neural sensory disorders in patients. Most neural sensory disorders, including tinnitus, phantom pain, and some types of chronic pain, are patient specific (Diatchenko et al. 2005; Møller et al. 2010; Whyte and Niven 2001), where each individual has a unique set of symptoms (e.g. different tinnitus pitch/location, different pain locations, throbbing vs. constant pain/tinnitus) which likely arise from unique groups of neurons and/or unique abnormal firing patterns. There is also an ongoing hypothesis that sensory disorders such as tinnitus and pain are driven by abnormal networks across multiple brain regions, and disrupting or modulating one or several nodes of this network could potentially treat the sensory disorder or corresponding symptoms (De Ridder et al. 2011a). Variabilities across patients make it difficult to treat all patients with the same approach, which is one reason why treatment outcomes for such neural disorders are inconsistent. However, with

the ability to target different groups of neurons with differential effects and different intensities of responses, and with an extremely large parameter space (there are many body locations, acoustic frequencies, tastes, smells, visual locations/colors/orientations, etc.), multisensory paired stimulation might be adaptable to each patient for more consistent outcomes and a higher percentage of successful treatments.

For example, tinnitus is characterized by hyperactivity and hyper-synchrony across neurons throughout the auditory system (Eggermont and Roberts 2004; Henry et al. 2014; Kaltenbach 2011; Lanting et al. 2008; Lanting et al. 2009; Levine et al. 2003), but variability across patients leads to inconsistent outcomes (Møller et al. 2010). Since each tinnitus patient hears different tinnitus pitches and types of sounds, it is likely that different groups of neurons in the auditory system are contributing to the percept in unique ways. Treatments which cannot target specific neurons can only have limited success in such a varied patient population, but a neuromodulation treatment with a large parameter space to target a variety of neurons in different ways might be more adaptable to individual cases. Furthermore, because symptoms vary, it's likely that different patterns of hyperactivity and hyper-synchrony exist across patients, so the ability to suppress with different intensities, or the ability to suppress some neurons while facilitating others to break up synchrony, might be useful. We have shown the ability to do this in every sensory cortical area in this study, which means multisensory neuromodulation might be helpful in many different sensory disorders, including tinnitus and pain. Additionally, because multisensory paired stimulation is simple and

noninvasive, it would be available to a large patient population without the safety and cost limitations of invasive treatments.

The concept of neural beamforming may also provide a noninvasive approach to treating neural sensory disorders. Neural beamforming involves the combination of many different stimuli at specific timing delays to converge on one particular brain target simultaneously. In this study, we have only combined two sensory stimuli at a time without testing different inter-stimulus delays, and our results are still relatively promising. If latencies and onset delays are investigated further, and if more sensory stimuli are combined (either by using more sensory systems or by combining multiple stimuli from the same sensory system), results may become even more controllable and/or adaptable to patients. Perhaps the concept of neural beamforming could be applied to other neuromodulation treatments as well, with the potential to shift patient outcomes, or at the very least, yield another stimulation option for patients that don't respond to other treatments.

Future Studies

In addition to future studies mentioned earlier in this discussion, more experiments could be performed to make this study more complete. Visual stimulation could be added as a paired stimulation option for all other sensory cortices. Olfactory stimulation could also be added for the auditory cortex plasticity study, as well as other somatosensory locations that were omitted in those preliminary experiments. Adding olfactory and gustatory stimulation to paired stimulation for S1 plasticity is trickier, as

electrical gustatory and olfactory stimuli naturally activate somatosensory receptors as well, and it becomes difficult to control the precise timing of natural non-electrical gustatory and olfactory stimuli. Additionally, different inter-stimulus delays should be attempted in all experiments, since latencies of different stimuli to the same cortical location vary. Previous studies have shown that the timing of bimodal stimulation can have a significant effect on whether activity is suppressed or facilitated (Caporale and Dan 2008; Markovitz et al. 2015; Tzounopoulos et al. 2007; Wu et al. 2015), and this needs to be considered if latency differences could be contributing to results and biasing our findings toward some stimulation combinations more than others. From a practical perspective, it is clear that the large parameter space provides many options to optimize treatment for each individual, but future studies will need to identify methods and biomarkers that can rapidly identify the best parameters, or at least reduce the parameter space to a manageable level for a more practical patient optimization.

Chapter 7: A Detailed Investigation of Somatotopic Trends in the Inferior Colliculus: Implications for Tinnitus Treatment

The long-term goal of mSync for treatment of sensory neural disorders is to systematically modulate specific populations of neurons that exhibit abnormal coding properties driving undesired symptoms. In this chapter, we investigated the ability to target specific neural subpopulations with mSync for the efficacy of treating patient-specific sensory disorder symptoms. We searched for a somatotopic map of body locations in the IC, a multisensory hub in the auditory pathway, which would enable us to target a variety of auditory neural subpopulations using somatosensory stimulation relevant for tinnitus treatment.

Summary

The inferior colliculus (**IC**) is an auditory structure in the midbrain functioning as a multisensory hub, integrating inputs from several auditory, visual, somatosensory, motor, limbic and cognitive nuclei. Although previous studies have demonstrated multimodal integration within the IC, especially in its outer shell, none have demonstrated a map of other sensory or motor features. Since we have better tools, multi-site arrays, and brain reconstruction techniques than those previous studies, we reinvestigated the existence of a somatotopic map in anesthetized guinea pigs. We initially focused on somatosensory inputs to the IC because they are excitatory, making them easier to characterize, and we have already investigated plasticity/modulatory effects of auditory/somatosensory interactions in auditory cortex in previous chapters of

this dissertation. Encouragingly, we discovered somatotopic trends across the dorsal surface of the IC, with a lateral-to-medial orientation of a nose-to-toe body representation and a rostral-to-caudal orientation of a left-to-right body representation. Latencies were also analyzed, and while there is no clear topography of somatosensory latency distribution across the IC, somatosensory stimulation locations with shorter latencies are more prevalent in lateral areas, which is where acoustic-driven latencies are shorter according to previous studies. Rate-level functions are generally linear for all somatosensory stimulation locations. Finally, an analysis of minimal-spread activation for somatosensory stimulation reveals that specific areas of the IC can be targeted by strategically choosing somatosensory stimulation locations. When combined with acoustic stimulation, somatosensory stimulation can also induce somatosensory stimulation location-specific facilitative or suppressive plasticity. Overall, these findings demonstrate that multisensory integration within a given sensory nucleus, such as somatosensory inputs into the auditory midbrain, are much more systematically organized than previously thought. In terms of clinical implementation, the ability to target specific sub-populations of neurons in the auditory system with multisensory stimulation while controlling facilitative and/or suppressive plasticity may be useful in treating neural sensory disorders such as tinnitus.

Introduction

The inferior colliculus (**IC**) is an auditory midbrain structure that acts as an auditory processing center and a relay between the cochlear nucleus and the thalamus

(Aitkin and Phillips 1984; Casseday et al. 2002; Ehret 1997). The IC consists of multiple sub-regions, including the central nucleus (**ICC**), the dorsal cortex (**ICD**), and the external nucleus (**ICX**), all of which have different roles and properties. The ICC is a part of the core auditory pathway and is characterized by tonotopy (Malmierca et al. 2008; Oliver 2005; Snyder et al. 2004) and short acoustic-driven latencies (Lumani and Zhang 2010). The ICD and ICX have broader tuning, longer latencies, and larger latency jitter (Barnstedt et al. 2015; Lumani and Zhang 2010), and are often tied to sound localization (Binns et al. 1992; Huffman and Henson 1990; Knudsen and Knudsen 1983) and attention (Jane et al. 1965), with ICX neurons responding better to broadband noise than pure tone stimulation (Aitkin and Phillips 1984). The ICD has been found to be innervated by both ascending and descending auditory projections from the cochlear nucleus and thalamus (Coleman and Clerici 1987; Oliver 2005). Overall, ICX neurons have shorter latencies than ICD neurons (Syka et al. 2000), and in general, lateral neurons have shorter latencies than other non-ICC areas (Langner et al. 2002; Schreiner and Langner 1988). In fact, in addition to an acoustic-driven threshold map (Stiebler 1986), the IC has been characterized with a latency map (Hattori and Suga 1997) and maps for duration and latency jitter (Straka et al. 2014).

Despite the detailed characterization of acoustic-driven activity in the IC, little has been done to characterize somatosensory-driven activity in the auditory system in general. Studies have shown multimodal integration in the IC (Aitkin et al. 1978; Aitkin et al. 1981b), including inputs from the somatosensory, visual, and limbic systems (Coles

and Aitkin 1979; Gruters and Groh 2012; Schofield et al. 2011b; Winer 2005), but little has been done to understand the organization of such interactions. Despite the existence of somatotopic maps in other subcortical sensory areas like the superior colliculus (Meredith and Stein 1986), such a map has not been found in the IC during simple preliminary studies (Aitkin et al. 1978; Aitkin et al. 1981b), but no one has investigated this thoroughly or recently.

Multisensory integration, which is essential for comprehending sensory inputs into a single perception, has been found in many places in the brain (Ghazanfar and Schroeder 2006; Murray and Wallace 2011; Stein and Stanford 2008). For example, interactions between the auditory, visual, and somatosensory systems have been shown to affect neural activity in the visual cortex (Dehay et al. 1988; Miller and Vogt 1984; Sadato et al. 1996), the superior colliculus (Drager and Hubel 1975; King and Palmer 1985; Wallace et al. 1998), and the occipital temporal cortex (Beauchamp 2005), all of which are traditional yet integrative visual areas. Other interactions have been shown to modulate activity in the gustatory and olfactory systems (de Araujo and Simon 2009; Demattè et al. 2006; Simon et al. 2008). Specifically in the auditory system, visual stimulation has been shown to activate auditory cortex (Calvert et al. 1997), especially when interacting with an auditory stimulus (Ghazanfar et al. 2005), and olfactory stimulation can also modulate auditory cortical activity (Cohen et al. 2011). The somatosensory system has been shown to have strong influences on the auditory system, as shown in Figure 1 in Chapter 1 of this dissertation. Somatosensory stimulation alone

has been shown to induce or change plasticity in auditory cortex (Ma and Suga 2003), and combined auditory and somatosensory stimulation has been shown to modulate auditory activity (Foxe et al. 2000; Foxe et al. 2002; Kayser et al. 2005), enhance neural firing (Murray et al. 2005), and induce plasticity in the auditory system (Gloeckner et al. 2013; Markovitz et al. 2015; Wu et al. 2015).

Based on all of this background, it is of no surprise that multisensory stimulation has been occasionally used to attempt to treat some neural disorders through plasticity induction. For example, mirror therapy for phantom limb pain takes advantage of visual and somatosensory/pain interactions to treat symptoms (Chan et al. 2007). Others have tried to take advantage of somatosensory and auditory interactions to treat tinnitus (Dehmel et al. 2008b; Gloeckner et al. 2013; Levine et al. 2003; Markovitz et al. 2015). Tinnitus is a neural sensory disorder characterized by a phantom sound perception (ATA 2010) and linked to hyperactivity and hyper-synchrony in the auditory system (Eggermont and Roberts 2004; Henry et al. 2014; Kaltenbach 2011; Lanting et al. 2008; Lanting et al. 2009; Møller et al. 2010). Previous studies have investigated the treatment of tinnitus with neuromodulation, including invasive deep brain stimulation (Cheung and Larson 2010), noninvasive transcranial magnetic stimulation (De Ridder et al. 2011b), and other invasive and noninvasive modalities (Vanneste and De Ridder 2012), all with varied results due to a high pathological variance across tinnitus patients. Neuromodulation has been used to successfully treat other neural disorders (Engineer et al. 2011; Johnson et al. 2013), and sensory stimulation is one attempted modality,

including trigeminal nerve stimulation for epilepsy (DeGiorgio et al. 2013) and depression (Cook et al. 2013). Perhaps neuromodulation that takes advantage of multisensory stimulation might be useful for tinnitus treatment with the correct modality and parameter selection.

However, in order to understand how to attack the tinnitus problem with multisensory neuromodulation, we need to better understand multisensory activity in the auditory system. As a primary location of somatosensory interactions, the IC could give clues for auditory plasticity induction if its somatosensory inputs are better characterized. Given the high variance in symptoms and outcomes in tinnitus patients, the ability to target specific neural sub-populations within the IC for a variety of plasticity outcomes may be essential for the treatment of a large portion of the tinnitus population. This study examines the possibility of a somatotopic map in the IC, characterizes somatosensory-driven excitation, investigates the ability to target sub-populations of neurons across the IC using different somatosensory stimulation locations, and attempts to induce controllable differential plasticity effects with paired auditory and somatosensory stimulation in IC neurons, all of which might be useful for understanding how multisensory neuromodulation can affect tinnitus.

Materials and Methods

Overview

Experiments were performed on 17 young female Hartley guinea pigs (400–450 g; Elm Hill Breeding Labs, Chelmsford, MA) anesthetized with an initial intramuscular injection of a ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL) mixture, with varying supplements every 45–60 minutes to maintain an areflexive state. Neural recordings were performed inside a small electrically-shielded and acoustic-attenuating booth using hardware from Tucker-Davis Technology (Alachua, FL), and neural signals were processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Surgery and Neural Recordings

A craniotomy was performed revealing the visual cortex on the right side of the guinea pig brain, and a 32-site recording electrode array (NeuroNexus Technologies, Ann Arbor, MI) was inserted through the visual cortex and into the IC. The recording electrode ground was either inserted into the neck of the animal or another part of the brain unrelated to sensory systems, depending on background electrical noise levels. Heart rate and blood oxygen content were monitored using an H100 pulse oximeter from EdanUSA (San Diego, CA), and body temperature was monitored using an Oakton Acorn series JKT thermocouple rectal probe (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C

using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN). The animal was fixed into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars.

Recording electrode arrays consisted of four 10-mm long shanks (site area approximately 413 μm^2) with eight recording sites per shank and a site spacing of 200 µm. Recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine wave. Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of neural spike activity. A detection threshold of 3.5 times the standard deviation of the voltage noise floor was used to determine when spikes occurred, and spike voltage waveforms were visually inspected to ensure that no noise was falsely detected.

Recording Electrode Placement

Recording electrode arrays were inserted through the visual cortex and into the IC (approximately 5-6 mm deep, depending on the animal). Broadband noise acoustic stimulation (50 ms duration, 0.5 ms rise/fall time, 70 dB SPL, equal energy between 625 Hz and 40 kHz) was performed using a speaker (Tucker-Davis Technology, Alachua, FL) coupled to the left ear bar, and functional responses were used to confirm placement in the IC. The speaker-ear bar system was calibrated using a 0.25 in. condenser microphone (ACO Pacific, Belmont, CA). Once neural responses to broadband noise could be observed, the electrode was removed and dipped in a red fluorescent dye (1, 1-

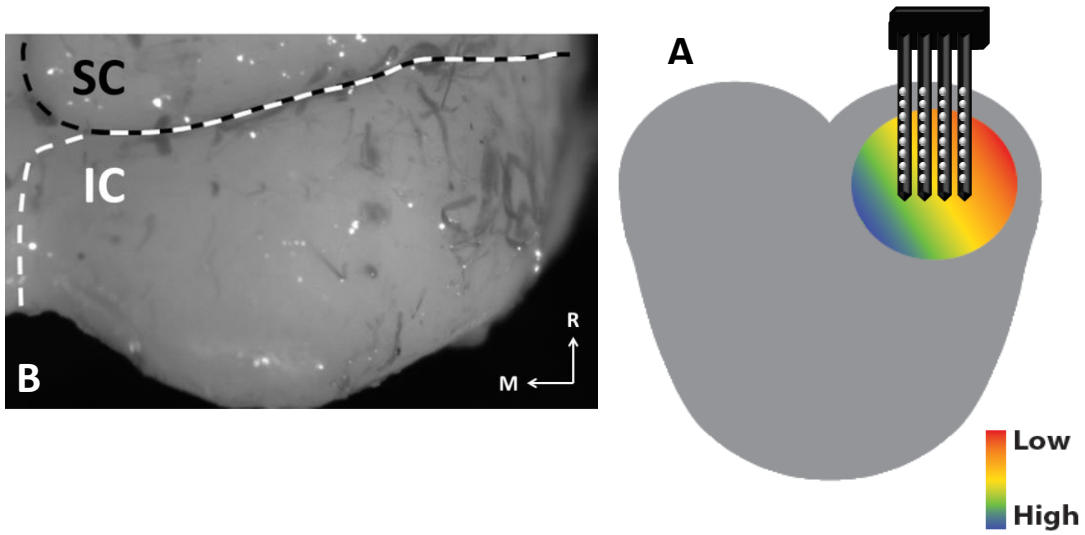


Figure 30: Recording methods

A: Michigan-style 4-shank electrode arrays were inserted into the right IC, spanning both the central nucleus (ICC, indicated in color) and external region (ICX, gray area surrounding ICC). The array was inserted in the dorsal/ventral direction, which is not parallel to the frequency axis (frequency bands are shown with different colors, where red represents the lowest frequencies and blue represents the highest frequencies). **B**: Responses to stimulation were mapped onto a picture of the dorsal IC surface, which shows the border between the IC and the superior colliculus (SC).

dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate; Sigma-Aldrich, St Louis, MO) such that electrode locations could be identified later through histology. Afterward, the electrode was repositioned in the same location and inserted until broadband noise induced responses on some recording sites but not others. The probe was then inserted further in 10 µm increments until broadband noise elicited responses in one additional recording site, at which point insertion ceased immediately. According to a recent study,

acoustic-driven responses can be found merely 40 µm from the surface of the IC (Ito et al. 2014), which is smaller than the error margin of our histological methods, so the border of the IC was deemed to be located at the shallowest recording site that exhibited responses at its current position, similar to methods used in previous studies (Markovitz et al. 2012; Markovitz et al. 2013; Offutt et al. 2014). The electrode was then inserted such that the shallowest recording site on each shank was located at the IC border. At this point, the shallow sites were located in the ICD (or possibly the ICX for the lateral-most IC locations), and deeper sites were either in the ICC, ICX, or deeper parts of the ICD depending on the location (example placement illustrated in Figure 30-A). This process was repeated for each IC electrode placement, and placements were completed in a grid-like fashion that spanned the entire IC, such that results could be mapped onto a picture of the surface of the IC (example surface picture in Figure 30-B).

The precise functional location of each recording site was verified using frequency response maps (FRMs) to determine which region each recording site resided, similar to previous studies (Lim and Anderson 2007a; Markovitz et al. 2013; Offutt et al. 2014; Straka et al. 2014). For FRMs, pure tone stimulation (1-40 kHz with 8 tones/octave, 0-70 dB-SPL in 10 dB steps, 4 trials each, 2/second in a random order) was presented to the animal's left ear to map the tuning and thresholds at each recording site. ICC sites exhibited a tonotopic gradient with sharp tuning (Snyder et al. 2004), while ICD and ICX sites exhibited broad tuning with no tonotopic gradient.

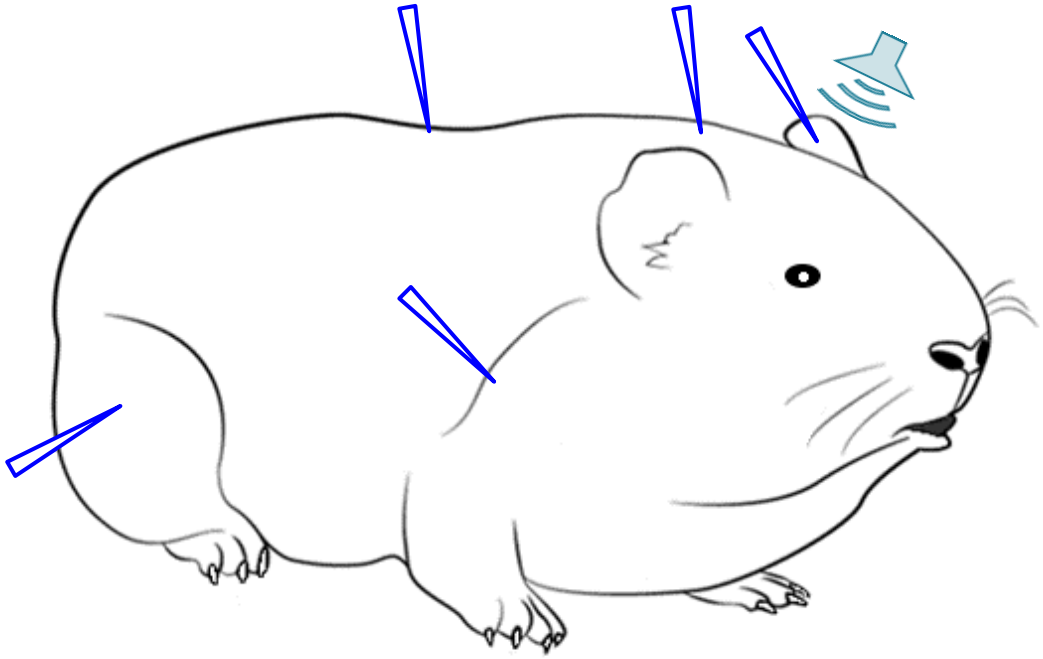


Figure 31: Stimulation methods

Electrical stimulation was performed on various somatosensory locations, including the neck, left and right shoulder, back, left and right leg, and left pinna (left shoulder and leg are not shown on this diagram). Auditory stimulation in the left ear was used to confirm locations in the IC.

Somatosensory Stimulation

Somatosensory simulation locations are shown in Figure 31 and include the left pinna, neck, left and right shoulders, back, and left and right legs (for all somatosensory stimulation locations that were stimulated on both sides of the body, the left sides are not shown in the figure). The pinna electrode was placed on the surface of the center of the pinna, and subcutaneous needle electrodes (Rhythmlink International LLC, Columbia,

SC) were used to stimulate other somatosensory locations. The pinna electrode was placed on the surface of the center of the pinna. The neck electrode was inserted subcutaneously halfway between the ears and the shoulder joints centrally. The shoulder electrodes were inserted dorsal of the shoulder joints, and the back electrode was inserted along the spine halfway between the neck electrode and the end of the spine. The hind leg electrodes were placed laterally halfway between the hip joint and the knee joint. For all stimulation locations, the stimulation ground was distributed between electrodes placed subcutaneously in the left and right arm and in the left and right hind leg, as spreading the ground across these four locations mitigated unintended activation of ground areas (control experiments were performed to confirm that no ground areas elicited activation in the IC at the highest stimulation current levels).

General Protocol for Mapping Study

At each IC recording location, an FRM and 100 trials of broadband noise were recorded to confirm the functional location of the electrode array. Afterward, electrical somatosensory stimulation (biphasic, 205 μs per phase, 50 trials, 2/second) of the skin was performed at each somatosensory location at nine different current levels (110-710 μA in 2 dB steps relative to 1 μA). Trials were randomized across all stimulation locations and all levels in order to mitigate cumulative effects, and 50 trials of spontaneous activity were also recorded within the randomization. Finally, another 100 trials of broadband noise were recorded, and broadband noise recordings before and after were compared to ensure that the IC was still functioning following the lengthy

somatosensory stimulation cycle. For each stimulation location/level, activity was windowed 5-55 ms after the stimulus and compared to spontaneous activity using Signal Detection Theory (Green and Swets 1966) to determine if spike activity was significantly increased (d'=1), similar to previous studies (Lim and Anderson 2007b; Offutt et al. 2014). The lowest level for which response activity was significant (and also for all higher levels) was determined to be the activation threshold for a given somatosensory stimulation location. 10 animals were used for this study, and the order of IC placement locations was randomized across animals to mitigate cumulative effects and the potential effects of time under anesthesia.

General Protocol for Plasticity Study

At each IC recording location, 100 trials of broadband noise were presented to the animal. Afterward, paired multisensory stimulation, in which electrical somatosensory stimulation (0.63 mA) preceded broadband noise acoustic stimulation (70 dB) by 5 ms, was performed for 1000 trials (2/second). Finally, 100 trials of broadband noise were presented again. To test for plasticity, acoustic-driven responses before paired stimulation were compared to those after to determine if paired stimulation had induced significant changes in spike activity (two-tailed, unequal variance, ranked t-test, $P<0.01$). Seven animals were used for this study.

IC Histological Reconstructions

We used a previously developed histological reconstruction method (Markovitz et al. 2012) to determine IC recording electrode placement locations and the relationships between locations across animals. Following each experiment, the animal was decapitated, and the head was submerged in a 3.7% paraformaldehyde solution for 2-3

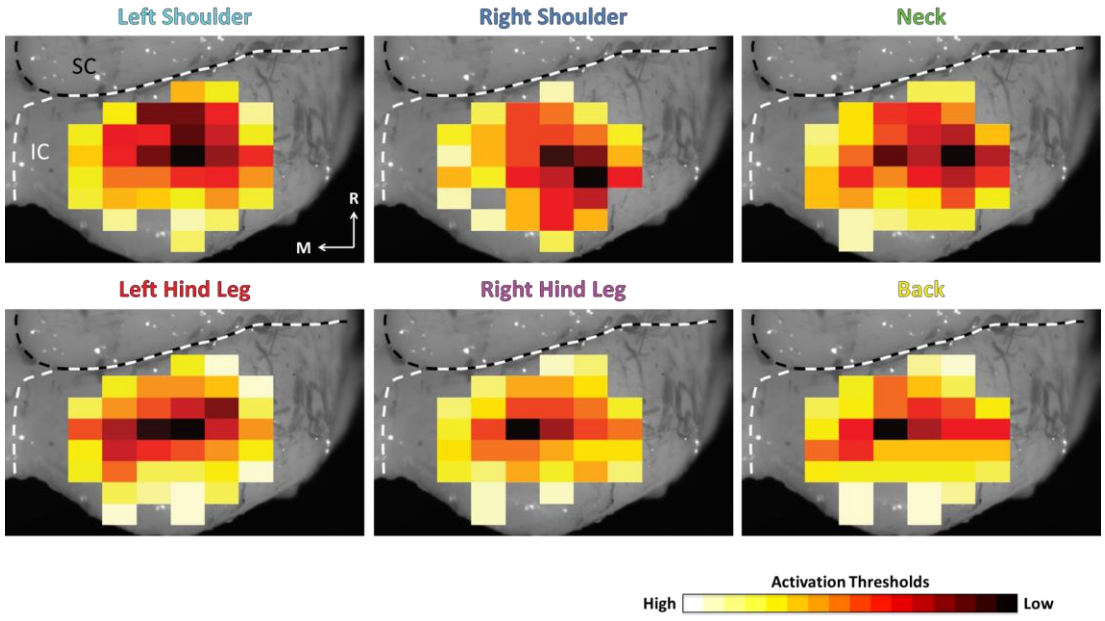


Figure 32: Threshold maps for individual somatosensory locations.

The threshold of activation, defined as the lowest electrical stimulation level that induces a significant excitatory response at a given recording location, is shown for each of six somatosensory stimulation locations: left shoulder, right shoulder, neck, left hind leg, right hind leg, and back. All six maps are normalized to the lowest threshold, such that the darkest colored IC recording location had the lowest threshold of all recording locations for a given stimulation location. All stimulation locations elicited responses in almost every IC recording location, but threshold maps were different for each stimulation location.

days. Afterward, the brain was removed from the skull and replaced in the solution for another 2-3 days before the IC was cut out and placed in sucrose. One day later, the IC was cryo-sliced (60 μm thick slices), and the slices were imaged under a fluorescent microscope. Using Rhinoceros software (Seattle, WA), a 3-D reconstruction of the brain and electrode placements was generated, and reconstructions of all brains were normalized based on previously established histological procedures, with a spatial error margin of approximately 100 μm (Markovitz et al. 2012). Adjustments for fixation shrinkage were included in this process.

Results

Mapping Study

Activation thresholds for each stimulation location at each IC location were calculated, and a summary map was positioned on the surface of the IC, with the lowest threshold on each recording electrode shank represented on the map. We created multiple identical rectangular column boundaries of the IC mapped in a grid-like fashion, such that one electrode shank location for each animal was included in each rectangular region based on normalized IC histological reconstructions, and thresholds were averaged across animals for each stimulation location in each rectangular IC region for the simplicity of visually presenting the data. The average thresholds of each region are shown for each stimulation location across the surface of the IC in Figure 32, where darker colors represent lower thresholds and lighter colors represent higher thresholds. Thresholds are

normalized to the lowest threshold across the entire IC for each stimulation location. IC locations that were not activated in any animals for a given stimulation location are omitted for each plot.

The threshold maps reveal two main points. First, with a high enough stimulation current, it seems that all somatosensory stimulation locations could activate nearly any given IC location, as only a few perimeter locations are omitted on some plots. Second, in

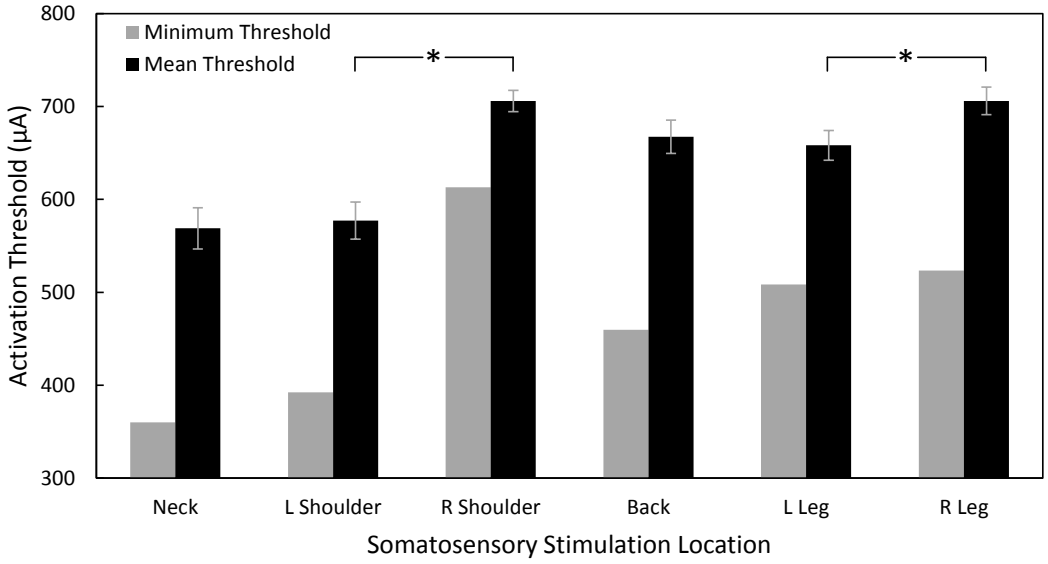


Figure 33: Minimum thresholds of activation for different somatosensory stimulation locations

Absolute minimum (grey) and average (black) thresholds across the entire IC are shown for each somatosensory stimulation location. Error bars show standard error across all IC locations. Left shoulder stimulation had a significantly lower mean threshold than right shoulder, and left leg stimulation had a significantly lower mean than right leg ($P<0.05$, indicated by asterisk).

general, central IC locations tended to have lower thresholds across all somatosensory stimulation locations on average, while perimeter locations generally had higher thresholds. However, while this general trend exists, threshold maps still exhibit some topographic trends across somatosensory stimulation locations. For example, neck stimulation induced lower thresholds in more lateral IC locations, while back and hind leg stimulation induced lower thresholds in more central and medial IC locations. Left shoulder stimulation activated more rostral IC areas at lower thresholds, while right shoulder stimulation with lower thresholds were focused more in caudo-lateral IC areas.

There were also trends in absolute lowest threshold across somatosensory stimulation locations (Figure 33, minimum thresholds all recording sites are shown in black, while the means across all recording sites are shown in grey). We observed that the neck generally had the lowest thresholds, while there was a wide variability in thresholds across the other somatosensory stimulation locations. Interestingly, the thresholds of left shoulder stimulation were statistically significantly lower than right shoulder (standard t-test, $P<0.05$), and the same is true for left and right hind leg stimulation.

Since somatosensory stimulation locations activate nearly all IC locations at some threshold, these trends cannot be easily detected with simple threshold maps. For a better visual representation of the data, we found the difference between left shoulder thresholds and right shoulder thresholds for each IC location and plotted them to determine if a trend exists (Figure 34-A). Red locations indicate that left shoulder stimulation had a lower normalized threshold than right shoulder stimulation, and blue

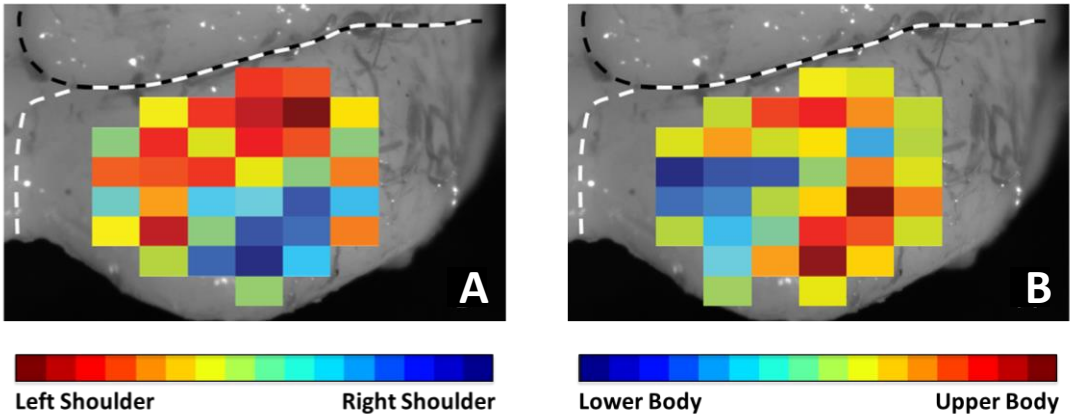


Figure 34: Trends in IC somatosensory stimulation threshold maps

Comparisons for left shoulder vs. right shoulder stimulation (**A**) and upper body vs. lower body stimulation (**B**) are shown. In A, for each IC recording location, the lowest threshold across all upper body stimulation locations (neck and shoulders) and the lowest threshold across all lower body stimulation locations (back and hind legs) was found. The difference between these thresholds was plotted for each IC location, and the colors represent the magnitude of this difference, such that blue locations indicate that the lower body threshold was lower than the upper body threshold, and vice versa for red locations. Similar calculations were performed for left shoulder vs. right shoulder in B. Upper body regions generally achieved lower thresholds of activation for more lateral IC sites, while lower body regions achieved lower thresholds in more medial IC sites. The right shoulder region achieved a lower activation threshold of IC sites caudal-lateral to those of the left shoulder. Overall, a map of the body of the guinea pig appears to be superimposed onto the IC in a head-to-toe orientation across the rostral-lateral to caudal-medial axis.

locations indicate that right shoulder stimulation had a much lower threshold than left shoulder stimulation. A spatial trend is evident in this visualization, as lower left shoulder thresholds dominate rostral IC areas while lower right shoulder threshold areas are more prevalent in caudo-lateral areas. We performed similar calculations for upper body versus lower body areas, where the lowest threshold across all upper body stimulation locations (neck and shoulders) was compared to the lowest threshold across all lower body stimulation locations (back and hind legs) for each IC recording location (Figure 34-B). Again, a spatial trend exists, as lower body stimulation locations had lower thresholds in medial IC regions while upper body locations dominated lateral IC areas.

Minimal-spread Activation Analysis

To further characterize the ability to target specific IC locations with somatosensory stimulation, we calculated what we have coined "minimal-spread activation". For a given IC location of interest, this term is defined as the overall total area of the IC that a given somatosensory stimulation location will activate if it is stimulated at its threshold for the IC location of interest. This calculation is illustrated in Figure 35. For a given IC location of interest (shown in yellow in A), we can determine the threshold for all somatosensory stimulation locations using the threshold maps in Figure 32 (for the example in Figure 35-B, only right shoulder and left hind leg are shown for simplicity). Note that it doesn't matter if we use absolute threshold or normalized threshold, since we will only be comparing these values to thresholds of the same somatosensory location. Using the threshold maps, we can then determine how

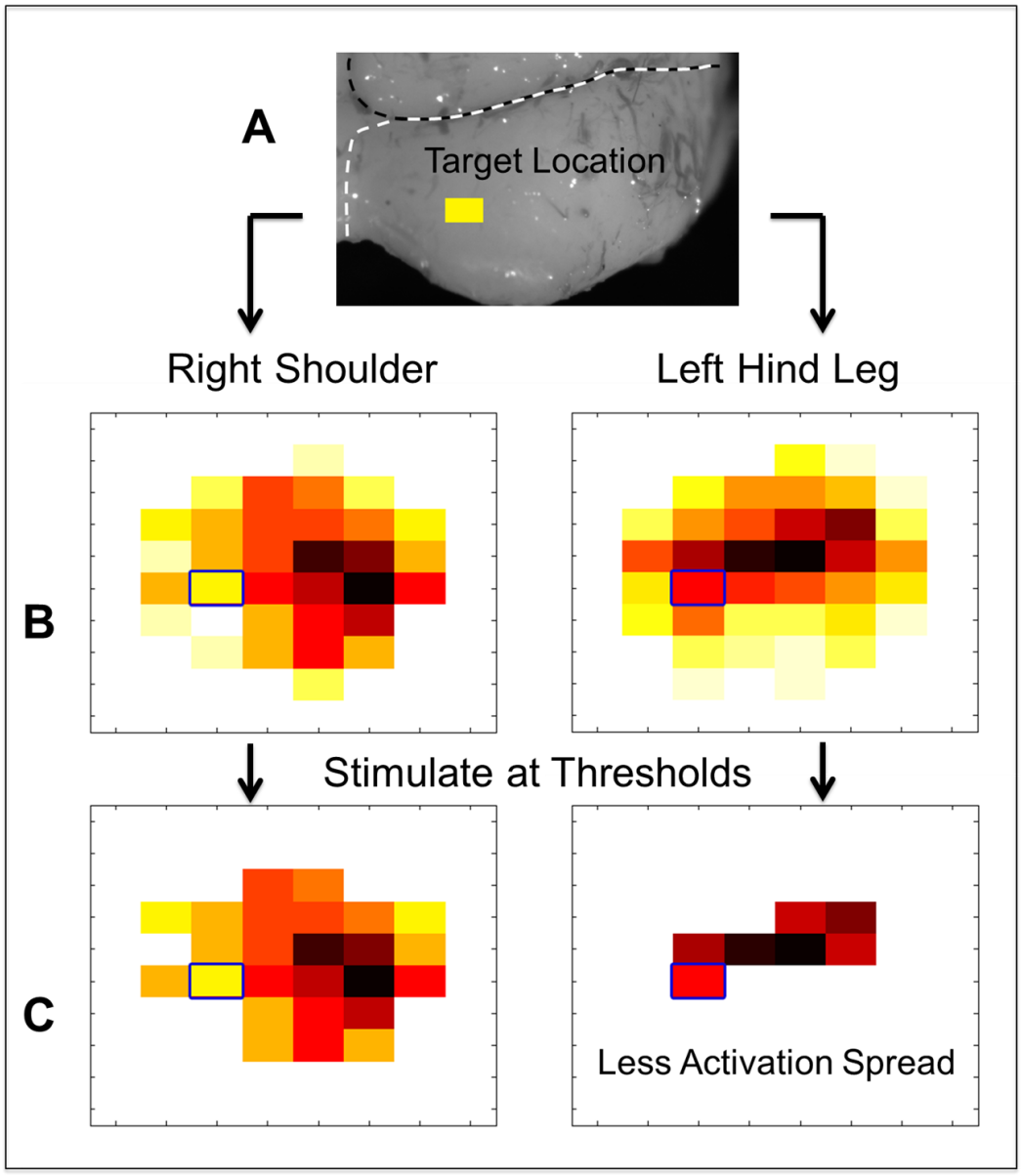


Figure 35: Calculation of minimal-spread activation

For a given recording location, the percentage of other recording locations across the IC that had activation thresholds at or below the threshold for a given somatosensory stimulation location was determined. This percentage indicates the fraction of recording locations that are activated if this somatosensory stimulation location is used to activate the desired

recording location at its threshold. For example, if the target recording location is indicated in **A**, and **B** shows the activation maps of two somatosensory stimulation locations for that location, then **C** shows which IC locations will be activated if the target recording location is activated at its threshold for each somatosensory stimulation location. For data analysis, we performed this calculation for all six of the stimulation locations shown in the threshold maps (only two are shown in the figure to illustrate the calculation).

many total IC locations will be activated by counting the number of locations with thresholds less than or equal to the threshold at the IC location of interest (shown in Figure 35-C, where only locations with a color equal to or darker than the location of interest are shown). From this, we can approximate the area of minimal-spread activation when the IC location of interest is activated using a given somatosensory stimulation location. In the Figure 35 example, left hind leg stimulation would activate a much smaller area (seven total IC locations) when targeting the location of interest than right shoulder stimulation (25 total IC locations activated).

We calculated minimal-spread activation for all somatosensory stimulation locations at each IC location, and we determined the "most selective" somatosensory stimulation location which had the smallest minimal-spread activation for each IC location. The most selective stimulation locations are shown in Figure 36. Two important observations can be taken from this plot. First, each somatosensory stimulation location is localized to a specific part of the IC. For example, neck stimulation is only observed in the lateral-most IC areas, and right shoulder stimulation is finely constrained to caudo-

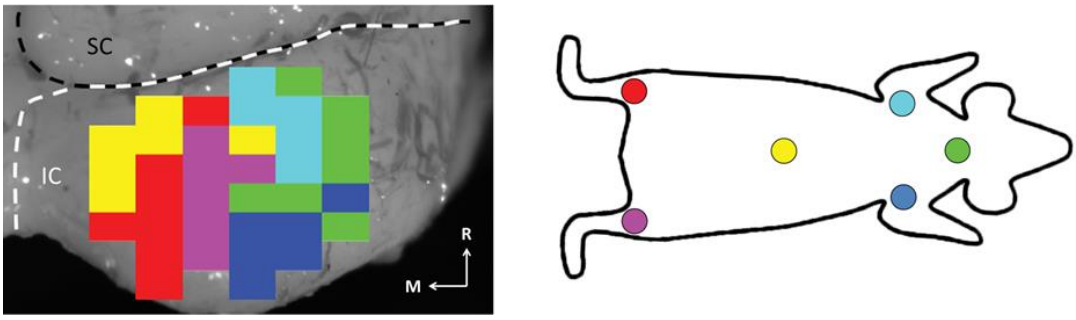


Figure 36: Minimal-spread activation map in the inferior colliculus

For each IC recording location, the somatosensory stimulation location with the lowest minimal-spread activation is shown (neck is green, left shoulder is cyan, right shoulder is blue, back is yellow, left hind leg is red, and right hind leg is purple). In general, recording sites with the same most selective minimal-spread activation stimulation location are localized to a specific area of the IC. Somatosensory stimulation locations are clustered together, indicating that specific somatosensory stimulation locations best correlate with specific areas in the IC. These clusters exhibit a somatotopic organization similar to those observed for the threshold maps, where the body of the guinea pig appears to be superimposed onto the IC in a head-to-toe orientation from the lateral portion of the IC to the medial portion.

lateral IC. Second, clear somatotopic trends can be observed, where upper body locations dominate lateral IC areas while lower body locations dominate medial IC, and the left shoulder is localized directly rostral of the right shoulder. In fact, a guinea pig schematic can be superimposed on top of the IC map, and it matches up well, although the lower body areas are slightly rotated. This rotation may be due to an actual rotation in the map

(the original S1 homunculus is not perfectly straight), or it could be due to overlap in electrical stimulation body activation.

To further characterize minimal-spread activation trends, we performed calculations similar to those for Figure 34 by comparing areas. Differences between minimal-spread activation areas for upper body (neck and shoulders) and lower body (back and hind legs) stimulation were calculated and plotted (Figure 37-A), and similarly for left and right shoulder stimulation locations (Figure 37-B). For these maps, colors indicate which somatosensory stimulation location(s) had smaller minimal-spread

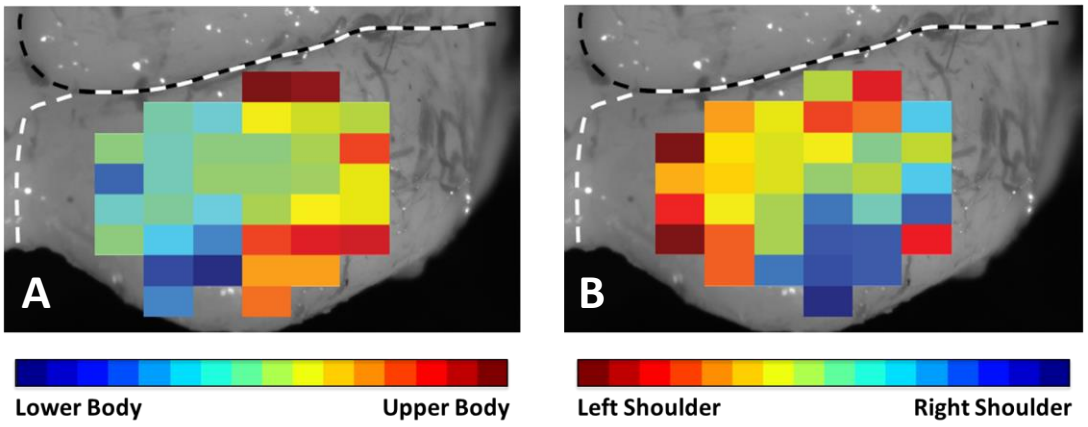


Figure 37: Minimal-spread activation trends in the inferior colliculus

Minimal-spread activation trends for upper body vs. lower body locations (**A**) and left shoulder vs. right shoulder (**B**) are shown, and calculations were the same as in Figure 31 except that the minimal-spread activation area was used instead of minimum threshold (colors indicate which body location(s) activated the smallest area of IC). Overall, a map of the body of the guinea pig appears to be superimposed onto the IC in a head-to-toe orientation across the rostral-lateral to caudal-medial axis, similar the results in Figure 31.

activations. The same trends from Figure 34 are seen here, as upper body regions have smaller minimal-spread activation areas in lateral IC locations while lower body regions have smaller areas in medial IC locations, and left shoulder dominates rostro-medial areas while right shoulder is localized to caudo-lateral areas.

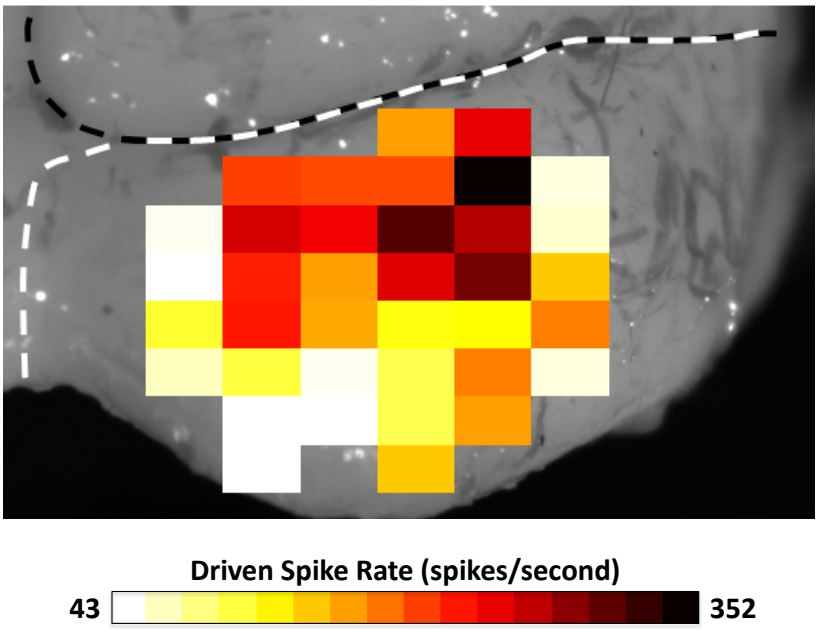


Figure 38: Driven spike rate map for left pinna stimulation.

An average driven spike rate (spontaneous activity is subtracted from total spike activity) was determined at each IC location in response to 710 µA stimulation. Spike rates tended to be greater in rostral and lateral IC locations, while there was less activation in medial and caudal locations. These results are consistent with what would be expected based on the somatotopic trends shown in previous figures, as left body locations better activated rostral IC locations and upper body locations better activated lateral IC locations.

Analysis of Spike Rates and Latencies

In addition to somatotopy, we also investigated somatosensory driven spike rates in the IC. In separate experiments, we investigated the driven spike rates of left pinna stimulation in the IC. We chose pinna stimulation because previous studies have shown vast pinna somatosensory inputs into non-cortical auditory areas (Kanold and Young 2001), and pinna somatosensory and proprioceptive inputs have been tied to sound

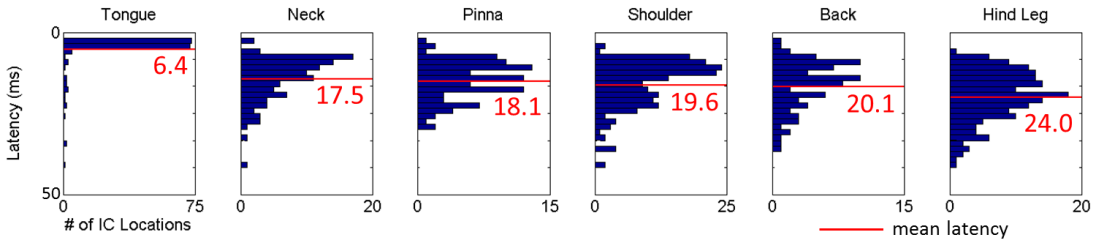
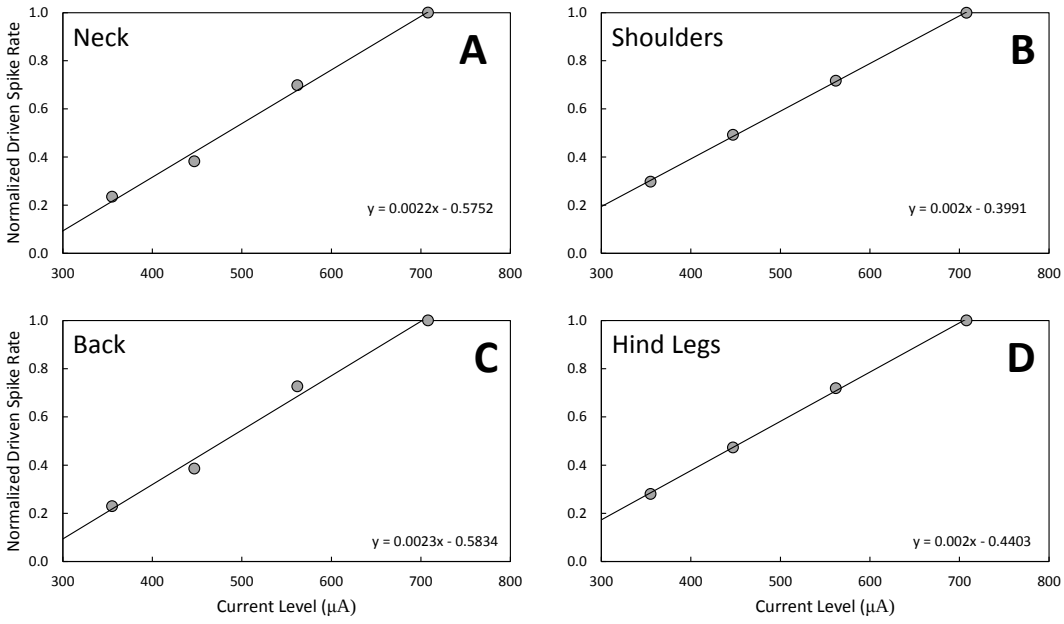


Figure 39: Latencies of somatosensory stimulation locations to the IC

Histograms of latencies for various somatosensory locations are shown. Each histogram includes one latency for each IC location, averaged across eight animals. The overall mean latency (labeled in red) is also shown for each stimulation location. Because there were no significant differences between the left and right shoulder latencies or between the left and right hind leg latencies, they are shown together. The distributions and means of latencies increased as the somatosensory location became further from the head of the animal (ANOVA F-test, $P<10^{-5}$). Tongue stimulation, which propagates through cranial nerves, had a much shorter latency than all other somatosensory locations, which project through the spinal cord.

localization (Neti et al. 1992; Young et al. 1996), to which the outer shell of the IC is linked in processing (Binns et al. 1992; Huffman and Henson 1990; Knudsen and Knudsen 1983). Driven spike responses to 710 μA pinna stimulation were analyzed across all IC locations, and the highest driven spike rate at each IC location is shown in Figure 38. Left pinna stimulation induced higher driven spike rates in rostro-lateral locations than anywhere else in the IC, and this matches somatotopic trends in IC from previous figures, as upper left body locations were localized in rostro-lateral areas.

Latencies and rate-level function were also characterized in the IC. While our data does not show a clear map of latency spatially across the IC, trends in latencies still exist. Histograms of latencies at different IC locations are shown for each somatosensory stimulation location in Figure 39, with mean values shown in red. Left/right shoulder and left/right hind leg histograms are combined together since they have similar latencies. Latencies for tongue stimulation (from a previous study) are short with low variance, and this is expected since responses to tongue stimulation travel through cranial nerves. For all other somatosensory stimulation locations that feed into the spinal cord, latencies exhibited a higher variance and the mean latency increased as somatosensory stimulation location distance from the brain increased. Interestingly, despite not exhibiting a clear latency map for any given somatosensory stimulus, IC somatosensory inputs with the shortest latencies (upper body areas) had lower thresholds in lateral IC regions, and previous studies have shown that lateral IC regions have shorter auditory-driven latencies (Langner et al. 2002; Schreiner and Langner 1988).



E

	Linear	Logarithmic	n = # Sites	RLF Slope
Neck	0.9933	0.9778	30	0.48
Shoulders	0.9997	0.9928	44	0.56
Back	0.9897	0.9811	12	0.51
Hind Legs	0.9994	0.9933	16	0.89

Figure 40: Rate-level functions for somatosensory stimulation in the IC

Average rate level functions for normalized driven spike rate are shown for stimulation of the neck (**A**), left and right shoulder (**B**), back (**C**), and left and right hind legs (**D**). IC recording sites were only chosen if they showed significant spike activity for at least the four highest current levels. For these sites, spike rates were normalized to the spike rate at the highest current level to reduce bias (differences in spike activity across levels in sites with greater spike rates would naturally receive more weight without normalization). These normalized rates were averaged across all sites for a given stimulation location. Because right shoulder and right hind leg had very few qualifying sites (n=2 for each), data for left and right shoulders and for left and right hind legs was combined. Spike rates for the highest

four current levels are plotted, and a linear trendline and its equation are shown for each stimulation location. R-squared fit values for the best linear and logarithmic fits are shown in **E**, along with the average slopes of the linear non-normalized rate-level functions (spike/μA after threshold). RLF intercepts are not shown since every location has a different threshold. The linear fit was better for all somatosensory stimulation locations, indicating that IC rate level functions for somatosensory stimulation are primarily linear.

Rate-level functions, which show the relationship between stimulation current level and driven spike rate, are shown for different somatosensory stimulation locations in Figure 40. IC recording locations were only included if stimulation elicited significant spike activity for at least the four highest current levels and showed increases in spike rate that were greater than one standard deviation of the noise floor. Driven spike rates of these IC locations were normalized to the spike rate at the highest current level to reduce bias (trends in sites with greater spike rates would naturally have more weight without normalization), and these normalized rates were averaged across all sites for a given stimulation location to produce the plots in Figure 40. Right shoulder and right hind leg stimulation locations had very few qualifying sites, so data for left and right shoulders and for left and right hind legs were combined. R^2 fit values for the best linear and logarithmic fits are shown in **E**, along with the number of IC locations used and the average slopes of the non-normalized rate-level functions (spike/μA after threshold). The linear fit resulted in higher R^2 values for all somatosensory stimulation locations, indicating that rate level functions for somatosensory stimulation in the IC are primarily

linear. In general, lower body regions had higher slopes than upper body regions, indicating that spike activity increased faster when stimulation current was increased for these stimulation locations, although these locations also had higher thresholds as seen in Figure 33.

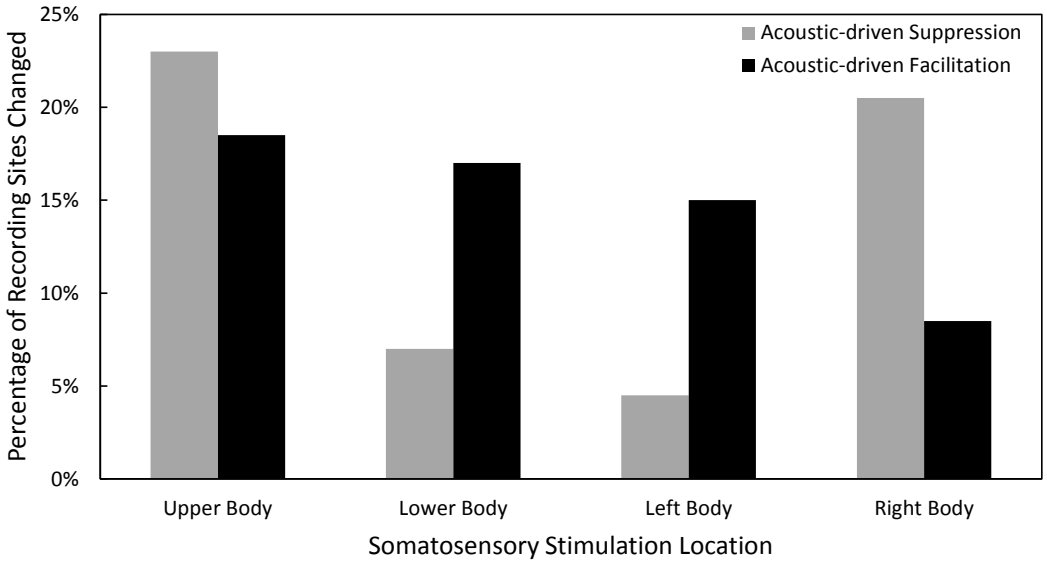


Figure 41: Plasticity effects of paired acoustic and electrical somatosensory stimulation

The percentage of recording sites (n=689 for upper body locations, n=213 for lower body locations, n=374 for left body locations, and n=352 for right body locations) in which acoustic-driven spike activity was suppressed (grey) or facilitated (black) after paired acoustic and somatosensory stimulation is shown. Contralateral (left) somatosensory locations induced facilitation in more recording sites than suppression, while ipsilateral (right) somatosensory locations induced more suppression than facilitation. Similarly, lower somatosensory locations were more facilitative while upper locations were more suppressive.

Plasticity Study

The plasticity effects of paired somatosensory and acoustic broadband noise stimulation were also investigated. Acoustic-driven spike activity before 1000 trials of paired stimulation was compared to acoustic-driven activity afterward to determine if significant plasticity occurred ($P<0.01$), and results are shown in Figure 41. Paired stimulation with upper body somatosensory stimulation locations induced suppression in more IC recording sites than facilitation, while lower body locations induced more facilitated sites than suppressed sites. Similarly, contralateral (left) somatosensory stimulation locations were more facilitative, while ipsilateral (right) somatosensory locations were more suppressive. These contralateral/ipsilateral findings are similar to those found in auditory cortex in previous chapters, and it is encouraging that they also exist in subcortical auditory regions. The observation that upper body and lower body stimulation areas exhibit plasticity trends may be relevant for targeting specific plastic changes in localized neural populations, considering that upper-lower body somatotopic trends exist in the IC.

Discussion

We have discovered somatotopy in the inferior colliculus, which was previously thought not to exist (Aitkin et al. 1978; Aitkin et al. 1981b). Although there is significant overlap in activation regions for different somatosensory stimulation locations (this could be due to the use of electrical stimulation, as outlined in Chapter 5), consistent spatial trends exist in threshold maps, and somatotopic maps can be determined if activation

thresholds are used (based on minimal-spread activation calculations). In previous studies, even S1 homunculus maps have some overlap (Földiák 1993; Schott 1993), which is also what we observed in Chapter 5 of this dissertation, so it is not surprising to see such overlap in a multisensory brain region such as the IC. It is possible that even greater localization could be achieved if more somatosensory locations were tested. This brings up more questions about the relationship between somatosensory and auditory inputs in the midbrain. Is there a correlation between somatotopic and sound localization maps in the IC, and what implications would this have in auditory processing? Additionally, although most activation locations were found in the ICD and ICX, some were found in the ICC, although a lower percentage of ICC recording sites responded to somatosensory stimulation than in ICD and ICX. Are these ICC neurons affected by direct projections, or are they modulated by ICX and/or ICD neurons that receive somatosensory inputs? More studies, including tracing studies, are necessary to answer these questions.

The idea that contralateral (left) somatosensory stimulation locations had lower thresholds than ipsilateral (right) locations is not surprising, since somatosensory fibers decussate in the spinal cord and brainstem before reaching the midbrain. This probably means that at least some IC somatosensory inputs come from somatosensory regions after decussation. The observation that upper body locations generally have lower thresholds than lower body locations might be related to the relevance of those locations to the auditory system. Noises originating from upper body parts, especially those close to the

head, are more likely to be heard by the ears, making their somatosensory inputs more relevant to the auditory system as multisensory integration helps to connect multiple sensory inputs into one perception. Additionally, contralateral somatosensory stimulation generally elicits more activation than ipsilateral stimulation within the somatosensory system (Schallert and Whishaw 1984), so it is not surprising that paired stimulation with contralateral somatosensory locations is more facilitative than with ipsilateral locations.

Paired stimulation induces plasticity in the IC, which might be useful in treating tinnitus. Because tinnitus is characterized by abnormal firing patterns, including hyperactivity and hyper-synchrony across neurons (Eggermont and Roberts 2004; Lanting et al. 2008; Lanting et al. 2009; Møller et al. 2010), the ability to change firing rates could mitigate the tinnitus percept. Furthermore, the ability to induce differential effects may be crucial. Suppression of activity could reduce hyperactivity, so the observation that ipsilateral somatosensory stimulation is more suppressive could play a role in treatments. Additionally, because different somatosensory locations induce different amounts of suppressive/facilitative plasticity, multiple somatosensory locations could be selected to increase rates in some neurons while decreasing rates in others, which could break up hyper-synchrony. More studies in tinnitus animal models or human trials would be useful in investigating these potential effects.

Minimal-spread activation may be especially useful for targeting activation in the auditory system for neuromodulation. If variability across tinnitus patients is at least in part due to varying sub-populations of problematic neurons with abnormal firing patterns

in the auditory system, then targeting specific sub-populations may be important in treating tinnitus symptoms across patients using neuromodulation. Our plasticity study shows the ability to induce differential plasticity effects in the IC using paired stimulation, and pairing acoustic stimulation with a specific somatosensory stimulation location at its threshold might allow us to induce targeted plasticity in a small population of IC neurons. Moreover, the ability to calculate an area or volume of activation for a given set of stimulation parameters (through minimal-spread activation) may allow us to model what IC areas will be activated by stimulation before it is performed, which might improve outcomes and efficiency in treatment programs. Our results show ideal minimal-spread activation stimulation locations can be localized, which is promising for targeted stimulation.

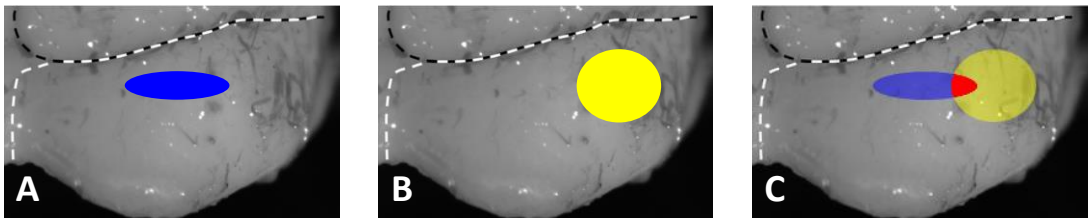


Figure 42: Targeting IC sub-populations with multiple stimulation locations

If the stimulation of one somatosensory location at a given current level activates a certain area of the IC (**A**), and the stimulation of a different somatosensory location at a given current level activates another area of the IC (**B**) with only a small amount of overlap between the two activated areas, then they could be stimulated simultaneously (**C**) to create diffuse activation in non-overlapping areas and more intense activation of the overlapped area (red) through current summation.

For further targeted stimulation/activation efficacy, multiple stimulation locations might be used. Figure 42 demonstrates this phenomenon. If the stimulation of one somatosensory location at its threshold activates a specific localized area of the IC (which we have shown), and the stimulation of a different somatosensory location activates a different specific localized area of the IC (which we have also shown), then the two can be stimulated together to elicit activation in both IC areas. However, if the two IC areas overlap slightly (but not completely), then the smaller overlapped area might be activated even more intensely through neural summation, while the remaining non-overlapped areas might only be activated diffusely. Such techniques could be applied to plasticity induction for neuromodulation.

Our latency analysis reveals an important notion when considering somatosensory activation of the IC and plasticity induction through paired stimulation. Stimulation of areas served by cranial nerves results in short, consistent latencies, while stimulation of areas served by the spinal cord – including the pinna – yields longer, highly variable latencies. As the somatosensory stimulation location gets further from the head, not only does the latency increase, but so does the variability based on the broadening of the histograms in Figure 39. This has implications on plasticity induction in IC, as inter-stimulus timing has been shown to be important in plasticity outcomes for paired stimulation, with different results at different inter-stimulus delays in our own studies (Chapters 3 and 4 of this dissertation) and other studies (Koehler and Shore 2013a; Tzounopoulos et al. 2007; Wolters et al. 2005; Wu et al. 2015). While broadband noise

stimulation has consistent short latencies to the IC (Langner et al. 1987), varying somatosensory stimulation location resulted in different latencies, which will change the onset timing of paired stimulation. Furthermore, the high variance of somatosensory latencies within the same stimulation location will lead to varying onset delays across the IC for a given paired stimulation paradigm. These trends in variability could adversely affect plasticity outcomes. Given that latency variance is higher for lower body regions, it might be necessary to focus more on upper body locations for plasticity induction with paired stimulation for more consistent outcomes in treatments. These challenges in matching or optimizing the best latency between different sensory inputs will also occur for other auditory regions, such as A1 and other sensory brain regions that may be targeted by mSync.

Our findings on rate-level functions for somatosensory stimulation in the IC are somewhat curious. Previous studies have found acoustic stimulation-generated rate level functions in the IC to have varying shape, and continuously increasing functions are often logarithmic in nature with a slowing rise in spike rate at higher stimulation levels (Ramachandran et al. 1999). However, our somatosensory rate-level functions are mainly linear for all tested somatosensory locations. This may indicate that stimulation intensity is coded differently in the IC for somatosensory stimulation compared to acoustic stimulation. The linear slopes of these rate level functions also have implications for paired stimulation. Increasing current levels in lower body stimulation resulted in faster increases in spike rates than for upper body stimulation. If somatosensory driven spike

rate is important for controlling plasticity induction, then we need to be careful in the selection of the somatosensory location, as lower body areas may require a smaller range of stimulation levels to achieve driven spike rates in a desired range, if it is necessary. More studies should be completed to investigate this aspect of stimulation level effects.

One major drawback of this study is the use of anesthesia. Ketamine has been shown to inhibit excitation/enhancement of neural activity (Hu and Davies 1997), especially through the blocking of various neurotransmitter receptors (Kaltenbach et al. 2000), and has also been shown to distort sensory perception (Oye et al. 1992). This may have greatly affected our mapping study and all ensuing analysis, as the entire study was based on measuring excitation of spike activity elicited by sensory stimulation. If the ability to excite neurons was reduced by ketamine, then our latencies, rate-level functions, and absolute thresholds would have been affected. However, trends in each of these analyses would likely still be observed, assuming ketamine affected IC neurons in a mostly uniform fashion. For example, while thresholds may have been lower overall without ketamine, the trends in differences in thresholds across different IC locations would not change if all thresholds were lower. Similarly, while latencies may have been shorter without ketamine (since measured latency tends to decrease as spike rates increase for the same group of neurons), the trend that latencies increased as stimulation distance from the brain increased would still remain. In addition to the inhibition of excitation, ketamine has also been shown to inhibit plasticity induction (Forsythe and Westbrook 1988; Gonzales et al. 1995). This may have significantly affected our paired

stimulation results. Perhaps without ketamine, more recording sites would have exhibited changes in neural activity, and perhaps trends in plasticity outcomes with respect to stimulation location would have been even more apparent. These issues could be investigated in awake animals in future studies.

In addition to those already discussed, future studies should investigate whether or not such somatotopic maps exist in other regions of the IC, especially the ICC, or in other auditory brain regions such as auditory cortex and the medial geniculate body in the thalamus. If somatotopic trends can be characterized in areas where tonotopy also exists, there may be implications for further targeting specific sub-populations of neurons in the auditory system, in which mSync could include different pure tone acoustic stimuli combined with specific somatosensory stimulation locations for more selective targeting of auditory neurons sensitive to a both specific frequency and somatosensory stimulation location. In this case, modulating a specific frequency band in the auditory system could be useful for tinnitus treatment, and combining somatosensory stimulation with specific tones could even further provide specificity for targeting. This concept could also be expanded to incorporate other sensory features across the brain to improve the ability to target neurons with mSync in general.

Chapter 8: Conclusions

Summary of Results

The primary focus of this dissertation was to characterize modulatory and plasticity effects on neural activity in sensory systems using multisensory stimulation. The results of this characterization could be used to develop safe, noninvasive, patient-adaptable neuromodulation treatments for neural sensory disorders that yield consistent outcomes. A summary of each study in this work is given here.

Chapter 2

In a preliminary proof-of-concept study for mSync, we were able to show that electrical somatosensory stimulation can excite the auditory pathway, particularly the IC, in which activation of different somatosensory stimulation locations resulted in varying degrees of activation across the ICC and especially the ICX. When electrical somatosensory stimulation was paired with simultaneous acoustic broadband noise stimulation, differential modulatory effects were observed in recording sites in the ICX, ICC, and A1. In some cases, acoustic-driven activity was significantly suppressed, while it was facilitated in other cases, and both outcomes were present in all three auditory areas in at least some recording sites. The ability to induce differential effects in the auditory pathway with mSync by taking advantage of somatosensory-auditory interactions could potentially target abnormal neural firing patterns in tinnitus.

Chapter 3

To expand upon the findings from Chapter 2, we performed a more detailed study on the plasticity effects of mSync in A1, in which somatosensory stimulation was paired with acoustic broadband noise stimulation at different inter-stimulus delays. Generally, mSync elicited significantly greater plasticity effects than control (no stimulation), and the extent of facilitative or suppressive plasticity was dependent on the location of somatosensory stimulation. Interestingly, contralateral somatosensory stimulation locations induced facilitation of A1 spike activity in a higher percentage of recording sites than suppression, while ipsilateral stimulation locations induced more suppression than facilitation.

When the paired stimulation inter-stimulus delay was varied, we could sometimes observe different results for different delays depending on the somatosensory stimulation location that was used. For paired acoustic and ipsilateral mastoid stimulation, activity was consistently suppressed more often than facilitated regardless of inter-stimulus delay, although different delays had slightly different ratios of suppressed recording sites versus facilitated sites, and a significantly greater percentage of recording sites were changed compared to control regardless of the delay used. However, for both the contralateral and ipsilateral pinna stimulation locations, an inter-stimulus delay of +15 ms (where acoustic stimulation preceded somatosensory stimulation) induced significantly more suppressed recording sites than facilitated sites in all animals, while all other delays were inconsistent and showed no significant differences between suppression and facilitation.

This result indicates that there is a timing-dependent component to plasticity induction with paired stimulation in auditory cortex.

The ability to induce a specific type of plasticity (facilitation or suppression) in different A1 neurons with mSync by adjusting the somatosensory stimulation location or inter-stimulus delay between somatosensory and acoustic stimulation provides a potential approach for modulating or disrupting pathogenic neurons driving the symptoms of tinnitus.

Chapter 4

Anesthesia may have affected our ability to induce plasticity in Chapter 3, especially our ability to induce facilitation, so we repeated the study in awake animals. However, stress can also be a factor in plasticity induction, so high-stress and low-stress groups were tested to account for this problem. Low-stress animals were treated with HTA, and an elevated plus maze behavioral test showed that HTA-treated animals exhibited significantly lower stress levels than non-treated animals in both a preliminary study and in the awake chronically implanted animals. For plasticity induction with paired stimulation, the high-stress group showed inconsistent results across animals with no significant trends across inter-stimulus delays. However, for the low-stress group, the +15 delay was once again significantly suppressive in all animals, while its neighboring +5 delay was consistently facilitative in all animals. This mirrored effect in neighboring delays is similar to plasticity observations in previous invasive bimodal stimulation

studies. These results confirm that mSync can modulate A1 activity in a controlled way in an awake animal, in which the timing between somatosensory and acoustic stimulation is a critical and potentially powerful way to appropriately alter pathogenic neurons for tinnitus treatment. Furthermore, stress and anesthesia significantly disturbs systematic plasticity in the brain, and stress relaxation methods may need to be implemented in patients to improve mSync outcomes. This stress-relief concept could be applied to all neuromodulation techniques, especially those that rely on plasticity induction for therapeutic effects.

Chapter 5

To generalize the findings from Chapters 2 through 4 beyond just the auditory system or tinnitus treatment, we chose to perform experiments in other sensory systems. In Chapter 5, we first mapped somatosensory and auditory inputs to S1, and observed a similar somatotopy to that of rats, although we found greater overlap in response areas for specific somatosensory stimulation locations within the guinea pig homunculus. Interestingly, S1 neurons that responded to acoustic broadband noise stimulation were primarily located in areas that mapped to lower body locations. This may indicate that the guinea pigs used in this particular study have significant connections between real-world sounds and lower body somatosensation based on environmental factors.

Although acoustic stimulation alone elicited excitatory responses in S1 in only a small percentage of neurons, somatosensory stimulation paired with acoustic broadband noise stimulation induced modulatory effects fully across S1, demonstrating the extensive

interactions between these two sensory systems that has been underappreciated in previous studies. Most of the paired stimulation modulatory effects were facilitative, and upper body stimulation locations had much larger ratios of facilitated recording sites to suppressed sites than lower body locations. All paired stimulation paradigms induced more changes in recording sites than somatosensory stimulation alone. Overall, these findings demonstrate that different modulatory effects can be induced via mSync not only in the auditory system but also the somatosensory system, signifying that mSync may be useful for multiple sensory disorders, such as tinnitus and pain.

Chapter 6

To further generalize the concept of mSync and to identify additional ways to control the effects of mSync outcomes, the modulatory and plasticity effects of paired stimulation were investigated in five primary sensory cortices. In each cortical area, stimulation of its primary sensory input (e.g. visual stimulation for V1) was paired with the stimulation of another sense, and many combinations were attempted for each case.

- In A1, contralateral somatosensory stimulation locations induced more facilitative than suppressive plasticity, while ipsilateral locations induced more suppression than facilitation. Paired auditory and gustatory stimulation was also primarily suppressive. During stimulation, modulatory effects were suppressive for acoustic/somatosensory paired stimulation in general.

- In S1, no clear plasticity trends could be found when pairing somatosensory stimulation with acoustic stimulation, although different somatosensory locations yielded different results. Modulatory effects during stimulation were primarily facilitative for all stimulation locations and induced changes in more recording sites than control.
- In V1, paired visual/olfactory stimulation induced suppressive plasticity, while gustatory and auditory stimulation both had facilitative plasticity effects when paired with visual stimulation. For paired visual and somatosensory stimulation, contralateral locations were suppressive, while ipsilateral locations were facilitative. During stimulation, modulatory effects were facilitative for all paired stimulation paradigms.
- In GC, contralateral somatosensory stimulation was suppressive in plasticity induction when paired with gustatory stimulation, while ipsilateral locations were facilitative, and similar to V1, all combinations were facilitative during paired stimulation.
- In OC, olfactory stimulation paired with either gustatory or somatosensory stimulation induced facilitative plasticity (regardless of somatosensory location), while auditory and olfactory paired stimulation was suppressive, and these trends were similar during stimulation.

Overall, the type, amount, and sensory cortical location of plasticity could be controlled with specific parameters, as differential effects were consistently observed. Although the

findings are complex and can vary for different neurons and mSync parameters, we also view this complexity as an asset for treatment since a wide range of parameters could be adjusted to target specific neurons with varying types of changes in their firing patterns to ultimately disrupt pathogenic neurons and/or networks.

Chapter 7

Previous chapters focused mostly on demonstrating the ability of paired sensory stimulation to induce different types of plasticity across different sensory neurons, but an important long-term goal would be to show that this type of controlled plasticity could be induced in a targeted neural subpopulation. One way to achieve targeted plasticity would be to leverage spatial organizations of sensory inputs in different brain regions. We first characterized somatosensory inputs into the IC and discovered a somatotopic threshold map across the region. Through minimal-spread activation and the strategic selection of stimulation level and location, small subpopulations of neurons could be targeted with somatosensory stimulation. Contralateral somatosensory locations had lower average thresholds than ipsilateral locations, although trends in differences in contralateral/ipsilateral thresholds were clear across the IC. Latencies of somatosensory stimulation were short and consistent for cranial nerve areas, but locations served by the spinal cord had higher latencies and latency variances. Rate-level functions were linear for all stimulation locations across IC recording sites. When paired with acoustic stimulation, the stimulation of upper body locations and ipsilateral body locations were more suppressive than facilitative of neural firing, while lower body locations and

contralateral body locations were more facilitative. These findings provide initial evidence that cross-sensory maps exist in the brain and converging pathways may allow us to target pathogenic neurons using mSync with varying parameters to treat sensory disorders; however, we need to be careful about differences in latency and rate-level functions for different stimulation parameters in order to induce consistent plasticity results with mSync.

Scientific Significance

The discovery of a somatotopic map is a major neuroscience finding in this dissertation, as previous studies had concluded that such a map doesn't exist in the auditory system. Additionally, the S1 homunculus of the guinea pig had not been previously characterized, which may be beneficial to future guinea pig studies. An underappreciated finding from this work is the extensive multisensory interactions within primary sensory cortices. For example, we observed that acoustic stimulation could modulate somatosensory stimulation across all of S1, and this spatially extensive plasticity effect is not observable if only assessing auditory-induced excitatory effects since only a small percentage of neurons may be directly excited by auditory stimulation alone. Interestingly, excitatory auditory responses were primarily found in lower body areas of S1, and if our hypothesis that there is a behavioral/environmental significance to this is correct, then perhaps multisensory excitatory projections are more environmental or adaptation-based for survival.

For the plasticity studies across all five sensory cortices, the ability to induce controlled plasticity in every sensory cortex with multisensory paired stimulation shows the importance of systematic multisensory integration throughout the brain. The ability of the brain to be significantly changed in such systematic ways gives us a general idea of how we easily adapt to complex multisensory stimuli in everyday life. Sensory systems are historically thought to be systematically hardwired, but vast, quick plastic changes in these studies imply otherwise. Additionally, the power of timing-dependent plasticity in the auditory system is another interesting finding. Other studies have used invasive bimodal stimulation to induce such plasticity, but the fact that we were able to do so with noninvasive and more natural sensory stimulation is important in understanding how timing-dependent plasticity unfolds during natural adaptation by the brain.

Finally, the implications of stress and anesthesia on plasticity are important. Previous studies have shown that stress and anesthesia can affect the release of various neurotransmitters and inhibit certain receptors, but to our knowledge, no one has actually done a full in vivo study to demonstrate the effects of stress and/or anesthesia on plasticity induction using neuromodulation. With this data, we can now begin to investigate the mechanisms of stress effects on plasticity related to patient outcomes, which will be important for improving neuromodulation treatments in general.

Clinical Significance

The concept of mSync and multisensory stimulation seems to be a promising method of neuromodulation for inducing changes in sensory brain regions, which might be useful for treating neural sensory disorders like tinnitus and pain. The ability to consistently suppress activity by inducing timing-dependent plasticity could help reduce hyperactivity in tinnitus and some forms of pain, and while this may not eliminate the cause of these disorders, it may at least treat their symptoms by reducing undesirable sensations. Additionally, the ability to induce different effects by varying mSync parameters, in which some neurons are facilitated while others are suppressed, might help treat disorders where synchrony across neurons is a problem.

The ability to control plasticity by selecting paired stimulation locations and timing has important clinical implications. Many sensory disorders, including tinnitus and pain, are highly variable across patients, which means each patient may need a personal, specially optimized treatment for his/her particular symptoms. With so many different parameter options in paired multisensory stimulation that provide varying effects, it may be possible to adapt stimulation to each patient for more consistent neuromodulation outcomes. Patient specificity/variability is already a challenge for neuromodulation and especially for tinnitus and pain treatment, making multisensory neuromodulation an intriguing option. The ability to try many different parameters safely and noninvasively is also crucial, as it will be more feasible to explore a variety of changes and parameter sets with minimal risks to the patient compared to invasive neuromodulation modalities.

Additionally, patient variability may be reduced through stress relief, which is another key clinical finding for many different types of treatments for diseases and disorders of the brain.

Finally, the ability to target specific subpopulations of neurons may be paramount in multisensory neuromodulation. It is likely that different subpopulations of neurons are responsible for the majority of symptoms in individual tinnitus and pain patients, and it would be beneficial to only target those populations such that plasticity induction in "healthy" neurons does not create new problems or side effects. This is actually a challenge in tinnitus treatment today, as some patients report a shift in or worsening of their tinnitus percept after treatment. Using different multisensory stimulation parameters for targeted plasticity might be clinically useful in these challenging situations.

Future Work

We have characterized targeted plasticity in the IC and S1 through mapping studies, but such studies have not been completed for other sensory brain regions, including all other sensory cortices. Studies that characterize location-dependent plasticity trends might be helpful for better understanding multimodal integration, and they could be clinically relevant. Similarly, we have only investigated timing-dependent plasticity in the auditory system, but such data would be useful in other sensory cortices as well. Even in A1, a timing study with paired stimulation that includes other somatosensory locations or even other sensory modalities might reveal interesting

outcomes. There are many timing and sensory combinations that could be tested for different brain regions, all of which could have important clinical and scientific implications.

The effects of stress on plasticity and neuromodulation are introduced in this dissertation, but so much is unknown about its mechanisms. Studies could be performed to determine which neurotransmitters and/or receptors are primarily responsible for inconsistent results in stressed subjects, and these mechanisms could be targeted through drugs and/or alternative methods to resolve this issue in patients whose stress cannot be reduced due to anxiety about their disorder and/or their neuromodulation treatment.

In addition to these future studies, which could be done in animals, human studies for multisensory neuromodulation are needed to determine if the plasticity effects shown in this dissertation are capable of treating neural sensory disorders. The background work in these animal studies shows promise and gives us a starting point for pushing forward with human implementation, making this the next logical step. Fortunately, because of its noninvasive nature, mSync can be implemented easily and safely in human studies for the treatment of different neural sensory disorders.

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Appendix A: Spike Responses to Specific Receptor Stimulation in Primary Somatosensory Cortex

Summary

Spike responses to specific somatosensory mechanoreceptor stimulation of the hind paw in primary somatosensory cortex (S1) were investigated in anesthetized guinea pigs. Long single pulse tactile stimuli were used to activate stretch receptors, including Merkel disks, while tactile vibration stimuli at different frequencies were used to activate vibratory receptors, such as Meissner corpuscles. Single square-wave pulse stimulation onset responses exhibited shorter latencies, shorter durations, and tall, thin post-stimulus time histogram (PSTH) peaks, while sinusoidal vibratory stimulation resulted in onsets with longer latencies, longer durations, and wider PSTH peaks of less height. These observations are likely related to the ramp of the initial pulse of stimulation, which gives insight on how S1 codes for ramp features and changes in the deformation of the skin. When comparing onset responses of electrical stimulation to those of tactile stimulation, it can be inferred that electrical stimulation generally activates either receptors or innervating axons all at once, but occasionally multiple pathways/mechanisms may apply for multi-peak PSTH responses. Additionally, after onset, vibratory stimulation responses were sustained and uniform even for low frequencies with long periods, which means the somatosensory pathway processes vibratory signals to change them before they reach the cortex.

Introduction

Mechanoreceptors are somatosensory receptors found in the skin across the entire body, and they allow us to perceive different attributes of objects that we touch (Abraira and Ginty 2013; Andres and von Düring 1973; Johnson 2001). Currently, we are aware of four different mechanoreceptors, including Merkel disks, Meissner corpuscles, Pacinian corpuscles, and Ruffini endings. Merkel disks are slow-adapting receptors that respond to stretch stimuli, where a deformation of the skin stretches it, informing us of the timing and intensity of a touch stimulus (Abraira and Ginty 2013; Johnson 2001). Meissner corpuscles are rapidly-adapting receptors that respond to low-frequency vibrations (Bensmaïa 2002), primarily in the 10-100 Hz range with weaker responses up to 200 Hz (Dallmann et al. 2015). Pacinian corpuscles are rapidly adapting receptors that respond to high-frequency vibrations (Gray and Sato 1953; Sato 1961), primarily in the 250-500 Hz range with weaker responses down to 100 Hz (Dallmann et al. 2015). Each of these mechanoreceptors gives us different information about a stimulus, and the combined information leads to somatosensory perception, which is thought to occur in somatosensory cortex. The purpose and mechanisms of the fourth receptor, Ruffini endings, are not well understood.

Primary somatosensory cortex (**S1**) is characterized by a neural response map organization resembling a homunculus, where somatosensory stimulation of different body locations results in the activation of specific neural populations in S1. Previous studies have shown this S1 somatotopic representation in humans (Aminoff et al. 1985; Baumgartner et al. 1991; Hari et al. 1993; Itomi et al. 2000; Kakigi et al. 1995; Liguori et

al. 1991; Mogilner et al. 1994; Nakamura et al. 1998; Narici et al. 1991; Nobre 2001; Penfield and Boldrey 1937; Woolsey et al. 1979; Yang et al. 1994b), rats (Cho et al. 2007; Godde et al. 2002; Petersen et al. 2001; Welker 1976), cats (Celesia 1963; Davenport et al. 2010; Dykes et al. 1980; Iwamura and Tanaka 1978; Shigenaga et al. 1989), pigs (Craner and Ray 1991), monkeys (Pons et al. 1985), and other mammals (Schott 1993).

Previous studies have used electrical stimulation of the somatosensory system to test various neural features in different brain regions, including pain responses (Davis et al. 1995) effects on motor function (Conforto et al. 2007; Laufer and Elboim-Gabyzon 2011), and treatment for neural lesion patients (Schuhfried et al. 2012) and stroke patients (Celnik et al. 2007; Koesler et al. 2009). Additionally, electrical somatosensory stimulation has been used to induce plasticity in sensory regions of the brain (Chipchase et al. 2011; Gloeckner et al. 2013; Markovitz et al. 2015). In all of these cases, it would be beneficial to understand how electrical stimulation activates mechanoreceptors and/or their innervating axons. This is especially true in the induction of timing-dependent plasticity, which has been shown in S1 (Panzeri et al. 2001; Petersen et al. 2001; Wolters et al. 2005) and would be affected by different latencies of different receptors.

While peripheral nerve responses to the activation of different receptors has been investigated (Burgess and Perl 1973; Vallbo and Hagbarth 1968), no one has fully characterized differences in S1 responses to different receptors to our knowledge. This characterization would not only be useful for understanding mechanisms of electrical stimulation, but also any kind of peripheral stimulation, including that of ultrasound,

which has recently been considered for the neuromodulation treatment of various disorders (Tyler 2011). This study aims to investigate S1 responses to different mechanoreceptors through analysis of neural spike patterns.

The somatosensory stimulation location that has the highest density of all four receptor types is the hand, which is why many mechanoreceptor experiments utilize hand stimulation (Abraira and Ginty 2013; Johnson 2001). Guinea pigs do not use their front paws in the same ways that humans use their hands, as their front paws are not well-equipped for examining objects. Still, their front and hind paws are their most involved skin regions for somatosensory perception, making them the optimal target for a mechanoreceptor rodent study. Although no one to date has characterized S1 in guinea pigs, we have recently mapped the somatotopic organization and found that electrical stimulation of the hind paw generally activates the caudal-most areas of S1. In this study, we tactilely stimulate different mechanoreceptors on the bottoms of the hind paws of anesthetized guinea pigs and record spike responses from neurons in the area of S1 that best responds to hind paw stimulation.

Materials and Methods

Overview

Neurophysiology experiments were performed on three young female Hartley guinea pigs (400–500 g; Elm Hill Breeding Labs, Chelmsford, MA) anesthetized with an initial intramuscular injection of ketamine (40 mg/kg, Zoetis Inc., Kalamazoo, MI) and xylazine (10 mg/kg, Akorn, Decatur, IL), with supplements every 45–60 minutes to

maintain an areflexive state. Experiments were performed inside an electrically-shielded and acoustic-attenuating room using hardware from Tucker-Davis Technology (Alachua, FL), and neural data was processed using Matlab software (Natick, MA). All experiments were completed under protocols approved by the University of Minnesota Institutional Animal Care and Use Committee (IACUC).

Surgery and Neural Recordings

A craniotomy revealing the right somatosensory cortex of each animal was completed. The animal's head was held into place using a stereotaxic frame with micromanipulators (Kopf Instruments, Tujunga, CA) and custom-made hollow ear bars, while the rest of the body was allowed to rest. The animal's heart rate and blood oxygen content were continuously monitored using an H100 pulse oximeter from EdanUSA (San Diego, CA), and body temperature was monitored using an Oakton Acorn series JKT thermocouple rectal probe (Vernon Hills, IL) and maintained at 38.0 ± 0.5°C using a heating pad and an HTP-1500 heat pump (Adroit Medical Systems, Loudon, TN). A 32-site recording electrode array (NeuroNexus Technologies, Ann Arbor, MI) was inserted into the right S1 of the guinea pig brain where spike responses to electrical stimulation of the hind paw were most prevalent (Figure 43-A). This recording electrode array was comprised of four 5-mm long shanks separated by 500 μm with eight iridium sites linearly spaced at 200 μm along each shank (site area = 413 μm^2). The array was inserted to a depth such that the main input Layer IV could be observed at approximately the middle of the eight recording sites on each shank (determined based on locating the initial

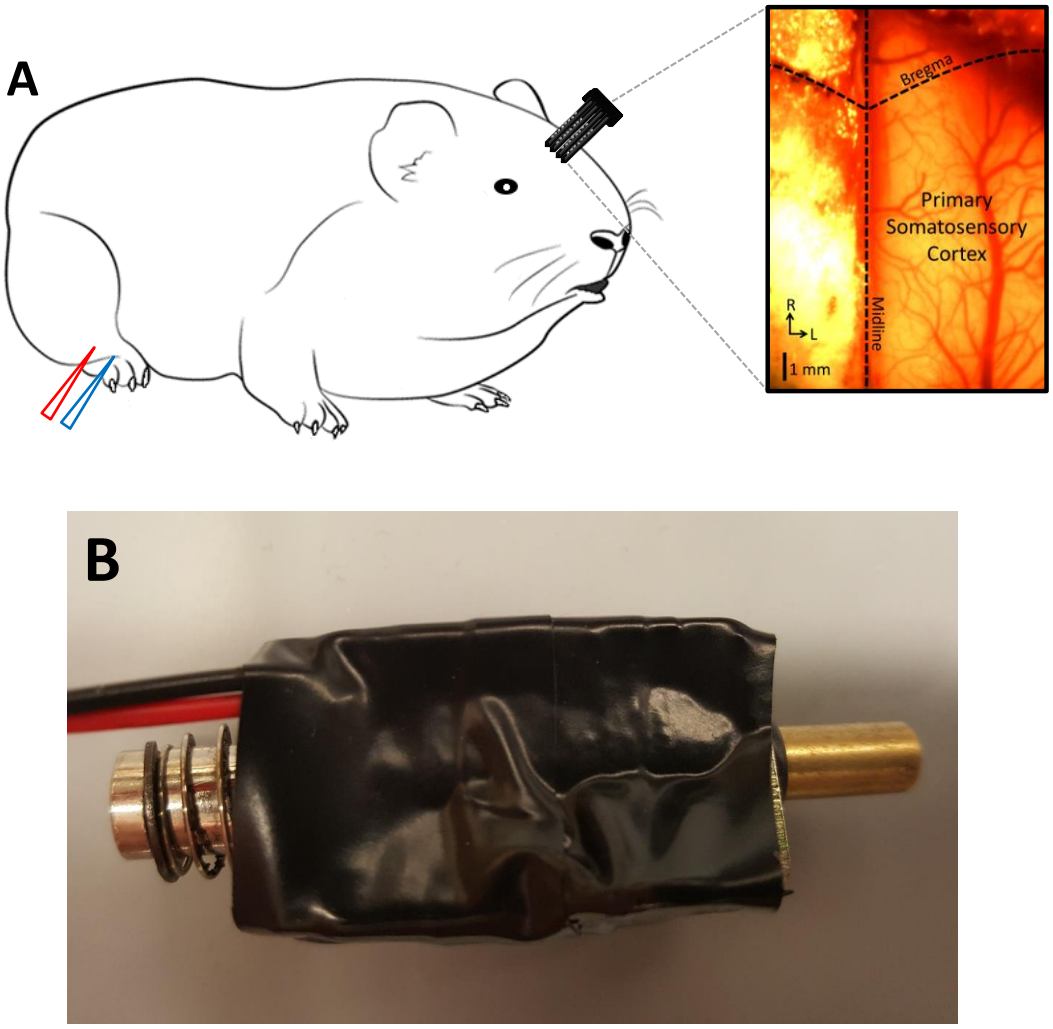

Figure 43: Recording and stimulation

Electrical stimulation of the hind paw was used to locate a suitable recording area in S1 (**A**, blue and red triangles represent the stimulation lead and ground). An actuator was used to mechanically stimulate somatosensory receptors in the hind paw of the guinea pig (**B**). The actuator used a solenoid to push a 0.5 cm cylinder into the hind paw at various frequencies. Stimuli, which were all 300 ms long, included a single pulse and pulse repetitions at 10, 20, 30, 50, 75, and 100 Hz.

sink using current-source density; (Gloeckner et al. 2013; Lim and Anderson 2007a; Markovitz et al. 2013)). This generally resulted in the tip sites being inserted 1.1-1.3 mm below the surface of the cortex. The recording ground for the electrode array was inserted into the upper neck of the animal. Recording electrode site impedances ranged between 0.3 and 0.8 MΩ when using a 1 kHz sine wave. Saline was routinely administered to the cortex after the probe was placed to limit the effects of dehydration.

Multiunit neural activity was sampled at a rate of 24.4 kHz, passed through an analog DC-blocking filter and an anti-aliasing filter up to 7.5 kHz, and then digitally filtered between 300 and 3000 Hz for analysis of spike activity. A detection threshold of 3.5 times the standard deviation of the voltage noise floor was used to determine when spikes occurred, and the timing of spikes relative to the beginning of a recording was used to construct post-stimulus time histograms (PSTHs). Only recording sites with significant excitatory responses to stimuli were used for analysis, and Signal Detection Theory (Green and Swets 1966) was used to determine significance (d'=1), similar to previous studies (Lim and Anderson 2007a; Markovitz et al. 2013; Offutt et al. 2014).

Somatosensory Stimulation

Electrical stimulation (biphasic, 205 µs per phase, 710 µA) was used to initially determine the location of the area of S1 that best responded to hind paw stimulation. For this stimulus, two subcutaneous needle electrodes (Rhythmlink International LLC, Columbia, SC) were placed within the left hind paw of the animal, such that one could be used as a stimulation lead and the other as a ground (Figure 43-A). For tactile

stimulation, we used a custom-made solenoid actuator to push a 0.5 cm cylinder into the bottom of the paw of the guinea pig (Figure 43-B). Seven different stimulus types were used, including a single 300 ms square-wave pulse and 300 ms of sinusoidal vibratory stimulation at 10, 20, 30, 50, 75, and 100 Hz. After the completion of all tactile stimuli, S1 responses to electrical stimulation of the left hind paw were recorded for comparison. In all cases, 100 trials (1 per second) of stimulation were performed consecutively for a given stimulus type.

Results

All seven tactile stimuli elicited significant excitatory activity in S1 (n=80 recording sites for single pulse stimulation, n=70 for 10 Hz vibratory stimulation, n=76 for 20 Hz, n=41 for 30 Hz, n=35 for 50 Hz, n=18 for 75 Hz, n=10 for 100 Hz). Unfortunately, our actuator created enough electrical noise for the 75 Hz and 100 Hz vibratory stimuli that the highest peaks of the noise were detected as spikes, and no method of filtering or spike detection could remedy this. This problem likely contributed to fewer recording sites showing significant excitatory activity for these two stimuli, as smaller increases in spike activity would be hidden by an increased noise floor. For sites with significant responses to these stimuli, analysis was performed, but PSTHs are not shown due to a visible increase in background noise during stimulation that masks the true shape of responses.

Typical PSTHs are shown for single pulse, 10 Hz, 20 Hz, and 50 Hz stimuli in Figure 44 for comparison of peak shapes (red lines indicate the beginning and end of a

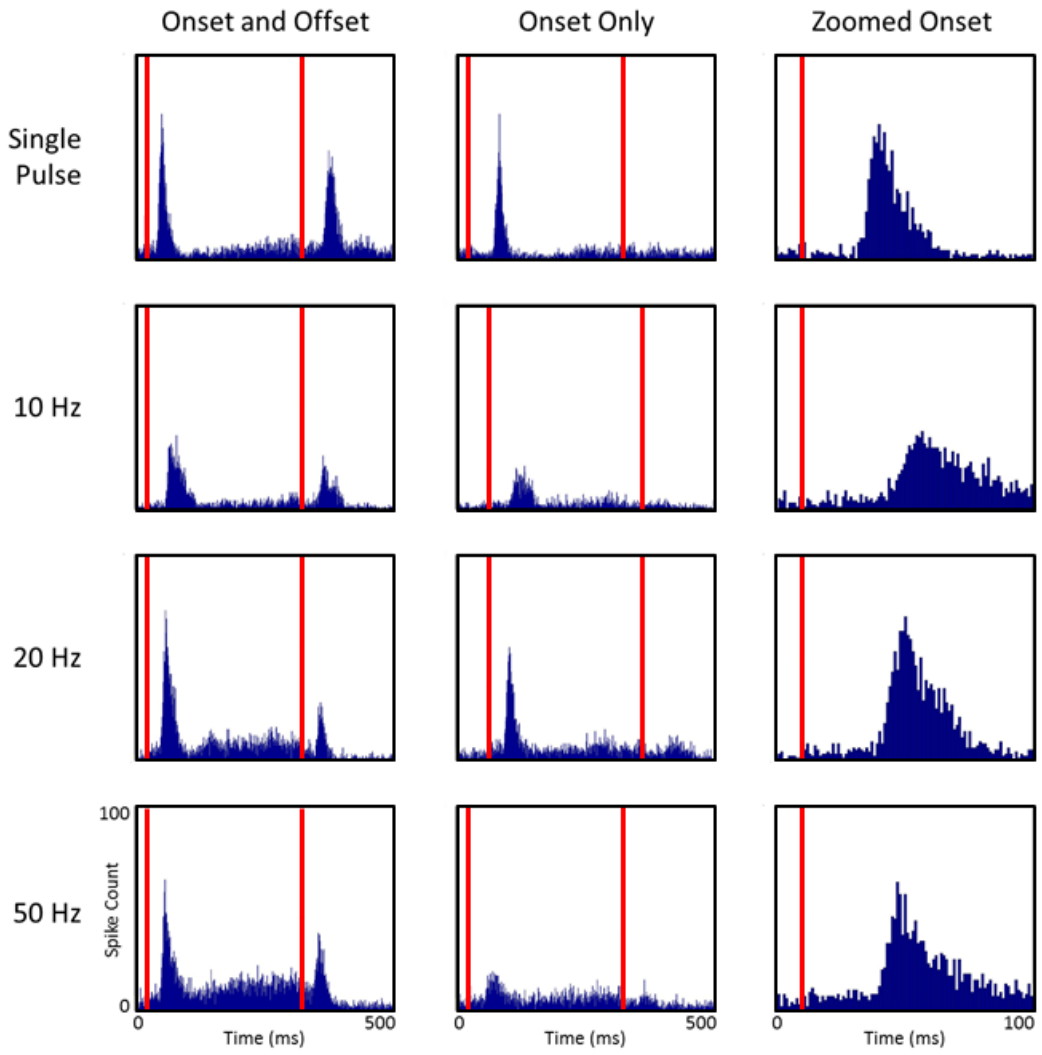

Figure 44: Post-stimulus time histogram examples

Post-stimulus time histograms (PSTHs) were constructed for visual representation of spike activity. Representative examples are shown for single pulse, 10 Hz, 20 Hz, and 50 Hz stimuli (75 and 100 Hz are not shown due to large artifacts). For each stimulus, examples with and without an offset response are shown, along with a zoomed-in version of an onset response. Red lines indicate the beginnings and ends of stimuli.

stimulus). PSTHs for 30 Hz stimuli are omitted as they look similar to the 20 Hz and 50 Hz PSTHs. Three PSTHs are shown for each stimulus, including one PSTH example with both onset and offset responses, one example with only an onset response, and one example zoomed-in on an onset response. Generally, single pulse onset response peaks were narrower and more uniform, while vibratory response onset peaks were wider with more of a decaying shape. Lower frequency vibratory stimulation PSTH peaks were generally wider with reduced heights, and had more of a decaying shape than those of higher frequency vibratory stimuli. Additionally, higher frequency vibratory stimuli consistently elicited more spike activity in between onset and offset peaks than lower frequency vibratory stimuli, and it was confirmed through raw data examination that this

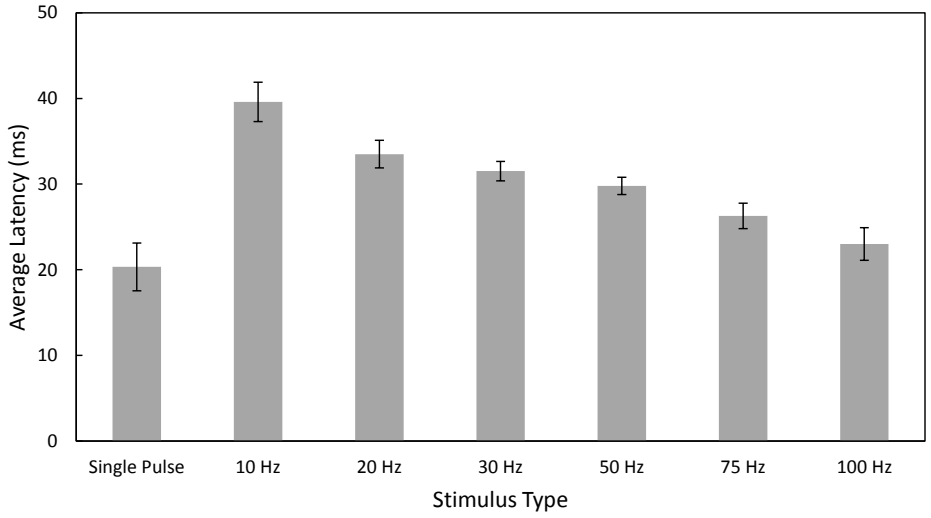

Figure 45: S1 latencies for mechanical stimulation of the hind paw.

Average latencies across all S1 locations are shown for all stimuli. The single pulse stimulus had the shortest latency, and latency decreased as frequency increased for vibratory stimuli.

was not due to increased electrical noise for all of the stimuli shown in this figure. When looking at PSTHs in the first column of the figure, this increase in spike activity starts earlier for the 50 Hz stimulus than for the 20 Hz stimulus. Single pulse offset peaks were generally much larger than those for vibratory stimuli, even in cases where onset peaks for the two stimuli were similar in size. In all cases where offset peaks existed, the onset peak was always larger than the offset peak.

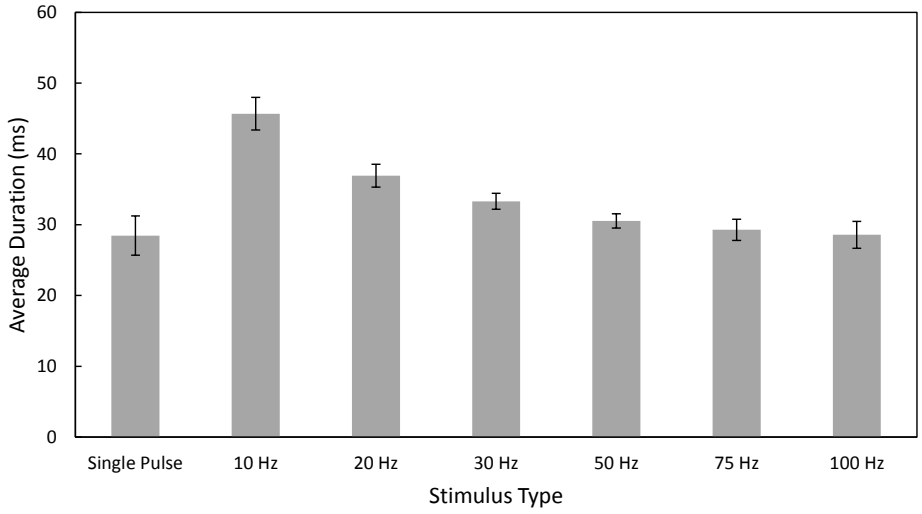

Figure 46: S1 durations for mechanical stimulation of the hind paw.

Average durations across all S1 locations are shown for all stimuli. The single pulse stimulus had the shortest duration, and duration decreased as frequency increased for vibratory stimuli.

Average onset latencies across all recording sites are shown for each tactile stimulus in Figure 45. Error bars show standard error across three animals. Onset latencies were shorter for the single pulse stimulus than for all vibratory stimuli, and

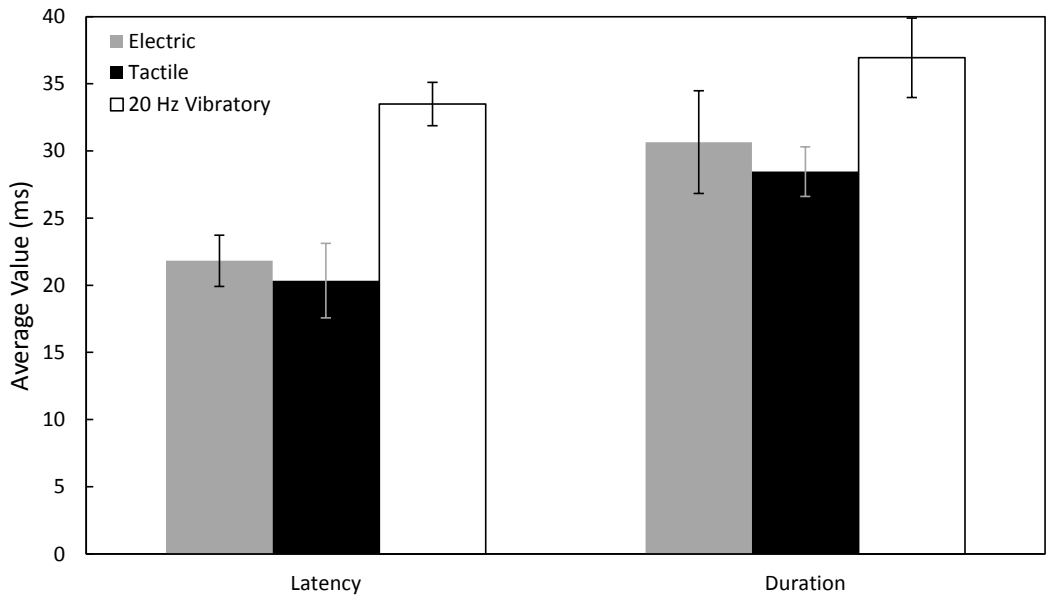

Figure 47: Comparison of electrical and tactile stimulation

Average latency and duration are shown for electrical stimulation (grey, n=66 recording sites), single pulse tactile stimulation (black), and 20 Hz vibratory stimulation (white). 20 Hz was used for vibratory stimulation because we expect to activate vibratory receptors at this frequency. Electrical stimulation seems most closely related to single pulse tactile stimulation, indicating that this may be the primary receptor typically activated.

lower frequencies of vibration yielded longer latencies than high frequencies. Similarly, onset durations were shorter for the single pulse stimulus than for vibratory stimuli, with lower frequencies of vibratory stimulation having longer durations (Figure 46). In both cases, values decreased less as the frequency of stimulation increased, following a general exponential decay relationship. These onset results are related to the initial pulse of stimulation, which is different for square-wave single pulses versus vibratory pulses,

and has a different ramp speed (initial velocity of the cylinder as it pushes into the skin) for vibratory pulses of different frequencies.

Figure 47 shows a comparison of latency and duration between electrical (n=66 recording sites in three animals), single pulse tactile, and 20 Hz vibratory stimulation. 20 Hz was chosen to represent vibratory stimulation because it is the lowest frequency within the primary frequency range of vibratory stimuli where we expect to substantially activate Meissner corpuscles, according to previous studies (Dallmann et al. 2015), and it had the greatest number of activated recording sites across all vibratory stimuli. Average latencies for electrical stimulation were more similar to those of single pulse tactile

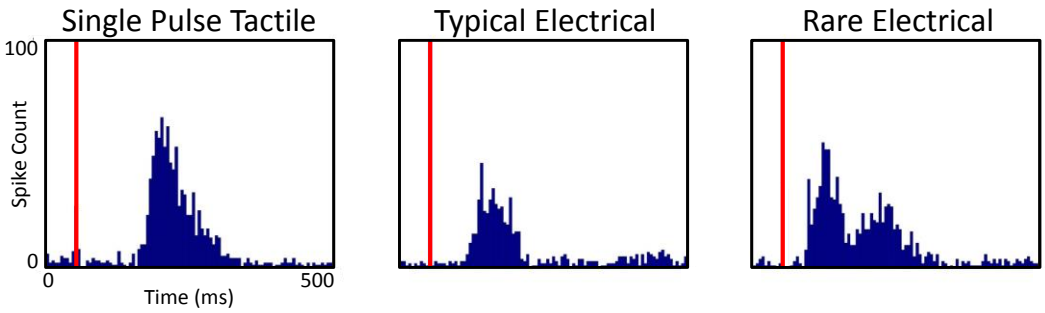

Figure 48: PSTH comparison of electrical and tactile stimulation

PSTH examples are shown for shape comparison, where the red lines indicate the times of stimuli. The shape of a typical electrical stimulation response has one narrow peak with a short latency, indicating that it may only activate stretch receptors like the single pulse tactile stimulus, or that receptors are activated simultaneously. However, we rarely also see electrical stimulation with multiple peaks, which could indicate the activation of both stretch and vibratory receptors.

stimulation than vibratory stimulation. Duration results are inconclusive, given that electrical stimulation provides only an instantaneous pulse, while both forms of tactile stimulation provide a sustained stimulus. Figure 48 shows typical single pulse tactile and electrical PSTH responses, as well as a rare electrical PSTH response which was observed in less than 5% of responding recording sites. Like single pulse tactile responses, the electrical stimulation PSTH has a more uniform shape than the logarithmic shapes of the vibratory responses shown in Figure 44. However, the rare electrical stimulation response shows two PSTH peaks, which was never observed in responses to tactile stimulation of any kind, even on the same recording sites. Considering that these rare responses have two distinguishable peaks and much longer durations than normal electrical stimulation responses, it is possible that multiple pathways/mechanisms are involved.

Discussion

Comparison of Tactile Stimulus Responses

Differences in onset latencies for vibratory stimulation frequencies are likely related to the sinusoidal nature of our vibratory stimulation. Given that the period lengths for our low-frequency vibratory stimuli are long, we can assume that S1 onset responses are primarily related to only the first cycle of vibration. For a sinusoidal stimulus, it takes longer for a low frequency stimulus to reach its maximum amplitude as it is ramped up. This means it may take a given stretch receptor longer to detect the 10 Hz stimulus than a square-wave single pulse stimulus, as each receptor requires a specific amplitude of

deformation before firing due to its own sensitivity or its location in the skin relative to the stimulus (Abraira and Ginty 2013; Hendry and Hsiao 2008). Although this means that latency alone doesn't tell us much about differences in neural onset from different receptor types, PSTH information can still provide insight. Low frequency response PSTH peaks are wider with reduced heights compared to single pulse response peaks, and this means S1 neurons do not fire as synchronously for low frequency stimulation, since activity is spread out over a greater period of time. Different stretch receptors have different amplitudes of activation (Abraira and Ginty 2013), so these receptors would also fire less synchronously in response to the first cycle of a slowly ramped sinusoidal stimulation. Additionally, if a certain threshold quantity of receptors must be activated in order to elicit a response in S1, then S1 latencies would be longer for stimuli that increase in amplitude more slowly and take longer to activate the necessary number of receptors for S1 activation.

This concept follows the latencies we see in Figure 46, so it appears that S1 onset responses to a ramped stimulus are actually dependent on the speed of the ramp, where onset shape and duration both code at least in part for the slope of the ramped stimulus. Higher frequency stimuli have steeper ramps, which explains why they have shorter latencies and durations than lower frequency stimuli, but still have longer durations and latencies than a square-wave single pulse stimulus. In this case, S1 activation does not occur until the ramped stimulus activates enough stretch receptors to reach a certain

threshold, and a wider range of receptor activation times yields a longer duration. Based on this, it might be possible to characterize a stimulus based on S1 onset responses.

The PSTHs in Figure 44 show that S1 neural firing does not necessarily increase in between onset and offset responses for single pulse and 10 Hz stimulation. We expect this, since vibratory receptors generally are not activated at frequencies below 20 Hz (Dallmann et al. 2015), but it also confirms that the S1 neurons we recorded from only code for onset and offset during a single sustained pulse stimulus (and do not fire during constant skin deformation). This is an interesting finding, as it is known that Merkel disks fire continuously during a constant deformation (Hendry and Hsiao 2008), indicating that some cortical or subcortical processing is transforming this signal into onset and offset responses. However, neural firing does increase during this time for 20 Hz and 50 Hz stimuli, and the increase begins earlier for the 50 Hz stimulus as shown in Figure 44. This observation is consistent across all recording sites. The period between stimulus peaks is 50 ms for a 20 Hz stimulus and 20 ms for a 50 Hz stimulus, and it is known that vibratory receptors fire once per cycle of vibration (Hendry and Hsiao 2008). Consequently, it makes sense that S1 vibratory responses would begin earlier for a 50 Hz stimulus, since it takes a full 50 ms for the receptors to generate a second action potential in response to a 20 Hz stimulus, while only 20 ms are necessary for a 50 Hz stimulus.

Accordingly, we can infer that this activity between onset and offset responses is actually S1 vibratory response activity. Additionally, the vibratory response for 20 Hz stimulation starts about 50 ms after the initial onset, and the vibratory response for the 50

Hz stimulation starts before the onset response dissipates, making its origin less than 40 ms after the initial onset (which is less than the length of two periods of stimulation). This means that vibratory S1 activity actually starts in response to receptor activation during the *second* period of stimulation. Furthermore, there is not an initial peak at the beginning of the vibratory activity for 20 Hz stimulation, and activity remains constant following its beginning, which means S1 is likely not coding the second cycle of vibration as a separate independent stretch stimulus. Therefore, S1 actually codes the second cycle as vibratory activity, which means the nervous system only requires two cycles of a vibratory stimulus to assign its type. This is a key finding, as one might expect the need for at least three cycles to establish a somatosensory input as a consistent vibratory stimulus, but this data shows that two is enough.

We also observed that this S1 vibratory response is continuous and uniform. Given that the period between cycles is 50 ms for a 20 Hz stimulus, we would expect vibratory receptors to fire somewhat synchronously with large time gaps in between action potentials. Even if different receptors are activated at different deformation amplitudes as discussed above, there would still be at least a 25 ms period where the stimulus amplitude is not increasing. Also, even if receptors begin coding for offset as the cylinder retracts, there would still be a time period of reduced velocity at the peaks of the sinusoidal wave, which would result in pulsatory receptor activity. Therefore, one might expect to see an increase or decrease in neural activity every 50 ms in S1 responses to 20 Hz stimulation. However, we observe continuous, uniform activity in our data for low

frequency stimulation of at least 20 Hz, which indicates that S1 codes for vibration differently than receptors and peripheral nerves (which would not fire uniformly). We hypothesize that processing at some level within the somatosensory pathway must be responsible for this.

Finally, we see that these vibratory responses are stronger for 50 Hz stimulation than for 20 Hz stimulation. Perhaps this means that firing rates of vibratory responses in S1 are directly related to the frequency of vibration, which is reasonable considering that vibratory receptors generally fire at the same frequency as their stimulus (Hendry and Hsiao 2008). However, a specific quantitative relationship cannot be drawn here, as stimulus amplitude likely also affects firing rate. Fortunately, based on our own observations of our solenoid stimulator, we know that the amplitude of stimulation decreases as frequency increases, so we can still establish a positive relationship between increased S1 firing and increases in stimulus frequency, regardless of amplitude. More studies which control for stimulation amplitude and an analysis on stimulation power are necessary to confirm this observation.

Implications on Electrical Stimulation Responses

Unfortunately, because our onset responses likely code for stimulus amplitude ramp and may have little to do with different types of receptor activation, not much can be concluded from Figure 47, which compares latencies and durations of electrical and tactile stimuli. Latencies between electrical and square-wave single pulse tactile stimuli are similar, indicating that electrical stimulation likely activates receptors or axons

immediately. Higher durations in electrical stimulation may be related to stimulation amplitude, and we cannot control for this since there is no way to quantitatively compare electrical stimulation amplitudes to tactile stimulation amplitudes.

PSTHs in Figure 48 show that common S1 electrical stimulation responses have a normal uniform shape that is somewhat similar to that of single pulse tactile stimuli. This isn't surprising, since electrical stimulation doesn't provide a vibratory component. However, based on the argument above, PSTH shape is likely tied to how synchronously receptors are activated. Stimuli that activated receptors at a wider range of times yielded PSTH shapes that were wider and more logarithmic in shape, while stimuli that activated receptors in a narrower time frame yielded more uniform PSTH shapes. Since electrical stimulation response PSTHs are even more uniform in shape than single pulse tactile stimuli, it could be inferred that electrical stimulation activates all receptors/axons simultaneously. In the rare occasion that electrical stimulation response PSTHs exhibited multiple peaks, it is possible that stimulation activated different types of receptors at different times, or that stimulation activated both receptors and post-synaptic axons simultaneously, which would elicit two separate signals with different latencies.

Future Studies

One major drawback of this study is that only one recording electrode placement was attempted per animal, for a total of three placements. S1 responses to tactile and electrical stimulation may vary across S1, as a previous study shows that different frequencies of stimulation can project to different S1 locations (Mogilner et al. 1994).

This might explain why some recording sites responded to some vibratory stimuli and not others in our study. If this study were repeated with more placements mapping the S1 in each animal, spatial trends may be discovered.

Additionally, a greater number of vibratory stimulation frequencies must be investigated, especially in the range of Pacinian corpuscles. Our stimulator produced electrical noise at high frequencies, and this needs to be remedied for future work. Square-wave vibratory stimuli might also be used in the place of sinusoidal waveforms for a more direct comparison of onset responses, especially for stimuli of higher frequencies where the period of vibration could be significantly shorter than the length of the onset response. Finally, nerve recordings could be paired with S1 recordings to better understand the relationship between the timing of receptor activation and the timing of S1 firing.