Insights into the Influences of Sensory Experience and Serotonin on Multisensory Processing in the Superior Colliculus

by

LeAnne Renee Kurela

Dissertation

Submitted to the Faculty of the

Graduate School of Vanderbilt University

in partial fulfillment of the requirements

for the degree of

DOCTOR OF PHILOSOPHY

in

Neuroscience

May, 2017

Nashville, Tennessee

Approved:

Jon Kaas, Ph.D

Mark Wallace, Ph.D

Alex Maier, Ph.D

Joseph Neimat, M.D.

Dedication

To everyone who listened to me whine, grumble and complain for the past six years.

Thank you for your infallible support. No more complaining.

About this, at least.

Acknowledgements

This work would never have been possible without the advice and input of my advisor, Dr. Mark Wallace, as well as my thesis committee members Dr. Vivien Casagrande, Dr. Alex Maier, Dr. Joseph Neimat, and Dr. Jon Kaas. Thank you for your guidance and encouragement.

Words cannot express how thankful I am for the help, love and support I have received throughout this process. To my labmates, friends and family, I am forever grateful for the long, late-night conversations, helping hands, impromptu venting and brainstorming sessions, heartwarming surprises, hugs, laughter and support. Without you, none of this could have ever been dreamed of, let alone accomplished.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

5-HT ..... 5-hydroxytriptamine; serotonin

6+6 ..... normally reared through maturation, visually deprived in adulthood

ACh ..... acetylcholine

aCSF ..... artificial cerebrospinal fluid

AES ..... anterior ectosylvian sulcus

AMPA ..... α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

ANOVA ..... analysis of variance

ASD ..... autism spectrum disorder

AUC ..... area under the curve

cMRF ..... contralateral mesencephalic reticular formation

DOI ..... 1-[2,5-dimethoxy-4-iodophenyl]-2-amino-propane; 5-HT2a receptor agonist

DR ..... dark-reared

FEF ..... frontal eye field

FF ..... fano factor

GABA ..... gamma-aminobutyric acid

GAD ..... glutamate decarboxylase

Glu ..... glutamate

IC.....inferior colliculus

ii..... interactive index

LFP.....local field potential

LGN.....lateral geniculate nucleus

msc.....mean statistical contrast

MUA .....multi-unit activity

NMDA.....N-methyl-D-aspartate

NR .....normally-reared

NT .....neurotransmitter

PPRF.....paramedian pontine reticular formation

PSTH.....peristimulus time histogram

RF .....receptive field

rLS..... rostral lateral suprasylvian sulcus

SAI .....stratum album intermediale

SAP ..... stratum album profoundum

SC .....superior colliculus

SGI .....stratum griseum intermediale

SGP..... stratum griseum profoundum

SGS..... stratum griseum superficiale

SIV ..... somatosensory area IV

SO .....stratum opticum

SOA.....stimulus onset asynchrony

SRF .....spatial receptive field

SUA.....single-unit activity

SZ.....stratum zonale

V1..... primary visual cortex

Chapter I

Introduction

Multisensory processing – essential for everyday life

The processing of sensory information from multiple modalities is essential to guide perception and behavior. Multisensory integration improves a multitude of behaviors. These include target detection, whereby the detection of visual or auditory target stimuli is enhanced when the target is paired with a simultaneous cross-modal stimulus (Fransinetti et al., 2002; Lovelace et al., 2003). This enhancement of detection, as well as enhancement of response times, has been examined and determined in a variety of response systems, including saccades and manual responses (Hershenson, 1962; Hughes et al., 1994; Frens et al., 1995; Murray et al., 2001; Corneil et al., 2002; Molholm et al., 2002; Amlot et al., 2003; Deiderich et al., 2003). Eye movements toward multisensory (audiovisual) targets exhibit the accuracy of visual saccades with the fast reaction time of auditory saccades, and this enhancement of saccadic eye movements with multisensory stimuli is found in both simple and complex scene scenarios (Frens et al. 1995; Corneil et al. 2002). Multisensory integration improves additional behaviors, as well, such as orientation (Stein et al., 1988, 1989), guided movements (Ghazanfar and Schroeder 2006; Zhang et al. 2004), speech processing (Barraclough et al. 2005; Calvert et al. 2000; Stevenson and James 2009), motion processing (Lewis et al., 2000), communication signaling (Sugihara et al. 2006; Keysers et al. 2003), perceptual decision-making (Romo et al., 2004) and localization (Wilkinson et al., 1996). In the past, it was assumed that individual sensory information was first processed separately, only to be combined later and in higher order areas (Felleman and Van Essen 1991; Kuypers et al. 1965; Massopust et al. 1965). Evidence now exists to the contrary.

Studies have shown that sensory information is not processed individually in any location within the brain.

Convergence of sensory modalities onto individual neurons is found in primary sensory regions, including primary auditory (Allman et al. 2008a; Allman et al. 2008b; Brosch et al. 2005; Cappe and Barone 2005; Fu et al. 2003; Ghazanfar et al. 2005; Kayser et al. 2005; Schroeder and Foxe 2002; Schroeder et al. 2001), visual (Falchier et al. 2002; Morrell 1972; Rockland and Ojima 2003) and somatosensory (Zhou and Fuster 2004; 2000) cortices. Evidence of multisensory convergence has been found all throughout the brain, including thalamic (medial geniculate nucleus: Graham 1977; Linke 1999: pulvinar: Jones 1985; Benevento and Fallon 1975; Benevento and Standage 1983; Fitzgibbon et al. 1995; Jones 2007; Rodringo-Angulo and Reinoso-Suarez 1988; suprageniculate nucleus: Benedek et al. 1996 Benedek et al. 1997; Berkley 1973) and higher order cortical regions (anterior ectosylvian and lateral suprasylvian cortex: Jiang et al. 2002; Wallace and Stein 1994; superior temporal sulcus: Barraclough et al. 2005; lateral intraparietal area: Gifford and Cohen 2004; Linden et al. 1999; Mazzoni et al. 1996; Snyder et al. 1998; ventral intraparietal area: Avillac et al. 2005; Bremmer et al. 2002; Schlack et al. 2005; temporoparietal association cortex: Leinonen et al. 1980; ventrolateral prefrontal cortex: Sugihara et al. 2006; premotor cortex: Fogassi et al. 1996; Fuster et al. 2000; Graziano 1999; Graziano et al. 1994). Building a system in this way may have many advantages. For instance, the cross-modal processing of sensory information in early cortical areas and early brain regions of signal processing may be a more efficient method of integration. Multisensory integration occurring in the thalamus helps to relay integrated signals to early cortical

areas (Giard and Peronnet 1999; Molhom et al. 2002), which can aid in multisensory binding capabilities. While multisensory convergence and integration takes place in a variety of brain regions, one of the most studied structures is the midbrain superior colliculus (SC) (Meredith and Stein 1983).

The superior colliculus

The SC (particularly the cat SC) is a perfect model in which to study multisensory processing due to its high incidence of multisensory neurons (Meredith and Stein 1996). In addition, the SC is involved in orientation behaviors, containing sensory and motor maps that are both aligned and spatially oriented (Sprague and Meikle 1965; Stein et al. 1988). Unilateral lesioning of the SC results in profound sensory neglect in the hemisphere contralateral to the lesion (Sprague and Meikle 1965). The mammalian SC is a laminated structure composed of seven layers (Kanaseki and Sprague 1974; May 2006). Based on morphology and physiology of neurons, connectivity with other brain regions, and behavioral correlates, the SC is normally divided into two functional layers: the superficial and deep layers.

The superficial SC

The superficial SC is composed of three layers, the stratum zonale (SZ), stratum griseum superficiale (SGS) and stratum opticum (SO). SZ is quite small, comprised of a few very small neurons (Sterling 1971). SGS is a larger layer compared to SZ, encompassing a variety of differently-sized neurons as well as horizontal interneurons (Huerta and Harting 1983; May 2006). SO is made up of mainly fibers, including incoming fibers from retinal axons, with very few cells spread throughout (Huerta and

Harting 1984; May 2006). The neurons lying within the superficial SC have many afferent connections. Based on connection architecture alone, the superficial layers are thought to be purely visual in nature. Projections from the retina are mainly to the SGS, with few sent to the SO (Graybiel 1975, 1976; Zhang and Hoffmann 1993; Pollack and Hickey 1979; Lund et al. 1980). Visual cortical areas send projections to the superficial SC, as well. V1 layer V pyramidal neurons project to the superficial SC, in tight visuotopic register with the retinal inputs found there (Hollander 1974; Kawamura and Konno 1979; Updyke 1977; Graham and Casagrande 1980; Harting and Noback 1971; Martin 1968; Symonds and Kaas 1978). There is also a plethora of connections from visual association areas. For instance, the rostral pole of superficial SC receives crossed input from layer V neurons of areas 17, 18 and 19 (Gilbert and Kelly 1975; Hollander 1974; Powell 1976; Updyke 1977). Additionally, areas 20a, 20b and the frontal eye field (FEF) project to SGS and SO (Harting et al. 1992; Hollander 1974; Norita et al. 1991; Kunzle and Akert 1977; Kunzle et al. 1976). The corpus callosum (Antonini et al. 1978, 1979), ventral lateral geniculate nucleus (LGN) (Edwards et al. 1979; Edwards et al. 1974; Graybiel and Hartwieg 1974; Kawamura et al. 1978; Nakamura and Itoh 2004; Swanson et al. 1974), parabigeminal nucleus (Graybiel 1978; Stevenson and Lund 1982) and nucleus of the optic tract (Edwards et al. 1979; Weber and Harting 1980) can also be included in the list of visually-inclined regions connected to the superficial layers.

Many connections of the superficial SC are reciprocal, both efferent as well as afferent. For instance, neurons within SGS have not only afferent connections from the dorsal LGN, but also efferent connections back to that same region (Harrell et al. 1982;

Kawamura et al. 1980; Baldwin and Kaas 2012; Harting et al. 1991; Wilson et al. 1995). The same is true for the parabigeminal nucleus (Baldwin and Kaas 2012; Casagrande et al. 1972; Harting et al. 1973). In addition, as the primary efferent layer, the SGS also sends efferent projections to the pulvinar (Huerta and Harting 1983; Lin and Kaas 1979; May 2006) and lateral posterior complex (Caldwell and Mize 1981; Kawamura et al. 1980; Mooney et al. 1984; Raczkowski and Diamond 1981; Rodrigo-Angulo and Reinoso-Suarez 1988; Benevento and Standage 1983; Huerta and Harting 1983). These projection patterns are unique to the superficial layers of the SC; the connections of the layers that comprise what is known as the deep SC are quite different.

Neurons located within the superficial SC respond well to large objects moving at a wide range of velocities (Waleszczyk et al. 2007). Upon closer examination of the spatiotemporal frequency profiles of these neurons, the majority respond optimally to very low spatial frequencies, and exhibit bandpass temporal frequency tuning; these properties are much like those found in retinal Y and W motion detector neurons which suggest that these superficial SC neurons are also part of that motion detection behavioral process. Additionally, neurons within the superficial layers of the SC are thought to be important in the “where” pathway of the visual system. Superficial SC neurons have a preference for low spatial frequencies combined with a large range of temporal frequencies in their targets. A large population of neurons within the superficial layers have been shown to respond well to large objects moving at a wide range of velocities (Waleszczyk et al. 2007), suggesting that the superficial SC neurons are less concerned with the exact type of stimulus being presented (“what”) and more interested in whether a stimulus is presented and its location (“where”).

Superficial layers of the SC have also been implicated in other visuomotor behaviors, such as reflex adjustment of the head and eyes (Waleszczyk et al. 2007) and form discrimination (Casagrande et al. 1972; Berlucchi et al. 1972; Sprague 1991; Tunkl and Berkley 1977)**.** Convergence of excitatory input from various visual information channels that have complementary temporal properties on superficial SC neurons, combined with the short pathway length of retina to superficial SC to deep SC, initiates fast orienting eye and head movement responses to sensory, specifically visual, stimuli (Waleszczyk et al. 2007). Lesions of this region result in deficits in the learning and performing of form discrimination tasks (Casagrande et al. 1972; Berlucci et al. 1972; Tunkl and Berkley 1977). The inability of animals to perform this task after superficial SC lesioning may result from anterograde degeneration in the connecting visual thalamic region (Casagrande et al. 1972). This was found only with lesions of the superficial SC; ablation of deep SC layers results in a multitude of other, different, deficits.

The deep SC

It is traditionally agreed that four layers make up what is considered the deep SC: stratum griseum intermediale (SGI), stratum album intermediale (SAI), stratum griseum profoundum (SGP), and stratum album profoundum (SAP). These layers are home to large and medium-sized neurons, with the larger neuronal types found in the more lateral 2/3 of the deep SC and smaller, medium-sized neurons in medial regions (Norita 1980). The deep SC has many connections to various brain regions, both efferent and afferent.

The deep layers of the SC receive projections from a multitude of sensory- and non-sensory-related brain areas. Like the superficial SC, deep SC layers receive visual input; however most of this comes from extrastriate visual areas such as areas 20, 21 and the anterior ectosylvian sulcus (AES) (Baleydier et al. 1983; Berson and McIlwain 1983; Kawamura and Konno 1979; Segal and Beckstead 1984; Tortelly et al. 1980, Mucke et al. 1982). Additionally, unlike the superficial areas, the deep SC receives auditory-related afferents from the AES (Meredith and Clemo 1989), dorsomedial periolivary nucleus (Edwards et al. 1979), the inferior colliculus and nucleus of the lateral lemniscus (Henkel 1983; Kudo 1981; Kudo and Niimi 1980; Moore and Goldberg 1966). The deep SC also receives projections from somatosensory-related regions, such as area SIV (Clemo and Stein 1982; McHaffie et al. 1988; Stein et al. 1983), rostral suprasylvian sulcus (Clemo and Stein 1984; Stein et al. 1983), contralateral sensory trigeminal complex, dorsal column nuclei, and the spinal cord (Blomquist et al. 1978; Edwards et al. 1979; Huerta 1984). As a motor-related as well as a sensory structure, the deep SC also receives motor projections from various brain areas. The frontal eye field (FEF) sends ipsilateral projections to the deep SC layers (Fries 1984; Huerta and Kaas 1990; Huerta et al. 1986; Kawamura and Konno 1979; Komatsu and Suzuki 1985; Leichnetz and Gonzalo-Ruiz 1996; Schlag and Schlag-Rey 1970). Ipsilateral projections from the substantia nigra (Moschovakis and Karabelas 1985; Beckstead et al. 1981), nucleus of the posterior commissure (Christoff 1974; Huerta and Harting 1982), deep nuclei of the cerebellum (Edwards et al. 1979) are also sent to the deep SC. Additional afferents arrive to the contralateral SC from the hypothalamus,

locus ceruleus, raphe, parabrachial nucleus, reticular formation, and reticular nucleus of the thalamus (see May 2006 for review).

Along with the multitude of afferents to the deep SC, there are many efferent connections from these layers. The primary output of the deep layers lies within what is known as the predorsal bundle (Huerta 1984). These axons arise from SGI and SGP cells and run in the contralateral mesencephalic reticular formation (cMRF) before crossing the midline and descending to the cervical spinal cord (May 2006), targeting in the paramedian pontine reticular formation (PPRF) (Cowie and Holstege 1992; Grantyn and Grantyn 1982). These cells have also been shown to terminate in the pons (Cowie and Holstege 1992). Additional efferent targets of the deep SC include cerebellar nuclei, inferior olivary nuclei (Graham 1977; Mower et al. 1979), and the suprageniculate nucleus of the thalamus (May 2006; Stein and Meredith 1993). It is these thalamic connections that are thought to be involved in the deep SC's role in object tracking. Lesions of the deep SC result in an animal's inability to track objects (Casagrande et al. 1972). The ablation of these layers which causes this behavioral deficit may be due to degeneration of nonvisual thalamus and brainstem motor areas downstream of the SC.

A connection between superficial and deep SC layers?

Historically, it was viewed that the superficial and deep layers functioned as two separate entities and thus it was assumed that they did not interact (Sprague 1975; Ogasawara et al. 1984). However, reciprocal connections between the superficial and deep SC layers have since been found (Behan and Appell 1992; Behan and Kime 1996; Doubell et al. 2003). These connections are sparse, involving very few, very small

terminals mainly housed within the SO and SGI layers (Huerta and Harting 1982b; Huerta MF 1984; Edwards 1977; Huerta et al. 1981; Lee and Hall 1995). Though unclear, it may be that these reciprocal connections serve as pathways for more visual information to reach the deep, and multisensory information to reach the superficial, SC (Doubell et al. 2003).

The functional importance of the SC

Given the vast connectivity of the SC, it is unsurprising that this structure has been implicated in many important processes of the brain. One historical emphasis that has been placed on the SC is its role in oculomotor processing, specifically saccade generation. The generation of saccadic eye movements requires a team of different neuronal subtypes found in various brain regions; the SC is one region where some of these neurons are found. Saccade related burst neurons and fixation neurons are found in the deep layers of the SC (Stuphorn et al. 2000). Fixation neurons are associated with suppression of saccades through excitatory connections in the brainstem (Quaia et al. 1999; Munoz and Wurtz 1995; Pare and Guitton 1994), while burst neurons activate prior to the initiation of a specific saccade, after the release of inhibition with activity termination of fixation neurons (Munoz and Wurtz 1995a, 1995b). These neuronal types, in conjunction with other neurons of saccade initiation, are essential for normal saccadic eye movements, making the SC critical for the process of eye movement generation, as well as orientation and localization.

Lesion studies have established the essential role the SC has in spatial localization, orientation responsivity, and as such, the coding of objects in the visual

world (Casagrande et al. 1972; Harting et al. 1973; Stein et al. 1976a; Sprague and Meikle 1965; Schneider 1969; Sprague 1996). The SC is also important in motion perception (Benedek et al. 1988; Burke et al. 1998; Mucke et al. 1982) as well as visual form discrimination and perception (Anderson et al. 1971; Berlucchi et al. 1972; Sprague 1991; Sprague et al. 1970; Sprague et al. 1977; Tunkl and Berkley 1977). These SC-mediated behaviors are facilitated under multisensory conditions, with improvements in speed and accuracy of responses (Bell et al. 2005; Burnett et al. 2004; Burnett et al. 2007; Diederich and Colonius 2004; Frens et al. 1995; Gingras et al. 2009; Hughes et al. 1994; Jiang et al. 2002; Nozawa et al. 1994; Stein et al. 1988; Wilkinson et al. 1996). Lesions of the SC (specifically the deep layers) result in a loss of this multisensory facilitation of responses. While animals continue to properly orient to sensory and multisensory cues in their surrounding environment, no benefit in response from multisensory targets remains; increased accuracy of response to multisensory as compared to unisensory targets is no longer observed with the ablation of the SC (Burnett et al. 2004). Thus, one major procedure of the SC is multisensory processing and integration, and the sensorimotor transformation that allows for this multisensory processing to influence motor output behaviors. An important piece of this transformation may lie in the sensory and motor topography of the SC.

Organization of the SC: maps

Within the SC lie various sensory maps. A visual map exists throughout the entire SC, with more rostral SC cells exhibiting a sensory receptive field (RF) in the nasal visual space, and temporal SC cells representing more caudal locations (Feldon and Kruger 1970, Meredith and Stein 1993). Within this visual map, the representation

of the horizontal meridian of visual space runs from the front to rear of the SC, while the vertical meridian of visual space is represented along the medial aspect with the upper visual field medially and the lower visual field laterally represented. Neurons in the superficial SC have much smaller RFs compared to the deeper SC visually-responsive neurons (Meredith and Stein 1990). Thus, the visual map in the deep SC is much coarser compared to that found in the superficial layers. The deep SC visual map encompasses the entire contralateral visual field, while also extending into ipsilateral space approximately 40° (Meredith and Stein 1990, 1993). Additionally, visuotopy is the most secure at the rostral locations of the map, and becomes increasingly poorer moving caudally and laterally (Meredith and Stein 1990). The activity of a single neuron or a small group of neurons cannot predict the exact location of a stimulus, and a small stimulus activates a large number of neurons in the SC.

Somatosensory RFs of deep SC cells are large and organized into a topographic map (Stein et al. 1976). Due to these large RFs, there is a great amount of overlap of representations of body regions that are adjacent to one another (Stein and Meredith 1993). The head is the most represented bodily area, encompassing nearly the entire half of the rostral SC. The forelimb is represented in the lateral aspects of the caudal 2/3 of the SC, while the rest of the body representation is compressed into an area that overlaps a portion of the forelimb representation and extends into a small caudal zone (Meredith et al. 1991). This somatosensory map is aligned nicely with the visual map in the deep SC, as the face regions are aligned with visual RFs representing the area centralis. While the visual and somatosensory maps are in a spatial construct, the topography of auditory RFs is different.

Auditory neurons in the deep layers of the SC are selective for a location of a sound source, and this selectivity shifts as a function of neuron position within in SC in order to form a continuous map of auditory space. In essence, the neurons are differentially sensitive to interaural time and intensity differences (Gordon 1973; King and Palmer 1983, 1985; Middlebrooks and Knudsen 1984). Throughout this map, the horizontal dimension of space is mapped rostrocaudally, and the vertical dimension of space is mapped mediolaterally (Middlebrooks and Knudesen 1984). This mapping schema is quite similar to that of the visual mapping topography, making it conceivable and quite likely that auditory and visual stimulus presentations from similar spatial locations will activate the same neuron clusters.

Within the SC also exists a motor map, based on the role of the SC in saccadic eye movements. Saccade related burst neurons and fixation neurons, important for the initiation of proper eye movements, are found within deep layers of the SC, and a topographic map of saccadic eye movements exists within these layers (Stuphorn et al. 2000). Fixation neurons are located at the rostral poles (Quaia et al. 1999; Munoz and Wurtz 1995a). Saccade related burst neurons encoding large eye movements are located caudally, while neurons involved in smaller eye movement generation are located rostrally (Soetedjo et al. 2002). These populations of neurons discharge immediately preceding saccades of specific distance and direction, and the inactivation of these cells results in alterations of saccade speed, duration and trajectory (Wurtz and Goldberg 1971; Dorris et al. 1997; Lee et al. 1988; Colonius and Arndt 2001; Schiller et al. 1980).

Response properties of SC neurons

Sensory neurons within the SC are incredibly diverse in their responsivity to various sensory stimuli. Below briefly describes some of the response properties of each of these different neuronal types of neurons found in the superficial and the deep SC layers.

Superficial SC neurons

The population of visually responsive neurons within the superficial layers of the SC is diverse in its responsivity to stimuli. Visual neurons in these layers tend to respond more optimally to moving rather than stationary visual stimuli, though some will respond to flashing spots of light in an ON-OFF manner similar to responses of retinal ganglion cells (Kuffler 1953). Some neurons also have a preference for high temporal frequencies of movement (Waleszczyk et al. 2007; Sterling and Wickelgren 1969), though slow velocity and medium velocity-preferring neurons also exist (Dreher and Hoffmann 1973). Most superficial visual neurons are at least partially direction-selective, responding vigorously to stimuli moving through their excitatory RF region in one preferred direction but weakly when the stimuli are moving in the opposite direction (Dreher and Hoffmann 1973). Repeated stimulus presentations result in response habituation in these neurons. Visually responsive neurons within the superficial SC can have differing RF organizations, as well. While many of these neuronal types exhibit a single excitatory region within their RF, some have multiple, spatially segregated excitatory regions encompassed within their RF and can also have a suppressive, inhibitory region surrounding the excitatory regions (Dreher and Hoffmann 1973). Thus,

the architecture of the RF imposes restrictions on the size of the visual stimulus which will optimally stimulate the neuron; optimal stimuli must move through the excitatory, but not inhibitory, regions of the neuron's RF. Visual neurons of the superficial SC, while exhibiting complex response profiles and properties, are an important part of the visual processing data stream (Hicks et al. 1986).

Deep SC neurons

Sensory neurons located in the deep layers of the SC also have complex response profiles. This complexity encompasses RF properties, neuronal response types and even preferences for specific stimuli. Deep layer neurons receive inputs from multiple modalities, so it is no surprise that there exists multiple types of sensory neurons within these layers. The ratio of these different types of neurons depends on the species studied, due to the importance of the SC in spatial orientation. For example, visual-auditory neurons are more prevalent in the cat SC compared to the rodent SC because of the dependence the cat places on vision and audition for orientation purposes. Rodents, relying much more on tactile inputs for orientation with the world, have more somatosensory neurons in their SC (Meredith and Stein 1996; Drager and Hubel 1975; Weldon and Best 1992). However, visual, auditory and somatosensory-responsive neurons exist in both cat and rodent deep layer SC. Though these neurons may all be classified as sensory responsive neurons, their response profiles are vastly different from one another.

Visually-responsive neurons found in the deep layers of the SC are similar, but not identical, to visually-responsive neurons of the superficial SC layers. Like superficial

neurons, deep layer visual neurons are typically binocular and most optimally responsive to moving stimuli that are similar in shape to their RF, but they will also respond sub-optimally to stationary stimuli, and habituate responses to repeated stimulus presentations (Sterling and Wickelgren 1969; Meredith and Stein 1996). Additionally, like superficial neurons, most deep layer visual neurons exhibit at least partial direction selectivity (Meredith and Stein 1993, 1996). However, there are some differences to the response profiles of deep layer visual neurons. The mean latency of responses in deep layer neurons is longer than that of superficial neurons, by approximately 30 ms (Sterling and Wickelgren 1969; Meredith et al. 1987; Wurtz and Albano 1980). Deep layer visual neurons also have bigger RFs than superficial neurons, by approximately 50° (Meredith and Stein 1990; 1996).

Like most visual neurons, somatosensory neurons in the deep layers of the SC also have large, well-defined RFs and clear best regions of response (Stein and Arigbede 1972; Clemo and Stein 1991). Very few of these neurons have suppressive surrounds or directional selectivity, and they respond well to stimulation of the skin and hair, preferring higher velocity stimuli (Clemo and Stein 1987; Stein et al. 1976). In response to maintained stimuli, somatosensory neurons also show response habituation, indicating that these neurons are better suited to respond to novel stimuli that elicit orientation responses (Clemo and Stein 1986, 1984, 1991; Stein et al. 1976).

Deep layer SC neurons responsive to auditory stimulus presentations respond poorly to pure tones, preferring complex sounds composed of multiple frequencies instead. Like visual neurons, auditory neurons usually exhibit at least partial direction selectivity, prefer moving stimuli as compared to stationary stimuli, and habituate to

repeated stimulus presentations (Gordon 1973; Horn and Hill 1966; Wickelgren 1971; Rauschecker and Harris 1989; Wise and Irvine 1983). The majority of auditory neurons in the deep layers of the SC are binaural in nature, differentially sensitive to interaural time and intensity differences. These differing sensitivities allow auditory neurons to be categorized into four different neuronal groups based on preference for ipsilateral or contralateral ear stimulation (Wise and Irvine 1983, 1985). EO/I neurons are excited by inputs from the contralateral ear alone, and exhibit an inhibition of response when the two ears are stimulated together. EE/F neurons respond to stimulation of either ear alone and exhibit a facilitated response when both ears are simultaneously stimulated. EO/F neurons respond to stimulation of the contralateral ear alone and exhibit a facilitation of response when both ears are stimulated together. OO/F neurons do not respond to either ear stimulated alone, but exhibit a facilitation of response when both are stimulated simultaneously (Wise and Irvine 1983, 1985). By integrating inputs from the two ears, these auditory neurons build RFs that have regions of maximal response, termed ‘best areas.’ These best areas are different for each type of neuronal group. EE/F neurons are the exception, not having a best area because they will respond to stimuli anywhere in auditory space. EO/F and OO/F type neurons have RFs whose best areas are within 20° of the frontal midline, while EO/I neurons have RFs with best areas reflecting the contralateral hemifield regions (King and Palmer 1983; Middlebrooks and Knudesen 1984). Together, these auditory neurons have maximal regions that cover the whole of auditory space.

The convergence of inputs from different sensory modalities in deep SC neurons renders multisensory properties to a great number of these neurons, and there is a

large amount of correspondence in the visual, auditory and somatosensory maps of a neuron that is responsive to all three stimulus types (trimodal). The optimal stimulus locales and characteristics for these types of neurons are where the RFs and properties correspond with one another, where they are overlapping. In the overlap of unisensory maps, a multisensory map is found (Stein and Meredith 1993). This map, along with these neuronal response properties, is incredibly important for the process of multisensory integration.

Multisensory integration within the SC

To properly interact with the surrounding world, the brain must determine which stimuli are related to one another and which are not. Neuronal responses can be enhanced with certain stimulus combinations while their responses can be depressed in response to other combinations. Neurons in the SC exhibit enhancements and depressions to these types of stimulus combinations, which in turn influence and guide SC-related behaviors. This ability of the SC neurons to combine stimuli from multiple modalities is defined as multisensory integration. There are two main types of multisensory integration that take place in SC cells. Neurons that show outright spiking responses to stimuli from two or more sensory modalities are considered overt multisensory neurons. Covert multisensory neurons are those which overtly respond to stimuli from only one sensory modality, but the response to that stimulus modality is modulated by the presentation of a sensory stimulus of a second modality. These are also known as modulatory multisensory neurons. Both overt and covert multisensory neurons engage in multisensory integration. The response of a multisensory neuron to a multisensory stimulus is drastically different compared to a response of that same

neuron to a unisensory stimulus presentation. If this multisensory response is statistically significantly greater than the best unisensory response, it is defined as a multisensory response enhancement. A multisensory response that is statistically significantly lower than the best unisensory response is defined as a response depression. Both response enhancements and response depressions can be quantified using the interactive index metric and mean statistical contrast.

Quantifying multisensory integration

Interactive index (ii) is a method of quantifying multisensory integration. ii is defined as the percent difference in mean number of stimulus-driven action potentials evoked by a cross modal stimulus and that evoked by the most effective modality-specific stimulus component (Meredith and Stein 1986a, 1986b; Stein and Meredith 1993, Carriere et al. 2008; Perrault et al. 2003, 2005; Stanford et al. 2005). This magnitude of change is calculated by

$$[(CM - SMmax)/(SMmax)] \times 100 = \% \text{ interaction}$$

where CM is the mean response evoked by the combined modality (i.e., multisensory) stimulus and SMmax is the mean response evoked by the most effective single modality stimulus (Meredith and Stein 1983; Stein 1986). Spontaneous activity is always subtracted for this calculation. Mean statistical contrast (msc) is a quantification of multisensory integration which compares the responses of a neuron to a multisensory stimulus and the predicted addition of the unisensory responses (Meredith and Stein 1986a, 1986b; Stein and Meredith 1993, Carriere et al. 2008; Perrault et al. 2003, 2005; Stanford et al. 2005). This method takes into account the neuron's responses to all

unisensory stimuli, not only the best unisensory stimulus as in ii. As an example, msc for an audiovisual neuron is calculated by

$$msc = \sum[(SA - A) - (V - VA)] \div n$$

where SA is the spontaneous activity, A is the response of the cell to auditory stimuli, V is the visual response of the cell, VA is the response of the cell to audiovisual multisensory stimulus presentations, and n is the number of trials. This model assumes independence of each sensory modality. When msc > 0, multisensory responses of the cell are greater than the sum of the unisensory responses. This is known as superadditive responsivity. When msc < 0, multisensory responses of the neuron are less than the sum of the unisensory responses, also known as subadditive responsivity (Carriere et al. 2008; Perrault et al. 2003, 2005; Stanford et al. 2005). It is possible for the same neuron to engage in both subadditive and superadditive multisensory integration (Stanford et al. 2005; Stanford and Stein 2007). Both ii and msc are important tools for the quantification of multisensory integration occurring at the single neuron level in the SC.

Integration factors - the principles of multisensory integration

Multisensory SC neurons are charged with the difficult task of binding and combining related stimuli while preventing the binding of unrelated stimuli. To do this properly, these SC neurons use specific aspects of the stimuli in order to determine which stimuli should be integrated and which should not. Early studies of the coding of multisensory integration have put these aspects into what is known as the principles of multisensory integration (Meredith et al. 1987; Meredith and Stein 1986).

There are three main principles of multisensory integration. The first is known as the spatial principle. Studies have shown that there exists a strong relationship between the proximity of multisensory stimuli and the interactions that result from their combination. More spatially coincident stimuli tend to result in response enhancements, and spatially disparate stimuli result in response depressions. These are dependent on the relationship of the sensory RFs to one another rather than the absolute spatial relationship of the specific stimuli to one another (Meredith and Stein 1996). The greatest amount of multisensory enhancement is produced by stimuli located in areas of sensory RF overlap. Increasing the spatial disparity between the sensory stimuli increases the incidence of multisensory inhibition generated when one stimulus is within its excitatory RF while the other stimulus is within its inhibitory RF, which will ultimately lead to response depression (Kadunce et al. 1997).

The second principle of multisensory integration is known as the temporal principle. Studies have shown that the largest gain of response to a multisensory stimulus compared to a unisensory stimulus is seen when the peak discharge periods of individual sensory responses overlap (Meredith et al. 1987). As the stimulus onset asynchrony (SOA) between auditory and visual stimulus presentations becomes larger, and the peak discharge periods of the stimuli become less overlapping, the magnitude of enhancement generally declines. In fact, if the SOA becomes large enough, the response enhancements seen for multisensory stimulus presentations can turn to response depressions. Most often, the maximum response enhancement is found when there is very minimal temporal disparity between stimuli. However, this is not true for all neurons. A preference for asynchronous combinations of stimuli can be accounted for

by the differences in input latencies for the two modalities. For example, in the SC, the average visual latency is 70 ms, which the average auditory latency is 15 ms, and the peak temporal tuning for many SC cells is around 50 ms, with the auditory lagging behind the visual stimulus presentation by 50 ms. This makes sense given the SC visual and auditory input latencies.

The third principle of multisensory integration is known as the principle of inverse effectiveness, stating that the weaker the component of unisensory stimuli eliciting a response, the larger the magnitude of multisensory integration (Meredith and Stein 1986b; Wallace et al. 1998; Wallace and Stein 1996). As the effectiveness of a single modality to elicit a response from the neuron increases, the gain of response from a multisensory stimulus presentation decreases. So, minimally effective unisensory stimulus combinations produce the largest multisensory enhancement gains. Although there is a large amount of evidence supporting the principle of inverse effectiveness, there are some constraints and limitations. Biologically, neurons cannot produce negative numbers of spikes or have a response rate higher than a certain threshold, thus there are floor and ceiling effects of neuronal responses. So, if a unisensory response is equal to the minimum possible response, then the multisensory response can only be the same as or higher than that unisensory response (Holmes 2009). Similarly, if a unisensory response is equal to the highest possible response rate of a neuron, the multisensory response could only be equal to or lesser than that unisensory response and cannot be improved. This produces confounds in the interpretation of magnitudes of multisensory enhancements and depressions. However, this can easily be accounted for by considering the dynamic range of the responsiveness of the

neurons at the start, altering the parameters of the stimuli accordingly to avoid these floor and ceiling effects (Alvarado et al. 2007; Perrault 2003, 2005).

Integration factors - neuronal factors important for multisensory integration

In addition to these three principles of multisensory integration, more recent studies have suggested that other neuronal factors play into integrative capacities. One such factor is the level of neuronal spontaneous activity. Spontaneous activity of a neuron, as well as its dynamic range, is an important determinant of the amount of multisensory integrative capacities it has. There is an inverse relationship between the level of spontaneous activity of a neuron and the magnitude of multisensory integration that it can produce (Perrault et al. 2003, 2005). Neurons with lower spontaneous activity tend to show higher magnitudes of multisensory enhancements compared to those with higher spontaneous activity. However, unisensory responsiveness is still a more reliable measure of multisensory integration of a neuron than spontaneous activity.

Integration factors - circuit-level factors important for multisensory integration

Another important factor which plays into the integrative capacities of SC neurons is the impact of cortical neurons on SC multisensory neurons. The influence of the AES on SC neurons is imperative for proper multisensory integration to occur. Deactivation of the AES via cooling results in the loss of some SC neuron's ability to integrate cross-modal cues while maintaining normal unisensory responsiveness (Wilkinson et al. 1996; Wallace and Stein 1994). Cooling of the rostral lateral suprasylvian sulcus (rLS) does much the same for some SC neurons, often a different subset of SC neurons compared to those affected by AES cooling (Jiang 2000; Jiang et

al. 2002). The ablation of either of these regions in neonatal life disrupts the maturation of multisensory integrative abilities in SC neurons (Jiang et al. 2006; Wallace and Stein 1997). These studies show that there are many factors contributing to proper multisensory integration, which in turn influence SC-mediated behaviors. An essential factor that is critical for normal multisensory integration is proper sensory experience throughout development.

The importance of sensory experience

Visual (Wallace et al. 2004, 2007) or auditory (Xu et al. 2014) deprivation during maturation obstructs the capacity of multisensory SC (Wallace et al. 2007; Xu et al. 2014) and cortical (Carriere et al. 2007) neurons to integrate information from different modalities. Any altered sensory experience during this stage of development drastically modifies these neurons' integrative capacities. For example, the rearing of animals in a spatially discordant environment results in the development of cells with spatially offset RFs (Wallace et al. 2007). The multisensory integrative system is incredibly plastic throughout developmental maturation, and alterations in sensory experience here can radically change multisensory processing that manifest not only in SC neuron spiking, but also at the behavioral and perceptual levels (Collignon et al. 2009, Eimer 2004, Hotting and Roder 2009, Leo et al. 2008, Putzar et al. 2007, 2012, Roder et al. 2004, Guerreiro et al. 2015, Hauthal et al. 2015, Occelli et al. 2013). Restoration of visual experience in adulthood, after developmental stages, has minimal effects on multisensory processing, with most neurons continuing to lack integrative capacity (Royal et al. 2010). Not only is sensory experience important for multisensory integration during developmental periods, normal sensory experience is critical

throughout a lifetime. Previous research has also suggested that late-onset sensory deprivation, during adulthood, precipitates deficit effects at the behavioral and functional levels (Focker et al. 2015, Tao et al. 2015, Collignon et al. 2013, Champoux et al. 2011, Voss et al. 2004, Burton et al. 2002, Kujala et al. 1997), providing evidence for the existence of plasticity throughout life. For example, both congenitally blind and late-onset blind individuals show above-average spatial abilities, suggesting that the compensatory plasticity mechanisms behind these abilities can occur in the adult brain of late-onset blind, not just the developing brain of congenitally blind, individuals (Voss et al. 2004). Further evidence for plasticity in the adult brain was found when investigating event-related potentials in early- and late-onset blind compared to sighted individuals while detecting auditory pitch changes (Kujala et al. 1997). Results revealed that event-related potentials found in both early- and late- onset blind groups were different than those found in the sighted group, but not from each other. This suggests that cross-modal reorganization occurs even in mature human brains (Kujala et al. 1997). Evidence supporting the idea of plasticity throughout life can also be found at the single neuron level in the SC. Restricting and selectively altering experience shows that, even at maturity, multisensory neurons cannot integrate cross-modal stimuli if they do not have enough experience with these stimulus modalities, even if their unisensory responsivity is normal (Yu et al. 2010; Xu et al. 2014). Sensory experience is one very important part of development that, if altered in any way throughout life, can have drastic consequences on the multisensory integrative capacities of SC neurons. This can cascade into a wealth of dysfunctions in SC-mediated behaviors. However, sensory experience is only one element of healthy development that influences multisensory

integration. Proper neurotransmitter balance is also essential for sensory, and multisensory, processing.

The actions of neurotransmitters in the SC

The SC is an extremely diverse brain region in its connections, functions, and neurotransmitter (NT) expressions. Many various NTs are expressed and play important roles in the SC. One such NT is glutamate (Glu). Glu is found throughout the SC, most abundantly in the superficial layers, specifically within SGS and SO (Binns 1999; Fonnum et al. 1979). A major transmitter in the retino-collicular and cortico-collicular pathways, Glu is found postsynaptically, within retinal and cortical terminals in the SC, and is greatly reduced in instances of retinal and/or cortical deafferentiation (Fosse et al. 1984; Sakurai et al. 1990; Sakurai and Okada 1992; Jeon et al. 1997; Mize and Butler 1996). Studies utilizing cultured SGS neurons from SC have shown that responses to stimulation are blocked in the presence of Glu receptor antagonists (Grantyn et al. 1987). Additionally, injections of Glu receptor antagonists into either superficial or deep layers limits the responsiveness of the non-injected layers to visual stimuli, indicating both that the reciprocal connections between superficial and deep SC layers are essential for proper visual responsiveness in general, and that this system is dependent on Glu (Isa et al. 1998). Visual response habituation of SC cells depends on Glu, as this intrinsic circuit is activated by both NMDA and non-NMDA receptor types and is blocked in the presence of antagonists activated at these receptor sties (Binns 1999). These studies implicate Glu involvement in SC visual responsivity (Binns and Salt 1984; Isa et al. 1998).

As alluded to previously, Glu is also found within the deep SC layers. Large neurons in the deep SC express NMDA receptors (Mize and Butler 1996), and these receptors may play a role in the multisensory processing occurring here. Iontoporetic injection of an NMDA receptor antagonist, AP5, reduces both unisensory and multisensory responsivity of deep SC neurons, decreasing multisensory responsiveness to a greater extent than it decreases unisensory responsiveness (Binns and Salt 1996). Thus, it is possible that the NMDA receptors are critical not only for unisensory, but also multisensory processing. It is also possible that the increased importance of NMDA receptors for multisensory responses arises because there exists a high density of receptors at synaptic locations with inputs from the cortical region AES, this input being essential for integrative responses in the SC (Binns 1999).

The transmitter Glu has also been suggested to play roles in the SC outside of sensory processing. Motor feedback to the SC is an excitatory projection that is dependent on Glu (Ghitani et al. 2014). Long term potentiation and the addition of new, functional synapses within at least the superficial layers requires Glu transmission (Zhao et al. 2006). NMDA receptors have been shown to play a crucial role in activity-dependent remodeling of synaptic connections in the fetal SC, important for axonal growth and regeneration (Sakata et al. 2006; Turner et al. 2005). Even tectotectal projections between SC are Glu-dependent (Olivier et al. 2000). Glu is an essential transmitter for the SC, however it is not the only neurotransmitter important for proper SC functioning. Gamma-aminobutyric acid (GABA) is also highly important. Among other functions, tectotectal connections are equally dependent on both Glu and GABA (Olivier et al. 2000).

The SC is abundant with GABA, having one of the highest levels of glutamate decarboxylase (GAD), the enzyme responsible for decarboxylation of Glu to GABA, in the entire brain (Binns 1999; Bowery et al. 1987). Along with Glu, GABAergic postsynaptic currents are found as early as postnatal day 1 in the SC of rodents (Juttner et al. 2001). Approximately 45% of cells within the SGS and 30% in the SGI are GABAergic (Mize 1988), receiving these inhibitory inputs from LGN and pretectum in the superficial SC layers, and the substantia nigra, zona incerta and brainstem into the deep SC layers (Appell and Behan 1990; Araki et al. 1984; Ficalora and Mize 1989). Three main types of neurons in the SC have been found to accumulate GABA: horizontal neurons, pyriform neurons and stellate neurons (Mize 1992; Meredith and Ramoa 1998). These neurons have wide dendritic fields and their GABA receptors are found both pre- and post-synaptically (Endo et al. 2003; Calabresi et al. 1990; Price et al. 1987). The GABA transmitter fulfills extremely important functions throughout the SC, including disinhibition (Mize 1992), a maintenance of activity balance between the two colliculi (Mize 1992) and between the superficial and deep SC layers (Katsuta and Isa 2003), disinhibition of information projection to the thalamus and brainstem (Schmidt et al. 2001) and the regulation of spontaneous activity (Chevalier et al. 1985; Buee et al. 1986).

GABA is incredibly important in the generation of sensory inhibitory surrounds and response habituation (Binns 1999). The iontophoretic application of GABA receptor antagonists reduces response habituation, and reduces the degree of surround inhibition of neurons, allowing neurons to have a greater response than normal to sensory (visual) stimulus presentations (Binns 1999). These intrinsic inhibitory circuits

are found all throughout the SC including deep layers, as many of the GABAergic neurons in SGS have long-range dendritic fields innervating deeper SC layers (Meredith and Ramoa 1998). In addition, GABA has been implicated in sensory processing occurring in the SC, specifically in situations of sensory deprivation.

Visual deprivation alters (multi)sensory responsivity of neurons within the SC, and some of this alteration may be mediated by changes in GABA expression in this brain region. Early visual experience has been shown to maintain GABAergic inhibition, and without this sensory experience, GABA receptor type B-mediated mechanisms such as paired-pulse depression increase (Balmer and Pallas 2015). This suggests that visual experience maintains GABAergic inhibition and prevents alterations of short-term depression in the SC (Balmer and Pallas 2015). Additionally, visual deprivation causes a loss of RF refinement, and it is suggested that this is partially due to the weakened inhibitory surround due to a loss of GABAergic inhibition (Carrasco et al. 2011). GABA plays a vital role in shaping activation fields, topographical maps and long-range inhibition within the SC; proper development of these important functions is dependent on both sensory experience and normal GABA mediation (Sooksawate et al. 2011).

Another abundant neurotransmitter with modulatory processes important for SC functioning is acetylcholine (ACh). ACh is distributed throughout most SC layers, with increased concentration in SGS, patches throughout SGI, and areas of SGP (McHaffie et al. 1991). While possibly important for other important functions, a vast majority of the work completed with ACh in the SC has involved sensory processing. The primary cholinergic input to the superficial SC arises from the parabigeminal nucleus, reciprocally connecting specifically visually-responsive areas in both brain regions

(Sefton and Martin 1984). Cholinergic neurons of the parabrachial region project to both dorsal lateral geniculate nucleus of the thalamus as well as the intermediate SC layers. The application of nicotinic agonists reduces visual responsiveness of these SC neurons, while application of antagonists potentiates responsiveness (Binns 1999), suggesting that the neurons in these regions relay visuosensory information to the cortex with the onset of orienting movements (Billet et al. 1999). It is thought that ACh may directly facilitate the release of GABA via nicotinic receptors on inhibitory neurons, reducing visual responsivity (Binns 1999).

Cholinergic input to the deep SC layers arrives from the pendunculopontine tegmental nucleus, innervating sensory and non-sensory related neurons in these regions (Krauthamer et al. 1995). Cholinergic activity has been suggested to influence saccadic activity (Krauthamer et al. 1995; Aizawa et al. 1999), tactile-evoked responses (Bezdudnaya and Castro 2014), and sensory map formation (Wang et al. 2009). Microinjection of nicotine into the SC increases short latency saccades for those that are represented in the location of injection site, suggesting that the activation of nicotinic ACh receptors in the SC can facilitate initiation without playing a very influential role in the dynamics of visually-guided saccades (Aizawa et al. 1999). Cholinergic neurons in this region may be involved in the relay of visuosensory information to the cortex with the onset of orientation movements (Billet et al. 1999). These nicotinic ACh receptors are also involved in the regulation of GABAergic inhibition, modulating visual processing. These receptors are extremely important in sensory map formation. A lack of the β2 subunit of ACh receptors results in the development of imprecise visual maps within V1 and the SC. Visually-responsive neurons within the SC have enlarged RFs

and decreased orientation and directional selectivity (Wang et al. 2009). This is true throughout the entire visual system; without β2 subunits in ACh receptors, the visual sensory system cannot anatomically and functionally develop normally (Rossi et al. 2001). The cholinergic system is important in the SC, and is necessary for its normal function.

Serotonin (5-HT) is the most widely distributed neurotransmitter in the brain (Dalhstrom and Fuxe 1964; Steinbusch 1981; Hay-Schmidt 2000), so it is no surprise that it is also found throughout the layers of the SC. Serotonergic innervation of the SC originates in the dorsal raphe nuclei and is essential for the proper development of the sensory representations that provide the SC with its distinctive spatiotopic organization. More generally, 5-HT has been shown to play an important role in the development of representations across the brain (Dalhstrom et al. 1964; Lesch 2011; Ueda et al. 1985; Janusonis et al. 1999; Mize et al. 1988; Villar et al. 1988; Arce et al. 1992; Huang et al. 1993; Binns et al. 1999; Lauder et al. 1982; Mize and Horner 1989; Rhoades et al. 1990). 5-HT innervation of the SC is completely established well before sensory map formation and multisensory processing capabilities are fully developed (Rhoades et al. 1990; Mize and Horner 1989). Alterations in this innervation result in sensory processing-related changes. For example, increasing 5-HT in the developing SC affects sensory RF size, resulting in stimulus responsivity alterations (Ke et al. 1999). Outside of the SC, 5-HT has been shown to play an important role in sensory processing in other areas of the brain. Visual and somatosensory cortical regions, along with subcortical regions such as the inferior colliculus (IC), rely on 5-HT for proper filtering

and processing of stimuli (Waterhouse et al. 1990; Jitsuki et al. 2011; Huang et al. 1993; Hurley 2011; Hurley and Pollack 2005; Hurley and Sullivan 2012; Hurley et al. 2005).

5-HT is also a mediator of cross-modal reorganization; sensory dysfunction in one modality due to sensory deprivation, for example, can lead to improvement of remaining modalities through 5-HT-mediated plasticity (Takahashi et al. 2013; Vetencourt et al. 2011). Visual deprivation increases extracellular 5-HT in the juvenile rat barrel cortex, which induces facilitation of synaptic delivery of AMPA-type Glu receptors in the cortex via the activation of 5HT2a receptors. This causes a sharpening of functional whisker barrel maps in the barrel cortex, consistent with previous research. Thus, sensory dysfunction of one modality leads to improvement of another modality by the refinement of cortical organization through 5-HT signal-mediated facilitation of synaptic remodeling (Takahashi et al. 2013). The 5-HT system has also shown involvement in plasticity mechanisms in the adult animal (Vetencourt et al. 2011). Adult rats treated with 5-HT1 receptor antagonist WAY-100635 restored visual cortex susceptibility to reorganization via monocular deprivation, suggesting that the 5-HT system underlies reactivation of plasticity in the visual system (Vetencourt et al. 2011).

The 5-HT system, along with the other neurotransmitter neuromodulatory systems outlined above, is incredibly important for proper function of the brain, sensory processing, plasticity, and the functions of the SC. It is as yet unknown how exactly these systems are intertwined and act in coordination with one another to allow proper functioning; such is a vast topic for future study. While a great deal more is known about how these systems work individually, there is still much more to be discovered.

Specific aims of this thesis

Previous work has helped us to learn a great deal about SC development, structure, and function, as well as the development of those processes such as multisensory integration. The work described here builds upon this foundational information in order to better understand the mechanistic bases of multisensory integration, first through the lens of visual experience involvement and then through investigation of the influence of the serotonergic neurotransmitter system.

As explained in detail above, the process of multisensory integration develops gradually in SC neurons, and normal development of this process requires sensory and multisensory experience. Without exposure to normal sensory stimuli, SC multisensory neurons maintain responsiveness to unisensory stimuli, yet never develop the capacity to integrate these sensory signals. Visual (Wallace and Stein 2007) or auditory (Xu et al. 2014) deprivation during maturation obstructs the capacity of multisensory SC and multisensory cortical (Carriere et al. 2007) neurons to integrate information from different modalities. However, the question of whether this system is still malleable after development at the level of SC multisensory neurons remains open. Previous work has shown that restoration of visual experience in adulthood after developmental visual deprivation has minimal success in restoring normal multisensory processing capabilities (Royal et al. 2010). This raised the possibility that there may exist a sensitive period for multisensory experience, outside of which (during adulthood, for example) this experience does not have the ability to drastically affect multisensory processing capacities. This formed the motivation for the study described in Chapter II of this thesis. To examine if multisensory processing remains susceptible to experiential

change throughout adulthood, we investigated unisensory and multisensory interactions in the SC of animals normally reared throughout development before being visually deprived in adulthood. By comparing interactions in these animals to those of completely visually deprived animals and normally raised animals, we found that the SC has layer-specific compensatory plasticity even during adulthood, and changes in the dynamics of multisensory integration are adaptive to visual deprivation even after normal development has occurred. This suggests that neurons within the SC maintain malleability after development and into adulthood, expanding our knowledge of the mechanisms by which multisensory processing in the SC occurs.

While the neurotransmitter makeup of the SC is understood, the functions and actions of these neurotransmitters and the receptor distribution on neuronal types is still largely unknown. The transmitter serotonin (5-HT) has been shown to play a critical role in the development of sensory and motor representations across the brain (Ueda et al. 1985; Villar et al. 1988; Rhoades et al. 1990; Arce et al. 1992; Huang et al. 1993; Gu and Singer 1995; Janusonis et al. 1999; Ke et al. 1999; Foehring et al. 2002; Hurley et al. 2002; Xiang and Prince 2003; Hurley et al. 2004; Lottem et al. 2016) and in the SC specifically (Lauder et al. 1982; Mize and Horner 1989; Rhoades et al. 1990). The 5-HT system is also incredibly important in shaping response profiles of neurons in auditory (Hurley and Pollak 1999; Hurley and Pollak 2001; Hurley 2006), visual (Waterhouse et al. 1986) and somatosensory (Jitsuki et al. 2011) systems. The motivation to expand these findings into the role of the 5-HT system in multisensory processing of SC neurons fueled the study described in Chapter III of this thesis. We found marked changes in both sensory (visual) and multisensory (audiovisual) processing with

manipulation of the 5-HT signalling in the SC. These results support the importance of the serotonin system in mediating facets of unisensory and multisensory signaling within the SC, expanding our knowledge both of the mechanisms by which multisensory integration occurs and also furthering our overall understanding of the SC and its multisensory integrative neurons.

References Cited

Aizawa, H., Kobayashi, Y., Yamamoto, M. and Isa, T. (1999). Injection of nicotine into the superior colliculus facilitates occurrence of express saccades in monkeys. *J Neurophysiol* **82,** 1642-1646.

Allman, B. L., Bittencourt-Navarrete, R.E., Keniston, L.P., Medina, A.E., Wang, M.Y. and Meredith, M.A. (2008). Do cross-modal projections always result in multisensory integration? *Cereb Cortex* **18**, 2066-2076.

Allman, B. L., Keniston, L. P. and Meredith, M.A. (2008). Subthreshold auditory inputs to extrastriate visual neurons are responsive to parametric changes in stimulus quality: sensory-specific versus non-specific coding. *Brain Res* **25,** 95-101.

Alvarado, J. C., Vaughan, J.W., Stanford, T.R. and Stein, B.E. (2007). Multisensory versus unisensory integration: contrasting modes in the superior colliculus. *J Neurophysiol* **97**, 3193-3205.

Amlot, R., Walker, R., Driver, J. and Spence, C. (2003). Multimodal visual-somatosensory integration in saccade generation. *Neuropsychologia* **41,** 1-15.

Anderson, M. E., Yoshida, M. and Wilson, V.J. (1971). Influence of superior colliculus on cat neck motoneurons. *J Neurophysiol* **34,** 898-907.

Antonini, A., Berlucchi, G., Marzi, C.A. and Sprague, J.M. (1979). Importance of corpus callosum for visual receptive fields of single neurons in cat superior colliculus. *J Neurophysiol* **42,** 137-152.

Antonini, A., Berlucchi, G. and Sprague, J.M. (1978). Indirect, across-the-midline retinotectal projections and representation of ipsilateral visual field in superior colliculus of the cat. *J Neurophysiol* **41,** 285-304.

Appell, P. P. and Behan, M. (1990). Sources of subcortical GABAergic projections to the superior colliculus in the cat. *J Comp Neurol* **302,** 143-158.

Araki, M., McGeer, P.L. and McGeer, E.G. (1984). Presumptive gamma-aminobutyric acid pathways from the midbrain to the superior colliculus studied by a combined horseradish peroxidase-gamma-aminobutyric acid transaminase pharmacohistochemical method. *Neuroscience* **13,** 433-439.

Arce, E. A., Bennett-Clarke, C.A., Mooney, R.D. and Rhoades, R.W. (1992). Synaptic organization of the serotoninergic input to the superficial gray layer of the hamster's superior colliculus. *Synapse* **11,** 67-75.

Avillac, M., Deneve, S., Olivier, E., Pouget, A. and Duhamel, J.R. (2005). Reference frames for representing visual and tactile locations in parietal cortex. *Nat Neurosci* **8,** 941-949.

Baldwin, M. K. and Kaas, J. H. (2012). Cortical projections to the superior colliculus in prosimian galagos (Otolemur garnetti). *J Comp Neurol* **520,** 2002-2020.

Baleydier, C., Kahungu, M. and Mauguiere, F. (1983). A crossed corticotectal projection from the lateral suprasylvian area in the cat. *J Comp Neurol* **214,** 344-351.

Balmer, T. S. and Pallas, S. L. (2015). Visual experience prevents dysregulation of GABAB receptor-dependent short-term depression in adult superior colliculus. *J Neurophysiol* **113,** 2049-2061.

Barraclough, N. E., Xiao, D., Baker, C. I., Oram, M. W. and Perrett, D. I. (2005). Integration of visual and auditory information by superior temporal sulcus neurons responsive to the sight of actions. *J Cogn Neurosci* **17,** 377-391.

Beckstead, R. M., Edwards, S. B. and Frankfurter, A. (1981). A comparison of the intranigral distribution of nigrotectal neurons labeled with horseradish peroxidase in the monkey, cat, and rat. *J Neurosci* **1**, 121-125.

Behan, M. and Appell, P. P. (1992). Intrinsic circuitry in the cat superior colliculus: projections from the superficial layers. *J Comp Neurol* **315**, 230-243.

Behan, M. and Kime, N. M. (1996). Intrinsic circuitry in the deep layers of the cat superior colliculus. *Vis Neurosci* **13**, 1031-1042.

Bell, A. H., Meredith, M. A., Van Opstal, A. J. and Munoz, D. P. (2005). Crossmodal Integration in the Primate Superior Colliculus Underlying the Preparation and Initiation of Saccadic Eye Movements. *Journal of Neurophysiology* **93**, 3659-3673.

Benedek, G., Fischer-Szatmari, L., Kovacs, G., Perenyi, J. and Katoh, Y. Y. (1996). Visual, somatosensory and auditory modality properties along the feline suprageniculate-anterior ectosylvian sulcus/insular pathway. *Prog Brain Res* **112**, 325-334.

Benedek, G., Mucke, L., Norita, M., Albowitz, B. and Creutzfeldt, O. D. (1988). Anterior ectosylvian visual area (AEV) of the cat: physiological properties. *Prog Brain Res* **75**, 245-255.

Benedek, G., Pereny, J., Kovacs, G., Fischer-Szatmari, L. and Katoh Y. Y. (1997). Visual, somatosensory, auditory and nociceptive modality properties in the feline suprageniculate nucleus. *Neuroscience* **78**, 179-189.

Benevento, L. A. and Fallon, J. H. (1975). The ascending projections of the superior colliculus in the rhesus monkey (Macaca mulatta). *J Comp Neurol* **160**, 339-361.

Benevento, L. A. and Standage, G. P. (1983). The organization of projections of the retinorecipient and nonretinorecipient nuclei of the pretectal complex and layers of the superior colliculus to the lateral pulvinar and medial pulvinar in the macaque monkey. *J Comp Neurol* **217**, 307-336.

Berkley, K. J. (1973). Response properties of cells in ventrobasal and posterior group nuclei of the cat. *J Neurophysiol* **36**, 940-952.

Berlucchi, G., Sprague, J. M., Levy, J. and DiBerardino, A. C. (1972). Pretectum and superior colliculus in visually guided behavior and in flux and form discrimination in the cat. *J Comp Physiol Psychol* **78**, 123-172.

Berson, D. M. and McIlwain, J. T. (1983). Visual cortical inputs to deep layers of cat's superior colliculus. *J Neurophysiol* **50**, 1143-1155.

Bezdudnaya, T. and Castro-Alamancos, M. A. (2014). Neuromodulation of whisking related neural activity in superior colliculus. *J Neurosci* **34**, 7683-7695.

Billet, S., Cant, N. B. and Hall, W. C. (1999). Cholinergic projections to the visual thalamus and superior colliculus. *Brain Res* **847**, 121-123.

Binns, K. E. (1999). The synaptic pharmacology underlying sensory processing in the superior colliculus. *Progress in Neurobiology* **59**, 129-159.

Binns, K. E. and Salt, T. E. (1996). Importance of NMDA receptors for multimodal integration in the deep layers of the cat superior colliculus. *J Neurophysiol* **75**, 920-930.

Blomqvist A., Flink, R., Bowsher, D., Griph, S. and Westman, J. (1978). Tectal and thalamic projections of dorsal column and lateral cervical nuclei: a quantitative study in the cat. *Brain Res* **141**, 335-41.

Bowery, N. G., Hudson, A. L. and Price, G. W. (1987). GABAA and GABAB receptor site distribution in the rat central nervous system. *Neuroscience* **20**, 365-383.

Bremmer, F., Klam, F., Duhamel, J. R., Ben Hamed, S. and Graf, W. (2002). Visual-vestibular interactive responses in the macaque ventral intraparietal area (VIP). *Eur J Neurosci* **16**, 1569-1586.

Brosch, M., Selezneva, E. and Scheich, H. (2005). Nonauditory events of a behavioral procedure activate auditory cortex of highly trained monkeys. *J Neurosci* **25**, 6797-6806.

Buee J., Deniau, J. M. and Chevalier, G. (1986). Nigral modulation of cerebello-thalamo-cortical transmission in the ventral medial thalamic nucleus. *Exp Brain Res* **65**, 241-4.

Burke, W., Dreher, B. and Wang, C. (1998). Selective block of conduction in Y optic nerve fibres: significance for the concept of parallel processing. *Eur J Neuroscience* **10**, 8-19.

Burnett, L. R., Stein, B. E., Chaponis, D. and Wallace, M. T. (2004). Superior colliculus lesions preferentially disrupt multisensory orientation. *Neuroscience* **124**, 535-547.

Burnett, L. R., Stein, B. E., Perrault, T. J. and Wallace, M. T. (2007). Excitotoxic lesions of the superior colliculus preferentially impact multisensory neurons and multisensory integration. *Exp Brain Res* **179**, 325-338.

Burton, H., Snyder, A. Z., Diamond, J. B. and Raichle, M. E. (2002). Adaptive changes in early and late blind: a fMRI study of Braille reading. *J Neurophysiol* **87**, 589-607.

Calabresi, P., Mercuri, N. B. and Bernardi, G. (1990). Synaptic and intrinsic control of membrane excitability of neostriatal neurons. I. An in vivo analysis. *J Neurophysiol* **63**, 651-662.

Caldwell, R. B. and Mize, R. R. (1981). Superior colliculus neurons which project to the cat lateral posterior nucleus have varying morphologies. *J Comp Neurol* **203**, 53-66.

Calvert, G. A., Campbell, R. and Brammer, M. J. (2000). Evidence from functional magnetic resonance imaging of crossmodal binding in the human heteromodal cortex. *Curr Biol* **10**, 649-657.

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

Carrasco, M. M., Mao, Y. T., Balmer, T. S. and Pallas, S. L. (2011). Inhibitory plasticity underlies visual deprivation-induced loss of receptive field refinement in the adult superior colliculus. *European Journal of Neuroscience* **33**, 58-68.

Carriere, B. N., Royal, D. W., Perrault, T. J., Morrison, S. P., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2007). Visual deprivation alters the development of cortical multisensory integration. *J Neurophysiol* **98**, 2858-2867.

Carriere, B. N., Royal, D. W. and Wallace, M. T. (2008). Spatial Heterogeneity of Cortical Receptive Fields and Its Impact on Multisensory Interactions. *Journal of Neurophysiology* **99**, 2357-2368.

Casagrande, V. A., Harting, J. K., Hall, W. C., Diamond, I. T. and Martin, G. F. (1972). Superior colliculus of the tree shrew: a structural and functional subdivision into superficial and deep layers. *Science* **177**, 444-447.

Champoux, F., Collignon, O., Bacon, B. A., Lepore, F., Zatorre, R.J. and Theoret, H. (2011). Early- and late-onset blindness both curb audiotactile integration on the parchment-skin illusion. *Psychol Sci* **22**, 19-25.

Chevalier, G., Vacher, S., Deniau, J. M. and Desban, M. (1985). Disinhibition as a basic process in the expression of striatal functions. I. The striato-nigral influence on tecto-spinal/tecto-diencephalic neurons. *Brain Res* **334**, 215-226.

Christoff, N. (1974). A clinicopathologic study of vertical eye movements. *Arch Neurol 31* **1**, 1-8.

Clemo, H. R. and Stein, B. E. (1982). Somatosensory cortex: a 'new' somatotopic representation. *Brain Res* **235**, 162-168.

Clemo, H. R. and Stein, B. E. (1984). Topographic organization of somatosensory corticotectal influences in cat. *J Neurophysiol* **51**, 843-858.

Clemo, H.R. and Stein, B.E. (1986). Effects of cooling somatosensory cortex on response properties of tactile cells in the superior colliculus. *J Neurophysiol* **55**, 1352-1368.

Clemo, H.R. and Stein, B.E. (1987). Responses to direction of stimulus movement are different for somatosensory and visual cells in cat superior colliculus. *Brain Res* **405**, 313-319.

Clemo, H.R. and Stein, B. E. (1991). Receptive field properties of somatosensory neurons in the cat superior colliculus. *J Comparative Neurology* **314**, 534-544.

Collignon, O., Charbonneau, G., Lassonde, M. and Lepore, F. (2009). Early visual deprivation alters multisensory processing in peripersonal space. *Neuropsychologia* **47**, 3236-3243.

Collignon, O., Dormal, G., Albouy, G., Vandewalle, G., Voss, P., Phillips, C. and Lepore, F. (2013). Impact of blindness onset on the functional organization and the connectivity of the occipital cortex. *Brain* **136**, 2769-2783.

Colonius, H. and Arndt, P. (2001). A two-stage model for visual-auditory interaction in saccadic latencies. *Percept Psychophys* **63**, 126-147.

Corneil, B. D., Van Wanrooij, M., Munoz, D. P. and Van Opstal, A. J. (2002). Auditory-Visual Interactions Subserving Goal-Directed Saccades in a Complex Scene. *Journal of Neurophysiology* **88**, 438-454.

Cowie, R. J. and Holstege, G. (1992). Dorsal mesencephalic projections to pons, medulla, and spinal cord in the cat: limbic and non-limbic components. *J Comp Neurol* **319**, 536-559.

Dahlstrom, A. and Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia* **20**, 398-399.

Diederich, A. and Colonius, H. (2004). Bimodal and trimodal multisensory enhancement: effects of stimulus onset and intensity on reaction time. *Percept Psychophys* **66**, 1388-1404.

Diederich, A., Colonius, H., Bockhorst, D. and Tabeling, S. (2003). Visual-tactile spatial interaction in saccade generation. *Exp Brain Res* **148**, 328-337.

Dorris, M.C., Pare, M. and Munoz D. P. (1997). Neuronal activity in monkey superior colliculus related to the initiation of saccadic eye movements. *J Neuroscience* **17**, 8566-8579.

Doubell, T.P., Skaliora, I., Baron, J. and King, A. J. (2003). Functional connectivity between superficial and deeper layers of the superior colliculus: an anatomical substrate for sensorimotor integration. *J Neuroscience* **23**, 6596-6607.

Drager, U.C. and Hubel, D.H. (1975a). Physiology of visual cells in mouse superior colliculus and correlation with somatosensory and auditory input. *Nature* **253**, 203-204.

Drager, U.C. and Hubel, D. H. (1975b). Responses to visual stimulation and relationship between visual, auditory, and somatosensory inputs in mouse superior colliculus. *J Neurophysiol* **38**, 690-713.

Dreher, B. and Hoffmann, K. P. (1973). Properties of excitatory and inhibitory regions in the receptive fields of single units in the cat's superior colliculus. *Exp Brain Res* **16**, 333-353.

Edwards, S. B. (1977). The commissural projection of the superior colliculus in the cat. *J Comp Neurol* **173**, 23-40.

Edwards, S. B., Ginsburgh, C. L., Henkel, C. K. and Stein, B. E. (1979). Sources of subcortical projections to the superior colliculus in the cat. *J Comp Neurol* **184**, 309-329.

Edwards, S. B., Rosenquist, A. C. and Palmer, L. A. (1974). An autoradiographic study of ventral lateral geniculate projections in the cat. *Brain Res* **72**, 282-287.

Eimer, M. (2004). Multisensory integration: how visual experience shapes spatial perception. *Curr Biol* **14**, R115-117.

Endo, T., Yanagawa, Y., Obata, K. and Isa, T. (2003). Characteristics of GABAergic neurons in the superficial superior colliculus in mice. *Neurosci Lett* **346**, 81-84.

Essen, L. (2000). Corticocortical Connections of Visual, Sensorimotor, and Multimodal Processing Areas in the Parietal Lobe of the Macaque Monkey. *The Journal of Comparative Neurology* **428**, 112-137.

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

Feldon, P. and Kruger, L. (1970). Topography of the retinal projection upon the superior colliculus of the cat. *Vision Res* **10**, 135-143.

Felleman, D. J. and Van Essen, D. C. (1991). Distributed hierarchical processing in the primate cerebral cortex. *Cereb Cortex* **1**, 1-47.

Ficalora, A. S. and Mize, R. R. (1989). The neurons of the substantia nigra and zona incerta which project to the cat superior colliculus are GABA immunoreactive: a double-label study using GABA immunocytochemistry and lectin retrograde transport. *Neuroscience* **29**, 567-581.

Fitzgibbon, T., Tevah, L. V. and Sefton, A. J. (1995). Connections between the reticular nucleus of the thalamus and pulvinar-lateralis posterior complex: a WGA-HRP study. *J Comp Neurol* **363**, 489-504.

Focker, J., Holig, C., Best, A. and Roder, B. (2015). Neural plasticity of voice processing: Evidence from event-related potentials in late-onset blind and sighted individuals. *Restor Neurol Neurosci* **33**, 15-30.

Foehring, R.C., van Brederode, J. F., Kinney, G. A. and Spain, W. J. (2002). Serotonergic modulation of supragranular neurons in rat sensorimotor cortex. *J Neurosci* **22**, 8238-8250.

Fogassi, L., Gallese, V., Fadiga, L., Luppino, G., Mateli, M. and Rizzolatti, G. (1996). Coding of peripersonal space in inferior premotor cortex (area F4). *J Neurophysiol* **76**, 141-157.

Fonnum, F., Karlsen, R. L., Malthe-Sorenssen, D., Skrede, K. K. and Walaas, I. (1979). Localization of neurotransmitters, particularly glutamate, in hippocampus, septum, nucleus accumbens and superior colliculus. *Prog Brain Res* **51**, 167-191.

Fosse, V. M., Heggelund, P., Iversen, E. and Fonnum, F. (1984). Effects of area 17 ablation on neurotransmitter parameters in efferents to area 18, the lateral geniculate body, pulvinar and superior colliculus in the cat. *Neurosci Lett* **52**, 323-328.

Frassinetti, F., Bolognini, N. and Ladavas, E. (2002). Enhancement of visual perception by crossmodal visuo-auditory interaction. *Exp Brain Res* **147**, 332-343.

Frens, M. A., Van Opstal, A. J. and Van der Willigen, R. F. (1995). Spatial and temporal factors determine auditory-visual interactions in human saccadic eye movements. *Percept Psychophys* **57**, 802-816.

Fries, W. (1984). Cortical projections to the superior colliculus in the macaque monkey: a retrograde study using horseradish peroxidase. *J Comp Neurol* **230**, 55-76.

Fu, K. M., Johnston, T. A., Shah, A. S., Arnold, L., Smiley, J., Hackett, T. A., Garraghty, P. E. and Schroeder, C. E. (2003). Auditory cortical neurons respond to somatosensory stimulation. *J Neurosci* **23**, 7510-7515.

Fuster, J. M., Bodner, M. and Kroger, J. K. (2000). Cross-modal and cross-temporal association in neurons of frontal cortex. *Nature* **405**, 347-351.

Ghazanfar, A. A., Maier, J. X., Moffman, K. L. and Logothetis, N. K. (2005). Multisensory Integration of Dynamic Faces and Voices in Rhesus Monkey Auditory Cortex. *The Journal of Neuroscience* **25**, 5004-5012.

Ghazanfar, A. A. and Schroeder, C. E. (2006). Is neocortex essentially multisensory? *Trends in Cognitive Sciences* **10**, 278-285.

Ghitani, N., Bayguinov, P. O., Vokoun, C. R., McMahon, S., Jackson, M. B. and Basso, M. A. (2014). Excitatory synaptic feedback from the motor layer to the sensory layers of the superior colliculus. *J Neurosci* **34**, 6822-6833.

Giard, M. H. and Peronnet, F. (1999). Auditory-visual integration during multimodal object recognition in humans: a behavioral and electrophysiological study. *J Cogn Neurosci* **11**, 473-490.

Gifford, G. W., 3rd and Cohen, Y. E. (2004). Effect of a central fixation light on auditory spatial responses in area LIP. *J Neurophysiol* **91**, 2929-2933.

Gilbert, C. D. and Kelly, J. P. (1975). The projections of cells in different layers of the cat's visual cortex. *J Comp Neurol* **163**, 81-105.

Gingras, G., Rowland, B. A. and Stein, B. E. (2009). The differing impact of multisensory and unisensory integration on behavior. *J Neurosci* **29**, 4897-4902.

Gordon, B. (1973). Receptive fields in deep layers of cat superior colliculus. *J Neurophysiol* **36**, 157-178.

Graham, J. (1977). An autoradiographic study of the efferent connections of the superior colliculus in the cat. *J Comp Neurol* **173**, 629-654.

Graham, J. and Casagrande, V. A. (1980). A light microscopic and electron microscopic study of the superficial layers of the superior colliculus of the tree shrew (Tupaia glis). *J Comp Neurol* **191**, 133-151.

Grantyn, A. and Grantyn, R. (1982). Axonal patterns and sites of termination of cat superior colliculus neurons projecting in the tecto-bulbo-spinal tract. *Exp Brain Res* **46**, 243-256.

Grantyn, R., Perouansky, M., Lux, H. D. and Hablitz, J. J. (1987). Glutamate-induced ionic currents in cultured neurons from the rat superior colliculus. *Brain Res* **420**, 182-187.

Graybiel, A. M. (1975). Anatomical organization of retinotectal afferents in the cat: an autoradiographic study. *Brain Res* **96**, 1-23.

Graybiel, A. M. (1976). Evidence for banding of the cat's ipsilateral retinotectal connection. *Brain Res* **114**, 318-327.

Graybiel, A. M. (1978). A satellite system of the superior colliculus: the parabigeminal nucleus and its projections to the superficial collicular layers. *Brain Res* **145**, 365-374.

Graybiel, A. M. and Hartwieg, E. A. (1974). Some afferent connections of the oculomotor complex in the cat: an experimental study with tracer techniques. *Brain Res* **81**, 543-551.

Graziano, M. S. (1999). Where is my arm? The relative role of vision and proprioception in the neuronal representation of limb position. *Proc Natl Acad Sci U S A* **96**, 10418-10421.

Graziano, M. S., Yap, G. S. and Gross, C. G. (1994). Coding of visual space by premotor neurons. *Science* **266**, 1054-1057.

Gu, Q. and Singer, W. (1995). Involvement of serotonin in developmental plasticity of kitten visual cortex. *Eur J Neurosci* **7**, 1146-1153.

Guerreiro, M. J., Putzar, L. and Roder, B. (2015). The effect of early visual deprivation on the neural bases of multisensory processing. *Brain* **138**, 1499-1504.

Harrell, J. V., Caldwell, R. B. and Mize, R. R. (1982). The superior colliculus neurons which project to the dorsal and ventral lateral geniculate nuclei in the cat. *Exp Brain Res* **46**. 234-242.

Harting, J. K., Hall, W. C., Diamond, I. T. and Martin, G. F. (1973). Anterograde degeneration study of the superior colliculus in Tupaia glis: evidence for a subdivision between superficial and deep layers. *J Comp Neurol* **148**, 361-386.

Harting, J.K. and Noback, C. R. (1971). Subcortical projections from the visual cortex in the tree shrew (Tupaia glis). *Brain Res* **25**, 21-33.

Harting, J. K., Updyke, B. V. and Van Lieshout, D. P. (1992). Corticotectal projections in the cat: anterograde transport studies of twenty-five cortical areas. *J Comp Neurol* **324**, 379-414.

Harting, J. K., Van Lieshout, D. P. and Feig, S. (1991). Connectional studies of the primate lateral geniculate nucleus: distribution of axons arising from the thalamic reticular nucleus of Galago crassicaudatus. *J Comp Neurol* **310**, 411-427.

Hauthal, N., Debener, S., Rach, S., Sandmann, P. and Thorne, J. D. (2015). Visuo-tactile interactions in the congenitally deaf: a behavioral and event-related potential study. *Front Integr Neurosci* **8**.

Hay-Schmidt, A. (2000). The evolution of the serotonergic nervous system. *Proc Biol Sci* **267**, 1071-1079.

Henkel, C. K. (1983). Evidence of sub-collicular auditory projections to the medial geniculate nucleus in the cat: an autoradiographic and horseradish peroxidase study. *Brain Res* **259**, 21-30.

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

Hicks, T.P. Stark, C.A. and Fletcher, W. A. (1986). Origins of afferents to visual suprageniculate nucleus of the cat. *J Comparative Neurology* **246**, 544-554.

Hollander, H. (1974). On the origin of the corticotectal projections in the cat. *Exp Brain Res* **21**, 433-439.

Holmes, N. P. (2009). The principle of inverse effectiveness in multisensory integration: some statistical considerations. *Brain Topogr* **21**, 168-176.

Horn, G. and Hill, R. M. (1966). Responsiveness to sensory stimulation of units in the superior colliculus and subjacent tectotegmental regions of the rabbit. *Experimental Neurology* **14**. 199-223.

Hotting, K. and Roder, B. (2009). Auditory and auditory-tactile processing in congenitally blind humans. *Hear Res* **258**, 165-174.

Huang, X., Mooney, R. D. and Rhoades, R. W. (1993). Effects of serotonin on retinotectal-, corticotectal-, and glutamate-induced activity in the superior colliculus of the hamster. *J Neurophysiol* **70**, 723-732.

Huerta M.F. (1984). “Comparative Neurology of Optic Tectum.”

Huerta, M. F., Frankfurter, A. J. and Harting, J. K. (1981). The trigeminocollicular projection in the cat: patch-like endings within the intermediate gray. *Brain Res* **211**, 1-13.

Huerta, M. F. and Harting, J. K. (1982). The projection from the nucleus of the posterior commissure to the superior colliculus of the cat: patch-like endings within the intermediate and deep grey layers. *Brain Res* **238**,: 426-432.

Huerta, M. F. and Harting, J. K. (1983). Sublamination within the superficial gray layer of the squirrel monkey: an analysis of the tectopulvinar projection using anterograde and retrograde transport methods. *Brain Res* **261**, 119-126.

Huerta, M. F. and Kaas, J. H. (1990). Supplementary eye field as defined by intracortical microstimulation: connections in macaques. *J Comp Neurol* **293**, 299-330.

Huerta, M. F., Krubitzer, L. A. and Kaas, J. H. (1986). Frontal eye field as defined by intracortical microstimulation in squirrel monkeys, owl monkeys, and macaque monkeys: I. Subcortical connections. *J Comp Neurol* **253**, 415-439.

Hughes, H. C., Nelson, M. D. and Aronchick, D. M. (1998). Spatial characteristics of visual-auditory summation in human saccades. *Vision Res* **38**, 3955-3963.

Hughes, H. C., Reuter-Lorenz, P. A., Nozawa, G. and Fendrich, R. (1994). Visual-auditory interactions in sensorimotor processing: Saccades versus manual responses. *Journal of Experimental Psychology: Human Perception and Performance* **20**, 131-153.

Hurley, L.M., Thompson, A. M. and Pollak, G. D. (2002). Serotonin in the inferior colliculus. *Hear Res* **168**, 1-11.

Hurley, L.M., Devibiss, D.M. and Waterhouse, B. D. (2004). A matter of focus: monoaminergic modulation of stimulus coding in mammalian sensory networks. *Curr Opin Neurobiol* **14**, 488-95.

Hurley, L. M. and Hall, I. C. (2011). Context-dependent modulation of auditory processing by serotonin. *Hear Res* **279**, 74-84.

Hurley, L. M. and Pollak, G. D. (2005). Serotonin modulates responses to species-specific vocalizations in the inferior colliculus. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **191**, 535-546.

Hurley, L. M. and Pollak, G. D. (2005). Serotonin shifts first-spike latencies of inferior colliculus neurons. *J Neurosci* **25**, 7876-7886.

Hurley, L. M. and Sullivan, M. R. (2012). From behavioral context to receptors: serotonergic modulatory pathways in the IC. *Front Neural Circuits* **6**.

Isa, T., Endo, T. and Saito, Y. (1998). The visuo-motor pathway in the local circuit of the rat superior colliculus. *J Neurosci* **18**, 8496-8504.

Janusonis, S., Fite, K. V. and Foote, W. (1999). Topographic organization of serotonergic dorsal raphe neurons projecting to the superior colliculus in the Mongolian gerbil (Meriones unguiculatus). *J Comp Neurol* **413**, 342-355.

Jeon, C. J., Gurski, M. R. and Mize, R. R. (1997). Glutamate containing neurons in the cat superior colliculus revealed by immunocytochemistry. *Vis Neurosci* **14**, 387-393.

Jiang, H., Lepore, F., Poririer, P. and Guillemot, J. P. (2000). Responses of cells to stationary and moving sound stimuli in the anterior ectosylvian cortex of cats. *Hear Res* **139**, 69-85.

Jiang, W., Jiang, H. and Stein, B. E. (2002). Two corticotectal areas facilitate multisensory orientation behavior. *J Cogn Neurosci* **14**, 1240-1255.

Jiang, W., Jiang, H. and Stein, B. E. (2006). Neonatal cortical ablation disrupts multisensory development in superior colliculus. *J Neurophysiol* **95**, 1380-1396.

Jitsuki, S., Takemoto, K. Kawasaki, T., Takahashi, A., Becamel, C., Sano, A., Yuzaki, M., Zukin, R. S., Ziff, E. B., Kessels, H. W. and Takahashi, T. (2011). Serotonin mediates cross-modal reorganization of cortical circuits. *Neuron* **69**, 780-792.

Jones E.G. (1985). The thalamus. New York: Penum Press.

Juttner, R., Henneberger, C., Grantyn, R. and Rothe, T. (2001). Early onset of glutamatergic and GABAergic synaptic activity in the visual layers of the rodent superior colliculus. *Int J Dev Neurosci* **19**, 255-261.

Kadunce, D. C., Vaughan, J. W., Wallace, M. T., Benedek, G. and Stein, B. E. (1997). Mechanisms of Within- and Cross-Modality Suppression in the Superior Colliculus. *Journal of Neurophysiology* **78**, 2834-2847.

Kanaseki, T. and Sprague, J. M. (1974). Anatomical organization of pretectal nuclei and tectal laminae in the cat. *J Comp Neurol* **158**, 319-337.

Katsuta, H. and Isa, T. (2003). Release from GABA(A) receptor-mediated inhibition unmasks interlaminar connection within superior colliculus in anesthetized adult rats. *Neurosci Res* **46**, 73-83.

Kawamura, K. and Hashikawa, T. (1978). Cell bodies of origin of reticular projections from the superior colliculus in the cat: an experimental study with the use of horseradish peroxidase as a tracer. *J Comp Neurol* **182**, 1-15.

Kawamura, K. and Konno, T. (1979). Various types of corticotectal neurons of cats as demonstrated by means of retrograde axonal transport of horseradish peroxidase. *Exp Brain Res* **35**, 161-175.

Kawamura, S., Fukushima, N., Hattori, S. and Kudo, M. (1980). Laminar segregation of cells of origin of ascending projections from the superficial layers of the superior colliculus in the cat. *Brain Res* **184**, 486-490.

Kayser, C., Petkov, C. I., Lippert, M. and Logothetis, N. K. (2005). Integration of touch and sound in auditory cortex. *Neuron* **48**, 373-384.

Ke, M., Mooney, R. D. and Rhoades, R. W. (1999). Increased serotonin in the developing superior colliculus affects receptive-field size of retinotectal afferents but not that of postsynaptic neurons. *Vis Neurosci* **16**, 121-130.

Keysers, C. and Perrett, D. I. (2004). Demystifying social cognition: a Hebbian perspective. *Trends in Cog Sci* **8**, 501-7.

King, A. J. and Palmer, A. R. (1983). Cells responsive to free-field auditory stimuli in guinea-pig superior colliculus: distribution and response properties. *J Physiol* **342**, 361-381.

King, A. J. and Palmer, A. R. (1985). Integration of visual and auditory information in bimodal neurones in the guinea-pig superior colliculus. *Exp Brain Res* **60**, 492-500.

Komatsu H. and Suzuki, H. (1985). Projections from the functional subdivisions of the frontal eye field to the superior colliculus in the monkey. *Brain Res* **327**, 324-7.

Krauthamer, G. M., Grunwerg, B. S. and Krein, H. (1995). Putative cholinergic neurons of the pedunculopontine tegmental nucleus projecting to the superior colliculus consist of sensory responsive and unresponsive populations which are functionally distinct from other mesopontine neurons. *Neuroscience* **69**, 507-517.

Kudo, M. (1981). Projections of the nuclei of the lateral lemniscus in the cat: an autoradiographic study. *Brain Res* **221**, 57-69.

Kudo, M. and Niimi, K. (1980). Ascending projections of the inferior colliculus in the cat: an autoradiographic study. *J Comp Neurol* **191**, 545-556.

Kuffler, S.W. (1953). Discharge patterns and functional organization of mammalian retina. *J Neurophysiol* **16**, 37-68.

Kujala, T., Alho, K., Huotilainen, M., Ilmoniemi, R. J., Lehtokoski, A., Leinonen, A., Rinne, T., Salonen, O., Sinkkonen, J., Standertskjold-Nordenstam, C. G. and Naatanen, R. (1997). Electrophysiological evidence for cross-modal plasticity in humans with early- and late-onset blindness. *Psychophysiology* **34**, 213-216.

Kunzle, H. and Akert, K. (1977). Efferent connections of cortical, area 8 (frontal eye field) in Macaca fascicularis. A reinvestigation using the autoradiographic technique. *J Comp Neurol* **173**, 147-164.

Kunzle, H., Akert, K. and Wurtz, R. H. (1976). Projection of area 8 (frontal eye field) to superior colliculus in the monkey. An autoradiographic study. *Brain Res* **117**, 487-492.

Kuypers, H. G., Szwarcbart, M. K., Mishkin, M. and Rosvold, H. E. (1965). Occipitotemporal Corticocortical Connections in the Rhesus Monkey. *Exp Neurol* **11**, 245-262.

Lauder, J. M., Wallace, J.A., Krebs, H., Petrusz, P. and McCarthy, K. (1982). In vivo and in vitro development of serotonergic neurons. *Brain Res Bull* **9**, 605-625.

Laurienti, P. J., Perrault, T. J., Stanford, T. R., Wallace, M. T. and Stein B. E. (2005). On the use of superadditivity as a metric for characterizing multisensory integration in functional neuroimaging studies. *Exp Brain Res* **166**, 289-297.

Lee, C., Rohrer, W. H. and Sparks, D. L. (1988). Population coding of saccadic eye movements by neurons in the superior colliculus. *Nature* **322**, 357-360.

Lee, P. and Hall, W. C. (1995). Interlaminar connections of the superior colliculus in the tree shrew. II: Projections from the superficial gray to the optic layer. *Vis Neurosci* **12**, 573-588.

Leichnetz, G. R. and Gonzalo-Ruiz, A. (1996). Prearcuate cortex in the Cebus monkey has cortical and subcortical connections like the macaque frontal eye field and projects to fastigial-recipient oculomotor-related brainstem nuclei. *Brain Res Bull* **41**, 1-29.

Leinonen, L., Hyvarinen, J. and Sovijarvi, A. R. (1980). Functional properties of neurons in the temporo-parietal association cortex of awake monkey. *Exp Brain Res* **39**, 203-215.

Leo, F., Bertini, C., di Pellegrino, G. and Ladavas, E. (2008). Multisensory integration for orienting responses in humans requires the activation of the superior colliculus. *Exp Brain Res* **186**, 67-77.

Lesch, K. P. (2011). When the serotonin transporter gene meets adversity: the contribution of animal models to understanding epigenetic mechanisms in affective disorders and resilience. *Curr Top Behav Neurosci* **7**, 251-280.

Lewis, J.W. and Van Essen, D. C. (2000). Corticocortical connections of visual, sensorimotor, and multimodal processing areas in the parietal lobe of the macaque monkey. *J Comp Neurol* **428**, 112-37.

Lin, C. S. and Kaas, J. H. (1979). The inferior pulvinar complex in owl monkeys: architectonic subdivisions and patterns of input from the superior colliculus and subdivisions of visual cortex. *J Comp Neurol* **187**, 655-678.

Linden, J. F., Grunewald, A. and Andersen, R. A. (1999). Responses to auditory stimuli in macaque lateral intraparietal area. II. Behavioral modulation. *J Neurophysiol* **82**, 343-358.

Linke, R. (1999). Differential projection patterns of superior and inferior collicular neurons onto posterior paralaminar nuclei of the thalamus surrounding the medial geniculate body in the rat. *Eur J Neurosci* **11**, 187-203.

Lottem E., Lorincz, M. L. and Mainen, Z. F. (2016). Optogenetic activation of dorsal raphe serotonin neurons rapidly inhibits spontaneous but not odor-evoked activity in olfactory cortex. *J Neurosci* **36**, 7-18.

Lovelace, C. T., Stein, B. E. and Wallace, M. T. (2003). An irrelevant light enhances auditory detection in humans: a psychophysical analysis of multisensory integration in stimulus detection. *Cognitive Brain Research* **17**, 447-453.

Lund, R. D., Land, P. W. and Boles, J. (1980). Normal and abnormal uncrossed retinotectal pathways in rats: an HRP study in adults. *J Comp Neurol* **189**, 711-720.

Martin G.F. (1968). The pattern of neocortical projections to the mesencephalon of the opossum, Didelphis virginiana. *Brain Res* **11**, 593-610.

Massopust L.C., Wolin, L. R. and Meder, J. (1965). Spontaneous electrical activity of the brain in hibernators and nonhibernators during hypothermia. *Exp Neurol* **12**, 25-32.

May, P. J. (2006). The mammalian superior colliculus: laminar structure and connections. *Prog Brain Res* **151**, 321-378.

Mazzoni P., Bracewell, R. M., Barash, S. and Andersen, R. A. (1996). Spatially tuned auditory responses in area LIP of macaques performing delayed memory saccades to acoustic targets. *J Neurophysiol* **76**, 1439-56.

McHaffie, J. G., Beninato, M., Stein, B. E. and Spencer, R. F. (1991). Postnatal development of acetylcholinesterase in, and cholinergic projections to, the cat superior colliculus. *J Comp Neurol* **313**, 113-131.

McHaffie, J. G., Kruger, L., Clemo, H. R. and Stein, B. E. (1988). Corticothalamic and corticotectal somatosensory projections from the anterior ectosylvian sulcus (SIV cortex) in neonatal cats: an anatomical demonstration with HRP and 3H-leucine. *J Comp Neurol* **274**, 115-126.

Meredith, M., Nemitz, J. W. and Stein, B. E. (1987). Determinants of multisensory integration in superior colliculus neurons. I. Temporal factors. *The Journal of Neuroscience* **7**, 3215-3229.

Meredith, M. A. and Clemo, H. R. (1989). Auditory cortical projection from the anterior ectosylvian sulcus (Field AES) to the superior colliculus in the cat: an anatomical and electrophysiological study. *J Comp Neurol* **289**, 687-707.

Meredith, M. A., Clemo, H. R. and Stein, B. E. (1991). Somatotopic component of the multisensory map in the deep laminae of the cat superior colliculus. *J Comp Neurol* **312**, 353-370.

Meredith, M. A., Nemitz, J. W. and Stein, B. E. (1987). Determinants of multisensory integration in superior colliculus neurons. I. Temporal factors. *J Neurosci* **7**, 3215-3229.

Meredith, M. A. and Ramoa, A. S. (1998). Intrinsic circuitry of the superior colliculus: pharmacophysiological identification of horizontally oriented inhibitory interneurons. *J Neurophysiol* **79**, 1597-1602.

Meredith, M. A. and Stein, B. E. (1983). Interactions among converging sensory inputs in the superior colliculus. *Science* **221**, 389-391.

Meredith, M. A. and Stein, B. E. (1986). Spatial factors determine the activity of multisensory neurons in cat superior colliculus. *Brain Res* **365**, 350-354.

Meredith, M. A. and Stein, B. E. (1986). Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. *J Neurophysiol* **56**, 640-662.

Meredith, M. A. and Stein B. E. (1986). Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. *Journal of Neurophysiology* **56**, 640-662.

Meredith, M. A. and Stein, B. E. (1990). The visuotopic component of the multisensory map in the deep laminae of the cat superior colliculus. *J Neurosci* **10**, 3727-3742.

Meredith, M. A. and Stein, B. E. (1996). Spatial determinants of multisensory integration in cat superior colliculus neurons. *Journal of Neurophysiology* **75**, 1843-1857.

Middlebrooks, J. and Knudsen, E. (1984). A neural code for auditory space in the cat's superior colliculus. *The Journal of Neuroscience* **4**, 2621-2634.

Mize, R. R. (1988). Immunocytochemical localization of gamma-aminobutyric acid (GABA) in the cat superior colliculus. *J Comp Neurol* **276**, 169-187.

Mize, R. R. (1992). The organization of GABAergic neurons in the mammalian superior colliculus. *Prog Brain Res* **90**, 219-248.

Mize, R. R. and Butler, G. D. (1996). Postembedding immunocytochemistry demonstrates directly that both retinal and cortical terminals in the cat superior colliculus are glutamate immunoreactive. *J Comp Neurol* **371**, 633-648.

Mize, R. R. and Horner, L. H. (1989). Origin, distribution, and morphology of serotonergic afferents to the cat superior colliculus: a light and electron microscope immunocytochemistry study. *Exp Brain Res* **75**, 83-98.

Molholm, S., Ritter, W., Murray, M. M., Javitt, D. C., Schroeder, C. E. and Foxe, J. J. (2002). Multisensory auditory-visual interactions during early sensory processing in humans: a high-density electrical mapping study. *Brain Res Cogn Brain Res* **14**, 115-128.

Mooney, R. D., Fish, S. E. and Rhoades, R. W. (1984). Anatomical and functional organization of pathway from superior colliculus to lateral posterior nucleus in hamster. *J Neurophysiol* **51**, 407-431.

Moore, R. Y. and Goldberg, J. M. (1966). Projections of the inferior colliculus in the monkey. *Exp Neurol* **14**, 429-438.

Morrell, F. (1972). Visual system's view of acoustic space. *Nature* **238**, 44-46.

Moschovakis, A. K. and Karabelas, A. B. (1985). Observations on the somatodendritic morphology and axonal trajectory of intracellularly HRP-labeled efferent neurons

located in the deeper layers of the superior colliculus of the cat. *J Comp Neurol* **239**, 276-308.

Mower, G., Gibson, A. and Glickstein, M. (1979). Tectopontine pathway in the cat: laminar distribution of cells of origin and visual properties of target cells in dorsolateral pontine nucleus. *J Neurophysiol* **42**, 1-15.

Mucke, L., Norita, M., Benedek, G. and Creutzfeldt, O. (1982). Physiologic and anatomic investigation of a visual cortical area situated in the ventral bank of the anterior ectosylvian sulcus of the cat. *Exp Brain Res* **46**, 1-11.

Munoz, D.P. and Wurtz, R. H. (1995a). Saccade-related activity in monkey superior colliculus. I. Characteristics of burst and buildup cells. *J Neurophysiol* **73**, 2313-2333.

Munoz, D.P. and Wurtz, R. H. (1995b). Saccade-related activity in monkey superior colliculus. II. Spread of activity during saccades. *J Neurophysiol* **73**, 2334-2348.

Murray, M. M., Foxe, J. J., Higgens, B. A., Javitt, D. C. and Schroeder, C. E. (2001). Visuo-spatial neural response interactions in early cortical processing during a simple reaction time task: a high-density electrical mapping study. *Neuropsychologia* **39**, 828-844.

Nakamura, H. and Itoh, K. (2004). Cytoarchitectonic and connectional organization of the ventral lateral geniculate nucleus in the cat. *J Comp Neurol* **473**, 439-462.

Norita, M. (1980). Neurons and synaptic patterns in the deep layers of the superior colliculus of the cat. A Golgi and electron microscopic study. *J Comp Neurol* **190**, 29-48.

Norita, M., McHaffie, J. G., Shimizu, H. and Stein, B. E. (1991). The corticostriatal and corticotectal projections of the feline lateral suprasylvian cortex demonstrated with anterograde biocytin and retrograde fluorescent techniques. *Neurosci Res* **10**, 149-155.

Nozawa G., Reuter-Lorenz, P. A. and Hughes, H. C. (1994). Parallel and serial processes in the human oculomotor system: bimodal integration and express saccades. *Biol Cybern* **72**, 19-34.

Occelli, V., Spence, C. and Zampini, M. (2013). Auditory, tactile, and audiotactile information processing following visual deprivation. *Psychol Bull* **139**, 189-212.

Ogasawara, K., McHaffie, J. G. and Stein, B. E. (1984). Two visual corticotectal systems in cat. *J Neurophysiol* **52**, 1226-1245.

Olivier, E., Corvisier, J., Pauluis, Q. and Hardy, O. (2000). Evidence for glutamatergic tectotectal neurons in the cat superior colliculus: a comparison with GABAergic tectotectal neurons. *Eur J Neurosci* **12**, 2354-2366.

Pare, M., and Guitton, D. (1994). The fixation area of the cat superior colliculus: effect of electrical stimulation and direct connection with brainstem omnipause neurons. *Exp Brain Res* **101**, 109-122.

Perrault, T. J., Jr., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2005). Superior colliculus neurons use distinct operational modes in the integration of multisensory stimuli. *J Neurophysiol* **93**, 2575-2586.

Perrault, T. J., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2003). Neuron-Specific Response Characteristics Predict the Magnitude of Multisensory Integration. *Journal of Neurophysiology* **90**, 4022-4026.

Perrault, T. J., Vaughan, J. W. Stein, B. E. and Wallace, M. T. (2005). Superior Colliculus Neurons Use Distinct Operational Modes in the Integration of Multisensory Stimuli. *Journal of Neurophysiology* **93**, 2575-2586.

Pollack, J. G. and Hickey, T. L. (1979). The distribution of retino-collicular axon terminals in rhesus monkey. *J Comp Neurol* **185**, 587-602.

Powell, T. P. (1976). Bilateral cortico-tectal projection from the visual cortex in the cat. *Nature* **260**, 526-527.

Price, G. W., Kelly, J. S. and Bowery, N. G. (1987). The location of GABAB receptor binding sites in mammalian spinal cord. *Synapse* **1**, 530-538.

Putzar, L., Goerendt, I., Lange, K., Rosler, F. and Roder, B. (2007). Early visual deprivation impairs multisensory interactions in humans. *Nat Neurosci* **10**, 1243-1245.

Putzar, L., Gondan, M. and Roder, B. (2012). Basic multisensory functions can be acquired after congenital visual pattern deprivation in humans. *Dev Neuropsychol* **37**, 697-711.

Quaia C., Lefevre, P. and Optican, L. M. (1999). Model of the control of saccades by superior colliculus and cerebellum. *J Neurophysiol* **82**, 999-1018.

Rauschecker, J.P. and Harris, L. R. (1989). Auditory and visual neurons in the cat's superior colliculus selective for the direction of apparent motion stimuli. *Brain Res* **490**, 56-63.

Raczkowski, D. and Diamond, I. T. (1981). Projections from the superior colliculus and the neocortex to the pulvinar nucleus in Galago. *J Comp Neurol* **200**, 231-254.

Rhoades, R. W., Mooney, R. D., Chiaia, N. L. and Bennett-Clarke, C. A. (1990). Development and plasticity of the serotoninergic projection to the hamster's superior colliculus. *J Comp Neurol* **299**, 151-166.

Rockland, K. S. and Ojima, H. (2003). Multisensory convergence in calcarine visual areas in macaque monkey. *Int J Psychophysiol* **50**, 19-26.

Roder, B., Rosler, F. and Spence, C. (2004). Early vision impairs tactile perception in the blind. *Curr Biol* **14**, 121-124.

Rodrigo-Angulo, M. L. and Reinoso-Suarez, F. (1988). Connections to the lateral posterior-pulvinar thalamic complex from the reticular and ventral lateral geniculate thalamic nuclei: a topographical study in the cat. *Neuroscience* **26**, 449-459.

Romo, R., Hernandez, A. and Zainos, A. (2004). Neuronal correlates of a perceptual decision in ventral premotor cortex. *Neuron* **41**, 165-173.

Rossi, F. M., Pizzorusso, T., Porciatti, V., Marubio, L. M., Maffei, L. and Changeux, J.P. (2001). Requirement of the nicotinic acetylcholine receptor beta 2 subunit for the anatomical and functional development of the visual system. *Proc Natl Acad Sci U S A* **98**, 6453-6458.

Royal, D. W., Krueger, J., Fister, M. C. and Wallace, M. T. (2010). Adult plasticity of spatiotemporal receptive fields of multisensory superior colliculus neurons following early visual deprivation. *Restor Neurol Neurosci* **28**, 259-270.

Sakata, Y., Fujioka, T., Endoh, H. and Nakamura, S. (2006). In vivo optical recordings of synaptic transmission and intracellular Ca2+ and Cl- in the superior colliculus of fetal rats. *Eur J Neurosci* **23**, 1405-1416.

Sakurai, T., Miyamoto, T. and Okada, Y. (1990). Reduction of glutamate content in rat superior colliculus after retino-tectal denervation. *Neurosci Lett* **109**, 299-303.

Sakurai, T. and Okada, Y. (1992). Selective reduction of glutamate in the rat superior colliculus and dorsal lateral geniculate nucleus after contralateral enucleation. *Brain Res* **573**, 197-203.

Schiller, P.H. True, S. D. and Conway, J. L. (1980). Deficits in eye movements following frontal eye-field and superior colliculus ablations. *J Neurophysiol* **44**, 1175-1189.

Schlack, A., Sterbing-D'Angelo, S. J., Hartung, K., Hoffmann, K. P. and Bremmer, F. (2005). Multisensory Space Representations in the Macaque Ventral Intraparietal Area. *The Journal of Neuroscience* **25**, 4616-4625.

Schlag, J. and Schlag-Rey, M. (1970). Induction of oculomotor responses by electrical stimulation of the prefrontal cortex in the cat. *Brain Res* **22**, 1-13.

Schmidt M., Boller, M., Ozen, G. and Hall, W. C. (2001). Disinhibition in rat superior colliculus mediated by GABAc receptors. *J Neurosci* **21**, 691-699.

Schneider, G. E. (1969). Two visual systems. *Science* **163**, 895-902.

Schroeder, C. E. and Foxe, J. J. (2002). The timing and laminar profile of converging inputs to multisensory areas of the macaque neocortex. *Brain Res Cogn Brain Res* **14**, 187-198.

Schroeder, C. E., Lindsley, R. W., Specht, C., Marcovici, A., Smiley, J. F. and Javitt, D. C. (2001). Somatosensory input to auditory association cortex in the macaque monkey. *J Neurophysiol* **85**, 1322-1327.

Sefton, A. J. and Martin, P. R. (1984). Relation of the parabigeminal nucleus to the superior colliculus and dorsal lateral geniculate nucleus in the hooded rat. *Exp Brain Res* **56**, 144-148.

Segal, R. L. and Beckstead, R. M. (1984). The lateral suprasylvian corticotectal projection in cats. *J Comp Neurol* **225**, 259-275.

Snyder, L. H., Batista, A. P. and Andersen, R. A. (1998). Change in motor plan, without a change in the spatial locus of attention, modulates activity in posterior parietal cortex. *J Neurophysiol* **79**, 2814-2819.

Soetedjo, R., Kaneko, C. R. and Fuchs, A. F. (2002). Evidence against a moving hill in superior colliculus during saccadic eye movements in the monkey. *J Neurophysiol* **87**, 2778-2789.

Sooksawate, T., Isa, K., Behan, M., Yanagawa, Y. and Isa, T. (2011). Organization of GABAergic inhibition in the motor output layer of the superior colliculus. *Eur J Neurosci* **33**, 421-432.

Sprague, J.M. (1996). Neural mechanisms of visual orienting responses. *Prog Brain Res* **112**, 1-15.

Sprague, J. M. (1975). Mammalian tectum: intrinsic organization, afferent inputs, and integrative mechanisms. Anatomical substrate. *Neurosci Res Program Bull* **13**, 204-213.

Sprague, J. M. (1991). The role of the superior colliculus in facilitating visual attention and form perception. *Proc Natl Acad Sci U S A* **88**, 1286-1290.

Sprague, J. M., Berlucchi, G. and Di Berardino, A. (1970). The superior colliculus and pretectum in visually guided behavior and visual discrimination in the cat. *Brain Behav Evol* **3**, 285-294.

Sprague, J. M., Levy, J., DiBerardino, A., and Berlucchi, G. (1977). Visual cortical areas mediating form discrimination in the cat. *J Comp Neurol* **172**, 441-488.

Sprague, J. M. and Meikle, Jr., T. H. (1965). The Role of the Superior Colliculus in Visually Guided Behavior. *Exp Neurol* **11**, 115-146.

Stanford, T. R., Quessy, S. and Stein, B. E. (2005). Evaluating the Operations Underlying Multisensory Integration in the Cat Superior Colliculus. *The Journal of Neuroscience* **25**, 6499-6508.

Stanford, T. R., Quessy, S. and Stein, B. E. (2005). Evaluating the operations underlying multisensory integration in the cat superior colliculus. *J Neurosci* **25**, 6499-6508.

Stanford, T. R. and Stein, B. E. (2007). Superadditivity in multisensory integration: putting the computation in context. *Neuroreport* **18**, 787-792.

Stein, B.E. and Arigbede, M. O. (1972). Unimodal and multimodal response properties of neurons in the cat's superior colliculus. *Exp Neurol* **36**, 179-196.

Stein, B. E., Goldberg, S. J. and Clamann, H. P. (1976). The control of eye movements by the superior colliculus in the alert cat. *Brain Res* **118**, 469-474.

Stein, B. E., Magalhaes-Castro, B. and Kruger, L. (1976). Relationship between visual and tactile representations in cat superior colliculus. *Journal of Neurophysiology* **39**, 401-419.

Stein, B. E., Meredith, M. A. and Wallace, M. T. (1993). The visually responsive neuron and beyond: multisensory integration in cat and monkey. *Prog Brain Res* **95**, 79-90.

Stein, B. E., Scott Huneycutt, W. S. and Meredith, M. A. (1988). Neurons and behavior: the same rules of multisensory integration apply. *Brain Research* **448**, 355-358.

Stein, B. E., Spencer, R. F. and Edwards, S. B. (1983). Corticotectal and corticothalamic efferent projections of SIV somatosensory cortex in cat. *J Neurophysiol* **50**, 896-909.

Stein, B. E., Wallace, M. W., Stanford, T. R. and Jiang, W. (2002). Cortex governs multisensory integration in the midbrain. *Neuroscientist* **8**, 306-314.

Stein, B. E., Huneycutt, W. S. and Meredith, M. A. (1988). Neurons and behavior: the same rules of multisensory integration apply. *Brain Research* **448**, 355-358.

Stein, B. E. and Meredith, M. A. (1986). Visual, Auditory, and Somatosensory Convergence on Cells in Superior Colliculus Results in Multisensory Integration. *J Neurophysiology* **56**, 640-662.

Steinbusch, H. W. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* **6**, 557-618.

Sterling, P. (1971). Receptive fields and synaptic organization of the superficial gray layer of the cat superior colliculus. *Vision Res* **3**, 309-328.

Sterling, P. and Wickelgren, B. G. (1969). Visual receptive fields in the superior colliculus of the cat. *J Neurophysiol* **32**, 1-15.

Stevenson, J. A. and Lund, R. D. (1982). A crossed parabigemino-lateral geniculate projection in rats blinded at birth. *Exp Brain Res* **45**, 95-100.

Stevenson, R. A. and James, T. W. (2009). Audiovisual integration in human superior temporal sulcus: Inverse effectiveness and the neural processing of speech and object recognition. *Neuroimage* **44**, 1210-1223.

Stricanne, B., Andersen, R. A. and Mazzoni, P. (1996). Eye-centered, head-centered, and intermediate coding of remembered sound locations in area LIP. *J. Neurophysiol* **76**, 2071-2076.

Stuphorn, V., Bauswein, E. and Hoffmann, K. P. (2000). Neurons in the primate superior colliculus coding for arm movements in gaze-related coordinates. *J. Neurophysiol* **83**, 1283-1299.

Sugihara, T., Diltz, M. D., Averbeck, B. B. and Romanski, L. M. (2006). Integration of Auditory and Visual Communication Information in the Primate Ventrolateral Prefrontal Cortex. *The Journal of Neuroscience* **26**, 11138-11147.

Suzuki, K. (1985). Projections from the functional subdivisions of the frontal eye field to the superior colliculus in the monkey. *Brain Research* **327**, 324-327.

Swanson, L. W., Cowan, W. M. and Jones, E. G. (1974). An autoradiographic study of the efferent connections of the ventral lateral geniculate nucleus in the albino rat and the cat. *J Comp Neurol* **156**, 143-163.

Symonds, L. L. and Kaas, J. H. (1978). Connections of striate cortex in the prosimian, Galago senegalensis. *J Comp Neurol* **181**, 477-512.

Takahashi, T. (2011). Serotonin as a mediator of cross-modal cortical reorganization. *Commun Integr Biol* **4**, 459-461.

Tao, Q., Chan, C. C., Luo, Y. J., Li, J. J., Ting, K. H., Wang, J. and Lee, T. M. (2015). How does experience modulate auditory spatial processing in individuals with blindness? *Brain Topogr* **28**, 506-519.

Tortelly, A., Reinoso-Suarez, F. and Llamas, A. (1980). Projections from non-visual cortical areas to the superior colliculus demonstrated by retrograde transport of HRP in the cat. *Brain Res* **188**, 543-549.

Tunkl, J. E. and Berkley, M. A. (1977). The role of superior colliculus in vision: visual form discrimination in cats with superior colliculus ablations. *J Comp Neurol* **176**, 575-587.

Turner, J. P., Sauve, Y., Varela-Rodriguez, C., Lund, R. D. and Salt, T. E. (2005). Recruitment of local excitatory circuits in the superior colliculus following deafferentation and the regeneration of retinocollicular inputs. *Eur J Neurosci* **22**, 1643-1654.

Ueda, S., Ihara, N. and Sano, Y. (1985). The organization of serotonin fibers in the mammalian superior colliculus. An immunohistochemical study. *Anat Embryol* **173**, 13-21.

Updyke, B. V. (1977). Topographic organization of the projections from cortical areas 17, 18 and 19 onto the thalamus, pretectum and superior colliculus in the cat. *J Comp Neurol* **173**, 81-122.

Vetencourt, J. F., Tiraboschi, E., Spolidoro, M., Castren, E. and Maffei, L. (2011). Serotonin triggers a transient epigenetic mechanism that reinstates adult visual cortex plasticity in rats. *Eur J Neurosci* **33**, 49-57.

Villar, M. J., Vitale, M.L., Hokfelt, T. and Verhofstad, A. A. (1988). Dorsal raphe serotoninergic branching neurons projecting both to the lateral geniculate body and superior colliculus: a combined retrograde tracing-immunohistochemical study in the rat. *J Comp Neurol* **277**, 126-140.

Voss, P., Lassonde, M., Gougoux, F., Fortin, M. Guillemot, J. P. and Lepore, F. (2004). Early- and late-onset blind individuals show supra-normal auditory abilities in far-space. *Curr Biol* **14**, 1734-1738.

Waleszczyk W.J., Nagy, A., Wypych, M., Berenyi, A., Paroczy, Z., Eordegh, G., Ghazaryan, A. and Benedek, G. (2007). Spectral receptive field properties of neurons in the feline superior colliculus. *Exp Brain Res* **181**, 87-98.

Wallace, M. T., Meredith, M. A. and Stein, B. E. (1998). Multisensory integration in the superior colliculus of the alert cat. *J Neurophysiol* **80**, 1006-1010.

Wallace, M. T., Perrault, Jr., T. J., Hairston, W. D. and Stein, B. E. (2004). Visual experience is necessary for the development of multisensory integration. *J Neurosci* **24**, 9580-9584.

Wallace, M. T. and Stein, B. E. (1994). Cross-modal synthesis in the midbrain depends on input from cortex. *J Neurophysiol* **71**, 429-432.

Wallace, M. T. and Stein, B. E. (1996). Sensory organization of the superior colliculus in cat and monkey. *Prog Brain Res* **112**, 301-311.

Wallace, M. T. and Stein, B. E. (1997). Development of multisensory neurons and multisensory integration in cat superior colliculus. *J Neurosci* **17**, 2429-2444.

Wallace, M. T. and Stein, B. E. (2007). Early experience determines how the senses will interact. *J Neurophysiol* **97**, 921-926.

Wallace, M. T. and Stein, B. E. (2007). Early Experience Determines How the Senses Will Interact. *Journal of Neurophysiology* **97**, 921-926.

Wallace, M. T., Wilkinson, L. K. and Stein, B. E. (1996). Representation and integration of multiple sensory inputs in primate superior colliculus. *J Neurophysiol* **76**, 1246-1266.

Wang, L., Rangarajan, K. V., Lawhn-Heath, C. A., Sarnaik, R., Wang, B. S., Liu, X. and Cang, J. (2009). Direction-specific disruption of subcortical visual behavior and receptive fields in mice lacking the beta2 subunit of nicotinic acetylcholine receptor. *J Neurosci* **29**, 12909-12918.

Waterhouse, B. D., Azizi, S. A., Burner, R. A. and Woodward, D. J. (1990). Modulation of rat cortical area 17 neuronal responses to moving visual stimuli during norepinephrine and serotonin microiontophoresis. *Brain Res* **514**, 276-292.

Weber, J. T. and Harting, J. K. (1980). The efferent projections of the pretectal complex: an autoradiographic and horseradish peroxidase analysis. *Brain Res* **194**, 1-28.

Weldon, D.A. and Best, P. J. (1992). Changes in sensory responsivity in deep layer neurons of the superior colliculus of behaving rats. *Behav Brain Res* **47**, 97-101.

Wickelgren, B.G. (1971). Superior colliculus: some receptive field properties of bimodally responsive cells. *Science* **173**, 69-72.

Wilkinson, L. K., Meredith, M. A. and Stein, B. E. (1996). The role of anterior ectosylvian cortex in cross-modality orientation and approach behavior. *Exp Brain Res* **112**, 1-10.

Wilson, J. R., Hendrickson, A. E., Sherk, H. and Tigges, J. (1995). Sources of subcortical afferents to the macaque's dorsal lateral geniculate nucleus. *Anat Rec* **242**, 566-574.

Wise, L.Z. and Irvine, D. R. (1983). Auditory response properties of neurons in deep layers of cat superior colliculus. *J Neurophysiol* **49**, 674-685.

Wise, L.Z. and Irvine, D. R. (1985). Topographic organization of interaural intensity difference sensitivity in deep layers of cat superior colliculus: implications for auditory spatial representation. *J Neurophysiol* **54**, 185-211.

Wurtz, R.H. and Albano, J. E. (1980). Visual-motor function of the primate superior colliculus. *Annu Rev Neurosci* **3**, 189-226.

Wurtz, R.H. and Goldberg, M. E. (1971). Superior colliculus cell responses related to eye movements in awake monkeys. *Science* **171**, 82-84.

Xiang, Z. and Prince, D. A. (2003). Heterogeneous actions of serotonin on interneurons in rat visual cortex. *J Neurophysiol* **89**, 1278-87.

Xu, J., Yu, L., Rowland, B. A., Stanford, T. R. and Stein, B. E. (2014). Noise-rearing disrupts the maturation of multisensory integration. *Eur J Neurosci* **39**, 602-613.

Xu, J., Yu, L., Stanford, T. R., Rowland, B. A. and Stein B. E. (2015). What does a neuron learn from multisensory experience? *J Neurophysiol* **113**, 883-889.

Yu, L., Rowland, B. A. and Stein, B. E. (2010). Initiating the Development of Multisensory Integration by Manipulating Sensory Experience. *The Journal of Neuroscience* **30**, 4904-4913.

Zhang, H. Y. and Hoffmann, K. P. (1993). Retinal projections to the pretectum, accessory optic system and superior colliculus in pigmented and albino ferrets. *Eur J Neurosci* **5**, 486-500.

Zhang, M., Weisser, V. D., Stilla, R., Prather, S. C. and Sathian, K. (2004). Multisensory cortical processing of object shape and its relation to mental imagery. *Cogn Affect Behav Neurosci* **4**, 251-259.

Zhao, J. P., Phillips, M. A. and Constantine-Paton, M. (2006). Long-term potentiation in the juvenile superior colliculus requires simultaneous activation of NMDA receptors and L-type Ca2+ channels and reflects addition of newly functional synapses. *J Neurosci* **26**, 12647-12655.

Zhou, Y. D. and Fuster, J. M. (2000). Visuo-tactile cross-modal associations in cortical somatosensory cells. *Proc Natl Acad Sci U S A* **97**, 9777-9782.

Zhou, Y. D. and Fuster, J. M. (2004). Somatosensory cell response to an auditory cue in a haptic memory task. *Behav Brain Res* **153**, 573-578.

Chapter II

Visual experience influences sensory and multisensory processing in the superior colliculus across a lifetime

This chapter is a manuscript under preparation to be submitted as: Kurela, L. R., Krueger-Fister, J., Nidiffer, A. R. and Wallace, M. T. Visual experience influences sensory and multisensory processing in the superior colliculus across a lifetime.

Introduction

Multisensory integration, the process by which sensory stimuli converge and are combined, is imperative for proper orientation to and interaction with the world (Stein 1988; Frens and Van Opstal 1998; Bell, Corneil et al. 2001; Stein 2012). The superior colliculus (SC) is one structure important for this process. Sensory stimuli are converged and integrated by individual neurons within the SC (Meredith and Stein 1983; Meredith and Stein 1986; Meredith and Stein 1986; Meredith et al. 1987; Meredith and Stein 1996; Perrault et al. 2003; Perrault et al. 2005), although this is not an inherent cellular process.

The process of multisensory integration develops gradually in SC neurons. As in cortical and historically unisensory brain regions, this normal development is necessitated by sensory experiences. Without normal sensory experience, SC multisensory cells maintain responsiveness to unisensory stimuli but never develop the capacity to integrate these sensory signals. Visual (Wallace and Stein 2007) or auditory (Xu et al. 2014) deprivation during maturation obstructs the capacity of multisensory SC and cortical neurons (Carriere et al. 2007) to integrate information from different modalities. Any altered sensory experience during this stage of development drastically

modifies these cells' integrative capacities; for example, the rearing of animals in a spatially discordant environment results in development of cells with spatially offset receptive fields (Wallace and Stein 2007). The multisensory integrative system is incredibly plastic throughout developmental maturation, and changes in sensory experience here can radically change multisensory processing, which can manifest at the spiking level as well as the behavioral and perceptual levels (Eimer 2004; Roder et al. 2004; Putzar et al. 2007; Leo et al. 2008; Collignon et al. 2009; Hotting and Roder 2009; Putzar et al. 2012; Occelli et al. 2013; Guerreiro et al. 2015; Hauthal et al. 2015). However, the question of whether this system remains flexible throughout a lifetime remains.

While research has provided information on the effects of late-onset sensory deprivation at the behavioral and functional organizational levels (Kujala et al. 1997; Burton et al. 2002; Voss et al. 2004; Champoux et al. 2011; Collignon et al. 2013; Focker et al. 2015; Tao et al. 2015), to our knowledge no work has been completed to investigate this at the neuronal level within the SC. Previous work has shown that restoration of visual experience in adulthood after developmental visual deprivation has minimal effects on the altered multisensory processing, with most neurons continuing to lack integrative capacity (Royal et al. 2010). This suggests the possible existence of a sensitive period for multisensory experience, outside of which, for example during adulthood, this experience does not have the ability to drastically affect multisensory processing capacities. To examine if multisensory processing is susceptible to experiential change throughout adulthood, we compared the unisensory and multisensory interactions in the SC of normally-reared (NR) and completely dark-reared

(DR) animals to animals normally reared through maturation before being visually deprived in adulthood (6+6). Building on the prior work of Royal et al. 2010, we hypothesized that if there exists a sensitive period for multisensory experience during development, then changes in sensory experience only during adulthood should have little effect on multisensory processing, and multisensory interactions recorded in our 6+6 animals would be incredibly similar to those recorded in NR animals. This was, in fact, the opposite of what we encountered. Our results show that the SC has a layer-specific compensatory plasticity even during adulthood and changes in the dynamics of multisensory integration are adaptive to visual deprivation even after normal development.

Materials and methods

General procedures

All experiments were completed in anesthetized and paralyzed terminal preparations. Electrophysiological recordings were performed on adult cats (n=7; > 1-year-old) for 80-96 hours following a craniotomy procedure. The experiments consisted of multiunit extracellular recordings from the superior colliculus (SC). All surgical and recording procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals under a protocol approved by the Institutional Animal Care and Use Committee and Vanderbilt University Medical Center, which is accredited by the American Association for Accreditation of Laboratory Animal Care.

Animal housing and visual experience

Cats (n=7) were used in electrophysiological recording experiments for this study. These animals were divided into three groups, each group varying in their amount of visual experience. One group of cats (n=3) were dark-reared, completely deprived of all visual experience through rearing in a light-absent environment from birth through adulthood (DR). Daily care and observation procedures were conducted with the use of binocular infared goggles and monitoring cameras to avoid any visual experience. Sedation procedures in preparation for recordings took place in the animal's dark-room housing cages in order to eliminate as much confounding visual experience as possible. Additionally, occluding masks and covered carriers were used during transport of the cats from light-deprived housing to experimental rooms. A second group of cats (n=2) were reared in normal light/dark conditions throughout development (approximately 6 months) and were then deprived of all visual experience through housing in a light-deprived environment during adulthood (6+6). The light-deprived housing environment into which these animals were transferred is identical to the housing described for the DR animals (see above), and these animals remained in this housing for >6 months prior to recording procedures. A third group of cats (n=2) were raised and housed under standard housing conditions in a normal light/dark cycle (NR).

Implantation and recording procedures

Animals were induced with ketamine hydrochloride (20 mg/kg, administered intramuscularly (IM)) and acepromazine maleate (0.04mg/kg IM) as initial anesthesia for surgical procedures. Animals were intubated and artificially respired, and a stable plane

of anesthesia and paralysis was achieved using a constant rate infusion of ketamine (5mg/kg/hr intravenous (IV)) and rocuronium bromide (2.2-2.5mg/kg/hr IV) delivered through a cannula placed in the saphenous vein for the remainder of the procedure. Before inducing paralysis, a stable plane of anesthesia was verified for each animal. Body temperature, expiratory CO_2, blood pressure and heart rate were continuously monitored (VSM7, Vetspecs/SCIL), recorded and maintained within ranges consistent with a deep and stable plane of anesthesia. A craniotomy was made to allow access to the SC and a head holder was attached to the cranium using stainless steel screws and orthopedic cement to hold the animal in a stable and recumbent position during the recording session without obstructing the face and ears.

Following the craniotomy procedure and mapping the location of the SC using parylene-insulated tungsten electrodes (initial impedance at 1 kHz = 4-5 MΩ), electrophysiological recordings were performed for 80-96 hours using a multi-channel U-probe (24 channels, 125 µm inter-electrode spacing, Plexon). Multi-unit activity (MUA) and local field potential (LFP) recordings targeted the SC (Supplementary Figure 1). Neural activity was recorded (SortClient software, Plexon), amplified and routed to an oscilloscope, audio monitor and computer in order to perform online and offline analyses.

Stimulus presentation and search strategy

The top of SC was determined by its characteristic fast visual responses. Visual fields were mapped and receptive field (RF) boundaries were determined. Visual stimuli consisted of the illumination of stationary light emitting diodes (LEDs; 100ms duration)

and auditory stimuli consisted of broadband noise bursts (20 Hz-20 kHz; 100 ms duration; 67 dBSPL). LEDs and speakers were concurrently mounted on a rotatable hoop at azimuthal locations ranging from 0°- 90° in 10° increments on either side of the midline. This hoop was placed 60 cm in front of the cat, and its rotation allowed the sampling of multiple locations within and outside the RFs of the recorded cells. Visual (V), auditory (A) and audiovisual (AV) stimuli were presented in a pseudorandomized interleaved manner at multiple azimuthal locations along a single elevation at a time. Multisensory presentations always consisted of visual and auditory stimuli presented at the same spatial location with a temporal offset where the visual stimulus preceded the auditory stimulus by 50 ms. Stimuli were presented in this interleaved manner until a minimum of 60 trials were collected for a single location (20 visual, 20 auditory, 20 multisensory). Consecutive stimulus presentations were separated by at least 3 s to avoid response habituation. 2-3 elevations and 3-4 azimuths were chosen within and around the mapped RF boundaries for each electrode penetration of recordings. In order to ensure that the top of SC was captured, the first set of recordings began when visual responses characteristic of the superficial SC were seen in more than half of the lower electrode channels but not in at least two of the uppermost electrodes on the U-probe. To ensure complete capture of the entirety of the SC with each electrode penetration, these sets of RF mappings and LFP/MUA recordings were completed twice more, advancing the U-probe 2000 μm between each set. 7-8 recording penetrations of this nature were performed and spread throughout each SC.

Data acquisition and MUA analysis

A custom-built PC-based real-time data acquisition system controlled the structure of the trials and the timing of the stimuli (LabView, National Instruments). The analog waveforms picked up by each electrode were transferred to a Plexon MAP system where they were digitized and high-pass filtered at 40 kHz for spiking data and low-pass filtered a 1 kHz for LFPs. MUA was thresholded and sorted online using Sort Client software, and stored for further offline analysis. Neuronal responses were detailed through construction of peristimulus time histograms (PSTHs) for each condition (visual only (V), auditory only (A), paired visual-auditory (VA)) for each location tested. Baseline for each PSTH was calculated as mean firing rate during the 500 ms immediately preceding the stimulus onset for each of the 3 conditions. Response threshold was set at 2 SD above this baseline in order to delineate the stimulus evoked response. The time at which the PSTH crossed above the 2 SD line (and remained so for at least 30 ms) was determined to be response onset. Response offset was measured as the time at which the PSTH fell below the 2 SD line and stayed below this line for $\geq$30 ms. Response duration was defined as the time interval between response onset and response offset. Mean stimulus evoked response was calculated as the average number of spikes elicited during the defined response duration interval per trial. Mean spontaneous firing rate was subtracted from the responses. Analyses of variance (ANOVA) were completed to determine differences between experience groups, and follow-up Student's t-test were then performed.

Evaluation of multisensory integration

Interactive index (ii) was used to quantify multisensory integration. ii measures how the multisensory response differs from the best unisensory response. The magnitude of this change was calculated as

$$[(CM - SMmax)/(SMmax)] \times 100 = \% \text{ interaction}$$

where CM is the mean response evoked by the combined modality (i.e., multisensory) stimulus and SMmax is the mean response evoked by the most effective single modality stimulus (Meredith and Stein 1983). Statistical comparisons between the mean stimulus evoked responses of the multisensory and best unisensory conditions and the additive prediction were done using a Wilcoxon Rank Test. The measurements outlined above were averaged for all electrodes recorded within the superficial (or deep) SC layers showing response enhancements (or depressions), within each experimental group.

LFP analyses

Evoked LFP responses for each electrode and all stimulus locations showing response enhancements within the superficial SC layers were averaged to produce a grand average event-related potential. This was also performed for electrodes recording from deep SC layers, as well as for stimulus locations showing response depressions and no interactions. LFP amplitudes were compared pre- and post- stimulus onset; the mean voltage within a window of 150 ms pre-stimulus onset was used as a baseline and compared to peak voltage change within a 300 ms post-stimulus timeframe. These were compared between visual, auditory, and multisensory conditions using t-tests to determine if LFP amplitude differed between stimulus conditions. These were compared

across experimental groups, as well. Mean magnitude (AUC, area under the curve) for the averaged evoked LFPs were computed for each of the stimulus conditions (V, A, AV), each of the interactions (enhancement, depression, no interaction), and the experimental groups (NR, DR, 6+6) for each of the electrodes, divided into superficial or deep layer SC recordings. The AUC for each stimulus condition within the interaction types was compared using t-tests, and then also compared across experimental groups.

Results

Multisensory interactions were found in animals from all experience groups

Multiunit extracellular activity (MUA) recordings were performed throughout the superficial and intermediate/deep SC (Supplementary Figure 2). Multisensory interactions were classified as enhancements if responses to audiovisual stimulus presentations exceeded unisensory (i.e. visual, auditory) stimulus responses and the sum of the unisensory responses. Recordings were classified as response depressions if audiovisual responses were significantly less than unisensory responses.

Instances of multisensory enhancement and depression were encountered in all three experience groups (Figure 1). Both response enhancements and depressions were found in NR, 6+6 and DR animals however it was more likely to encounter these interactions in NR recordings. Of the 1296 recordings performed in NR animals, 67% exhibited multisensory interactions. 56% of the 2856 recordings performed in 6+6 animals exhibited multisensory interactions, compared to only 37% of 5688 recordings in DR animals. In addition, the ratio of interactions differed between experience groups. While NR animals exhibited more response enhancements than depressions, this trend

switched for the 6+6 and DR groups; response depressions were more prevalent than enhancements in the DR and 6+6 animals (Figure 1). These results show that while it was more likely to find instances of multisensory interactions in NR animals, both response enhancements and response depressions were encountered in all three experience groups of animals.

Instances of multisensory integration in superficial layers are similar between experience groups

1456 total recordings took place within the superficial SC; 244 in NR, 321 in 6+6, and 891 recordings in DR animals. ANOVA revealed a main effect of experience group on responses (Table 1). Overall, the most activity was recorded in the NR animals; superficial SC neurons were the most responsive to visual and auditory stimulus presentations compared to the 6+6 and DR recordings (Figure 2A). Average MUA response to auditory stimuli was 61.52 $\pm$ 10.16 spikes/trial in NR animals, 25.65 $\pm$ 3.38 spikes/trial in 6+6 animals, and 11.97 $\pm$ 1.56 spikes/trial in DR animals (Table 2). Visual responses also differed between groups; 84.5 $\pm$ 17.35 spikes/trial in NR, 17.87 $\pm$ 1.46 spikes/trial in 6+6, and 25.71 $\pm$ 2.83 spikes/trial in DR animals (Table 2). However, when examining all superficial SC recordings together, no statistically significant difference between the groups in audiovisual responses or the amount of multisensory integration were encountered (Figure 2A). When dividing the data by the type of multisensory interaction exhibited (i.e. multisensory enhancements or depressions), a similar trend was found. NR superficial SC neurons were more active and responsive compared to 6+6 and DR recordings showing multisensory enhancements (Figure 2B)

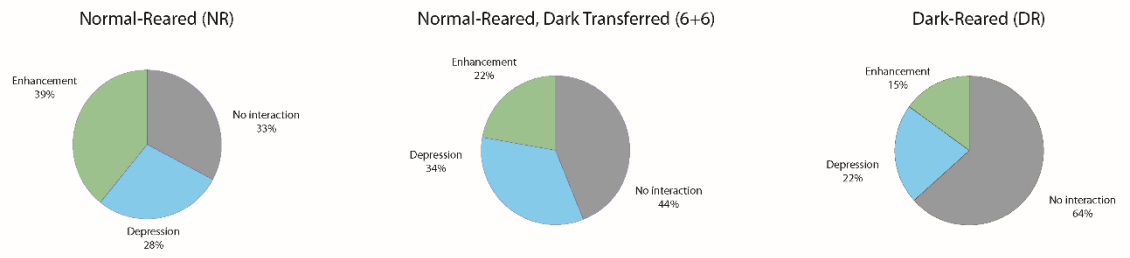


Figure 2-1. Multisensory interactions were encountered in all visual experience groups. Multi-unit activity (MUA) recordings were performed in NR (left), 6+6 (middle), and DR (right) animals, exhibiting multisensory response enhancements (green), response depressions (blue) or no interaction (grey). Instances of multisensory interactions (enhancement and depression) were encountered in all three experience groups. Of the interactions seen, response enhancements were more prevalent in NR animals, and depressions more prevalent in 6+6 and DR animals.

and depressions (Figure 2C). ANOVA revealed a main effect of experience group for superficial SC enhancements (Table 1). Auditory responses in NR recordings were greater than those from DR and 6+6 recordings (Table 2); 49.67 ± 16.17 spikes/trial (NR) compared to 14.86 ± 2.39 spikes/trial (DR) and 16.28 ± 3.67 spikes/trial (6+6). NR responses to audiovisual stimuli were also greater than responses in 6+6 animals (Table 2); 85.34 ±29.87 compared to 48.16 ± 12.21 spikes/trial. Visual responsivity was greater in the NR group compared to either of the two dark-reared groups; 72.92±28.00 spikes/trial in NR recordings compared to 14.88 ±2.75 spikes/trial in 6+6 and 22.58 ± 3.47 spikes/trial in DR animals (Figure 2B). Again, there were no statistically significant differences between the DR and 6+6 experience groups at the level of interactive index.

Similar patterns were found in instances of response depression in the superficial SC (Figure 2C). ANOVA revealed an effect of experience group (Table 1). Responses to auditory stimuli were greater in NR animals as compared to 6+6 and DR animals; 67.15 ± 12.99 spikes/trial compared to 34.92 ± 4.83 spikes/trial (6+6) and 5.22 ± 0.44 spikes/trial (DR) (Table 2). Visual responses were also greater in NR animals, 90.14 ± 22.2 spikes/trial compared to 20.87 ± 0.91 spikes/trial in 6+6 animals and 29.53 ± 4.68 spikes/trial in DR animals (Figure 2C). Similarly, audiovisual responses were greater in NR animals, as well (Table 2); 38.88 ± 8.09 spikes/trial in NR animals compared to 11.63 ± 1.08 spikes/trial (6+6) and 15.13 ± 1.94 spikes/trial (DR). The amount of response depression (ii) observed in these recordings did not differ significantly between experimental groups (Figure 2C).

	ANOVA results
Superficial MUA Response	F(3,271) = 12.92, p<0.001
Superficial MUA Response Enhancement	F(3,42)= 8.07, p<0.0001
Superficial MUA Response Depression	F(3,63)=5.83, p=0.025
Deep MUA Response	F(3,771)=2.24, p<0.0001
Deep MUA Response Enhancement	F(3,169)=2.01, p<0.0001
Deep MUA Response Depression	F(3,146)=1.26, p=0.045
NR Superficial MUA Recording Time	p>0.05
DR Superficial MUA Recording Time	F(2,80)=4.5, p=0.014
6+6 Superficial MUA Recording Time	p>0.05
NR Deep MUA Recording Time	p>0.05
DR Deep MUA Recording Time	F(2,455)=2.83, p=0.04
6+6 Deep MUA Recording Time	F(2,108)=5.98, p=0.003
Superficial LFP AUC	F(2,50)=8.04, p=0.0009
Superficial LFP Peak	p>0.05
Deep LFP AUC	F(2,50)=92.69, p<0.0001
Deep LFP Peak	p>0.05
NR Superficial LFP Recording Time	F(2,117)= 7.4, p=0.01
DR Superficial LFP Recording Time	F(2,141)= 12.74, p<0.0001
6+6 Superficial LFP Recording Time	F(1,253)= 5.4, p=0.021
NR Deep LFP Recording Time	F(2,237)= 4.88, p=0.0084
DR Deep LFP Recording Time	F(2,604)= 5.64, p=0.0037
6+6 Deep LFP Recording Time	F(2,185)= 32.73, p<0.0001

Table 2-1. Results of analysis of variance (ANOVA) tests.

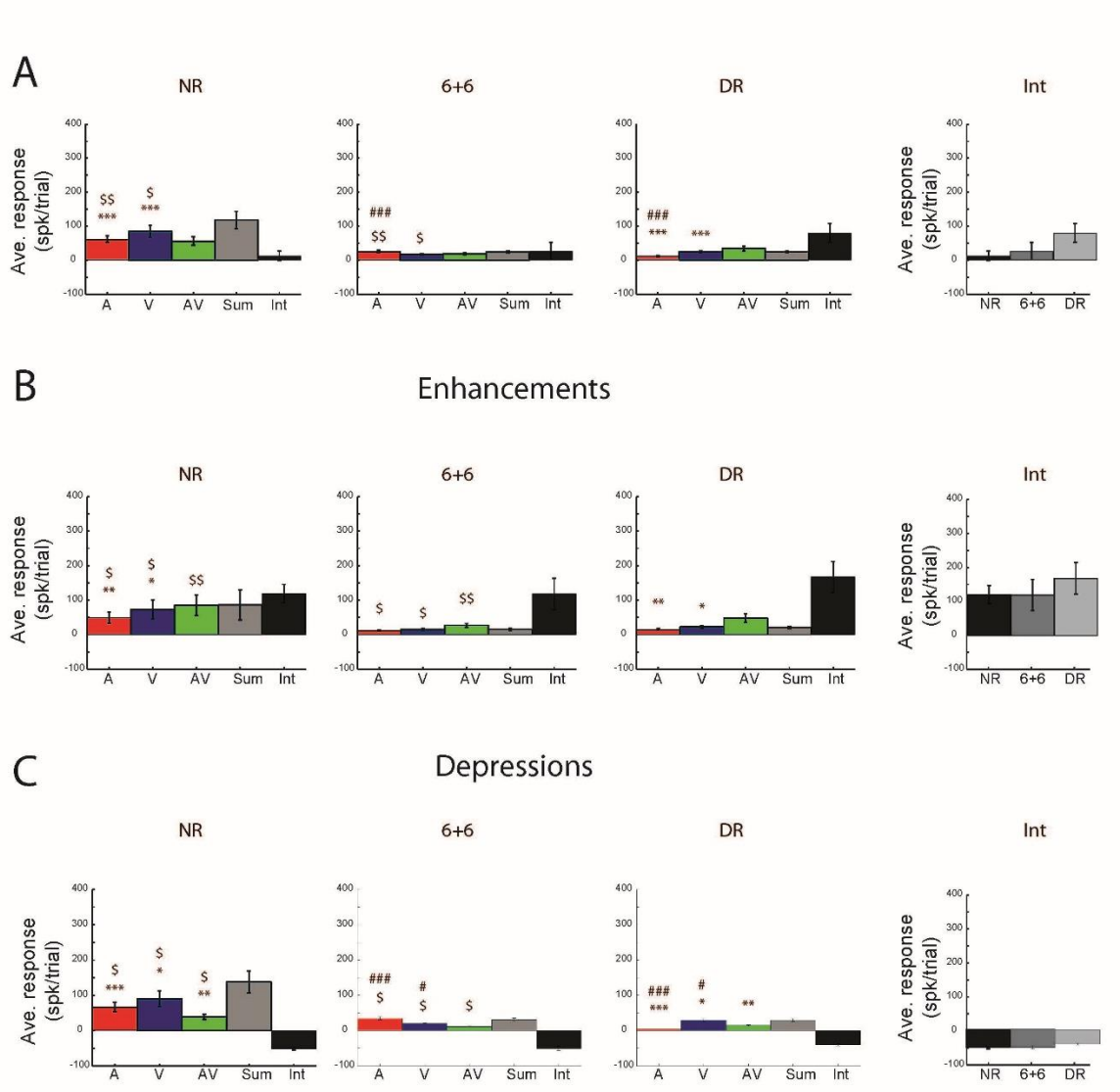


Figure 2-2. Multisensory integration in the superficial layers of the SC is similar between experience groups. (A) When examining all recordings within the superficial SC, NR recordings (left) exhibit the most activity, for auditory (red), visual (blue) and audiovisual (green) stimulus presentations, when compared with recordings from 6+6 (middle) and DR (right) recordings. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001 between NR and DR recordings (*), NR and 6+6 ($), and between DR and 6+6 recordings (#). While NR recordings showed more activity overall,

no difference was found in terms of the magnitude of intergration (black, far right graph) between the three groups. Similar trends were seen when examining only recordings exhibiting multisensory response enhancement (B) and multisensory response depression (C).

	NR to DR	NR to 6+6	DR to 6+6
Superficial MUA Auditory Response	t(627)=8.33, p<0.0001	t(267)=3.05, p=0.00249	t(618)=5.57, p<0.0001
Superficial MUA Visual Response	t(627)=5.21, p<0.0001	t(267)= 2.06, p=0.0405	t(618)=2.02, p=0.0438
Superficial MUA Audiovisual Response	p>0.05	p>0.05	p>0.05
Superficial MUA Response Gain (ii)	p>0.05	p>0.05	p>0.05
Superficial MUA Auditory Response Enhancement	t(108)=3.301, p=0.00131	t(40)=2.257, p=0.0339	p>0.05
Superficial MUA Visual Response Enhancement	t(108)=1.880, p=0.0428	t(40)=1.094, p=0.0281	p>0.05
Superficial MUA Audiovisual Response Enhancement	p>0.05	t(108)=1.361, p=0.0176	p>0.05
Superficial MUA Response Enhancement Gain (ii)	p>0.05	p>0.05	p>0.05
Superficial MUA Auditory Response Depression	t(103)=5.244, p<0.0001	t(61)=1.926, p=0.049	t(76)=6.197, p<0.0001
Superficial MUA Visual Response Depression	t(103)=2.46, p=0.0155	t(61)=2.87, p=0.0410	t(76)=2.01, p=0.0477
Superficial MUA Audiovisual Response Depression	t(103)=3.23, p=0.00165	t(61)=2.12, p=0.0384	p>0.05
Superficial MUA Response Depression Gain (ii)	p>0.05	p>0.05	p>0.05
Deep MUA Auditory Response	t(2428)=9.66, p<0.0001	t(769)=10.22, p<0.0001	t(2175)=36.97, p<0.0001
Deep MUA Visual Response	t(2428)=3.21, p=0.000133	t(769)=7.97, p<0.0001	t(2715)=5.97, p<0.0001
Deep MUA Audiovisual Response	t(2428)=2.40, p=0.0163	t(769)=7.27, p<0.0001	t(2715)=19.29, p<0.0001
Deep MUA Response Gain (ii)	t(2428)=1.21, p=0.0228	p>0.05	t(2715)=1.32, p=0.0186
Deep MUA Auditory Response Enhancement	p>0.05	t(167)=4.07, p<0.0001	t(570)=17.48, p<0.0001
Deep MUA Visual Response Enhancement	t(511)=3.99, p<0.0001	t(167)=4.91, p<0.0001	t(570)=3.89, p=0.000114
Deep MUA Audiovisual Response Enhancement	t(511)4.011, p<0.0001	p>0.05	t(570)=6.14, p<0.0001
Deep MUA Response Enhancement Gain (ii)	t(511)=1.87, p=0.042	p>0.05	t(570)=2.99, p=0.000291
Deep MUA Auditory Response Depression	t(441)=5.56, p<0.0001	t(144)=2.55, p=0.0117	t(441)=12.37, p<0.0001
Deep MUA Visual Response Depression	p>0.05	t(144)=3.704, p=0.000302	t(441)=3.63, p=0.000319
Deep MUA Audiovisual Response Depression	t(441)=4.57, p<0.0001	t(144)=3.63, p=0.000399	t(441)=11.68, p<0.0001
Deep MUA Response Depression Gain (ii)	p>0.05	t(144)=3.56, p=0.000509	t(441)=5.83, p<0.0001

Table 2-2. t-test results for MUA recordings, divided by experience experimental group.

Instances of multisensory integration differ between experience groups in deep SC layers

2505 total recordings took place within the deep SC; 411 in NR, 638 in 6+6, and 1456 in DR animals. Among deep SC layer recordings, responsivity to unisensory and multisensory stimuli differed between experience groups. ANOVA revealed an effect of experience group in deep layer responses (Table 1). Compared to NR recordings, 6+6 recordings in deep layer SC exhibited increased responsivity to auditory and audiovisual stimuli, while exhibiting a decrease in visual responses (Figure 3A). Auditory responses were 50.81 $\pm$ 6.99 spikes/trial in NR, 76.69 $\pm$ 4.98 spikes/trial in 6+6 and 22.98 $\pm$ 1.08 spikes/trial in DR animals (Table 2). Audiovisual responses were 62.54 $\pm$ 14.02 spikes/trial in NR animals, 80.97 $\pm$ 4.99 spikes/trial in 6+6, and 32.58 $\pm$ 2.01 spikes/trial in DR animals. 6+6 animals exhibited a decrease in visual responsivity compared to DR and NR recordings (Figure 3A). NR visual responses were, on average, 50.27 $\pm$ 6.76 spikes/trial, compared with 26.39 $\pm$1.42 spikes/trial in 6+6 recordings, and 27.04 $\pm$ 1.52 spikes/trial in DR recordings (Table 2). DR animals showed a decrease in responses to visual, auditory and audiovisual stimuli compared to NR recordings (Figure 3A). When examining multisensory interactions, recordings from DR animals exhibited a greater response gain compared to both NR and 6+6 (Figure 3A). NR recordings had a 38.82 $\pm$ 16.66% gain of response to audiovisual stimuli, compared to 68.77 $\pm$ 18.3% in 6+6 recordings and 118.34 $\pm$ 11.78% gain in DR recordings (Figure 3A).

When dividing the data by the type of multisensory interaction exhibited (i.e. response enhancement and response depression), we found that experience groups differed in responses to unisensory and multisensory stimuli as well as interactive index

(ii). Focusing on recordings exhibiting response enhancement from each experience group, ANOVA revealed an effect of experience group (Table 1). 6+6 animals showed greater responsivity to auditory unisensory stimuli compared to both NR and DR animals; 73.63 ± 6.54 spikes/trial in 6+6 recordings compared to 46.8 ± 9.45 spikes/trial in NR recordings and 14.77 ± 0.93 spikes/trial in DR recordings (Figure 3B). Both 6+6 and DR recordings showed a marked reduced responsivity to visual stimuli compared to NR animals; 38.39 ±8.08 spikes/trial in NR recordings compared to 19.6 ± 1.44 spikes/trial in 6+6 and 16.43 ± 1.26 spikes/trial in DR recordings (Figure 3B). Additionally, audiovisual responses were reduced in DR recordings as compared to NR and 6+6 recordings (Table 2); 92.69 spikes/trial in 6+6 recordings compared to 46.05 ± 3.41 spikes/trial in DR recordings. Importantly, there was also a great difference in ii between these groups; recordings from DR animals had significantly greater gain of response to multisensory compared to unisensory stimuli; 148.21 ±33.42% gain in NR recordings as compared to 131.7 ± 28.48% in 6+6 and 253.54 ± 19.05% gain in DR recordings (Figure 3B).

Focusing on recordings exhibiting response depression, we encounter very similar trends among and between our three experience groups. ANOVA revealed a main effect of experience group in deep recordings exhibiting response depression (Table 1). 6+6 animals showed greater responsivity to auditory unisensory stimuli and multisensory audiovisual stimuli compared to both NR and DR animals (Figure 3C). Auditory responses were 52.58 ± 9.75 spikes/trial in NR recordings, 81.39 ± 7.66 spikes/trial in 6+6 recordings and 30.89 ± 2.87 spikes/trial in DR recordings (Table 2). Audiovisual MUA responses were, on average, 30.94 ± 5.12 spikes/trial in NR

recordings, 62.66 ± 7.09 spikes/trial in 6+6 recordings and 15.9 ± 1.07 spikes/trial in DR recordings (Figure 3C). 6+6 animals also showed markedly reduced responses to visual stimuli compared to recordings from NR and DR deep layer SC; 59.08 ± 10.09 spikes/trial in NR recordings 32.36 ± 2.44 spikes/trial in 6+6 recordings, and 39.19 ± 3.07 spikes/trial in DR recordings. ii also differed between these three experience groups (Table 2). Recordings from 6+6 animals had blunted gain of response depression compared to DR and NR groups; -29.49 ± 2.78% gain in 6+6 animals compared to -43.6 ± 2.82% gain in NR recordings and -49.01 ± 1.38% gain in DR recordings (Figure 3C). Recordings from DR animals showed more drastic response depression compared to recordings from 6+6 animals (Figure 3C).

Time of recordings during the study did not affect multisensory integrative capacities in the superficial SC layers

As another method of analysis, we split the data from each individual animal into thirds, based on the time during the study in which they were recorded. This was performed to determine if the visual LED flash stimulus used during the recording session was sufficient visual experience to alter multisensory responsivity and processing; based on previous evidence suggesting that visual stimuli used during recording procedures is enough experience to alter responsivity in SC neurons of DR animals (Yu et al. 2012; Yu et al. 2013). Within the superficial SC layers, no changes in responsivity occurred in NR recordings over time (Figure 4A). In contrast, recordings from DR animals changed over time. ANOVA revealed an effect of recording time (first, middle, last third of recordings) on MUA responses (Table 1). The latest group of

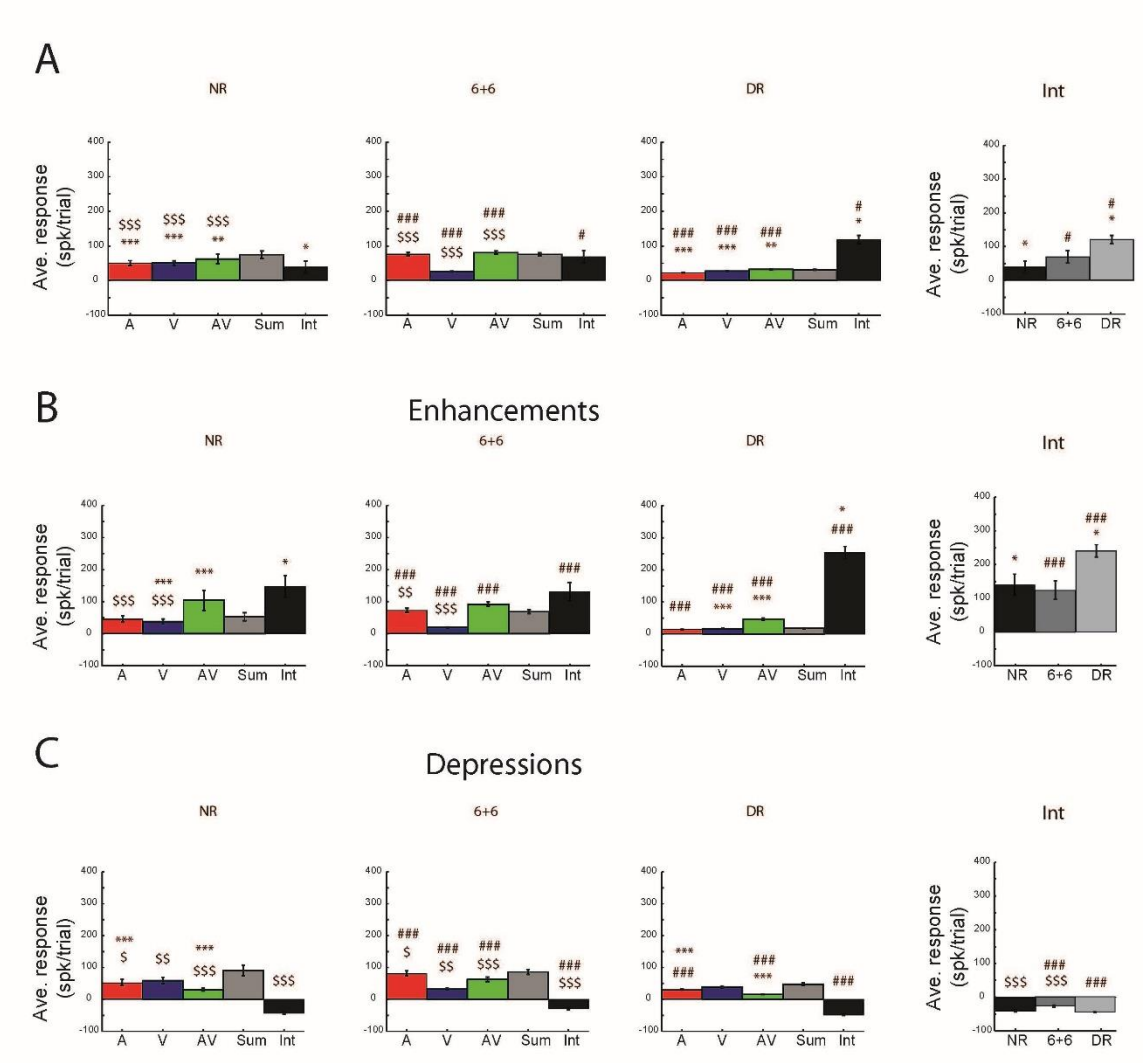


Figure 2-3. Greater multisensory gain of response is encountered in DR multi-unit recordings within the deep layers of the SC. (A) When examining all recordings within the superficial SC, NR recordings (left) and 6+6 recordings exhibit more activity in comparison to DR (right) recordings for auditory (red) and audiovisual (green) stimulus presentations. Visual (blue) responses were low for both experience groups (DR and 6+6). Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001 between NR and DR recordings (*), NR and 6+6 ($), and between DR and 6+6 recordings (#). DR recordings also showed a greater gain of response to multisensory

stimulus presentaitons (Int; far right graph) as compared to the NR and 6+6 groups. Similar trends were seen when examining only recordings exhibiting multisensory response enhancement (B) and multisensory response depression (C).

recordings exhibited increased visual and multisensory) responsivity in DR animals (Figure 4B, Table 3). While audiovisual responsivity changed over time, gain of response did not. Like recordings from NR animals, superficial SC recordings in 6+6 animals did not differ based on the time during the experiment in which they were recorded (Figure 4C).

Time of recordings during the study affects multisensory integrative capacities in the deep SC of 6+6 animals

Focusing on intermediate/deep layer SC recordings, we saw effects of stimulus presentations on neural responsivity. In recordings from NR animals, ANOVA did not reveal any effect of time of recording on visual, auditory, or audiovisual responses, or multisensory gain. ANOVA revealed an effect of time of recording on responsivity in data from DR animals (Table 1). Responsivity to visual and auditory stimuli remained unchanged across time. Audiovisual responses decreased in the middle group of recordings as compared to the first and last recordings; 50.53 $\pm$ 5.15 spikes/trial in the first group of recordings, 33.57 $\pm$ 2.22 spikes/trial in middle recordings, compared to 53.2 $\pm$ 10.76 spikes/trial in the final group of recordings (Figure 5B). This decrease in audiovisual responsivity rebounded in the last group of recordings (Figure 5B). However, there were no alterations in multisensory gain over time of recordings. ANOVA revealed an effect of recording time on 6+6 deep responses (Table 1). Recordings from 6+6 animals exhibited trends similar to what we saw in the DR group; unisensory and multisensory responsivity increased during recordings occurring in the middle of sessions, and then reduced again in the last group of recordings (Figure 5C).

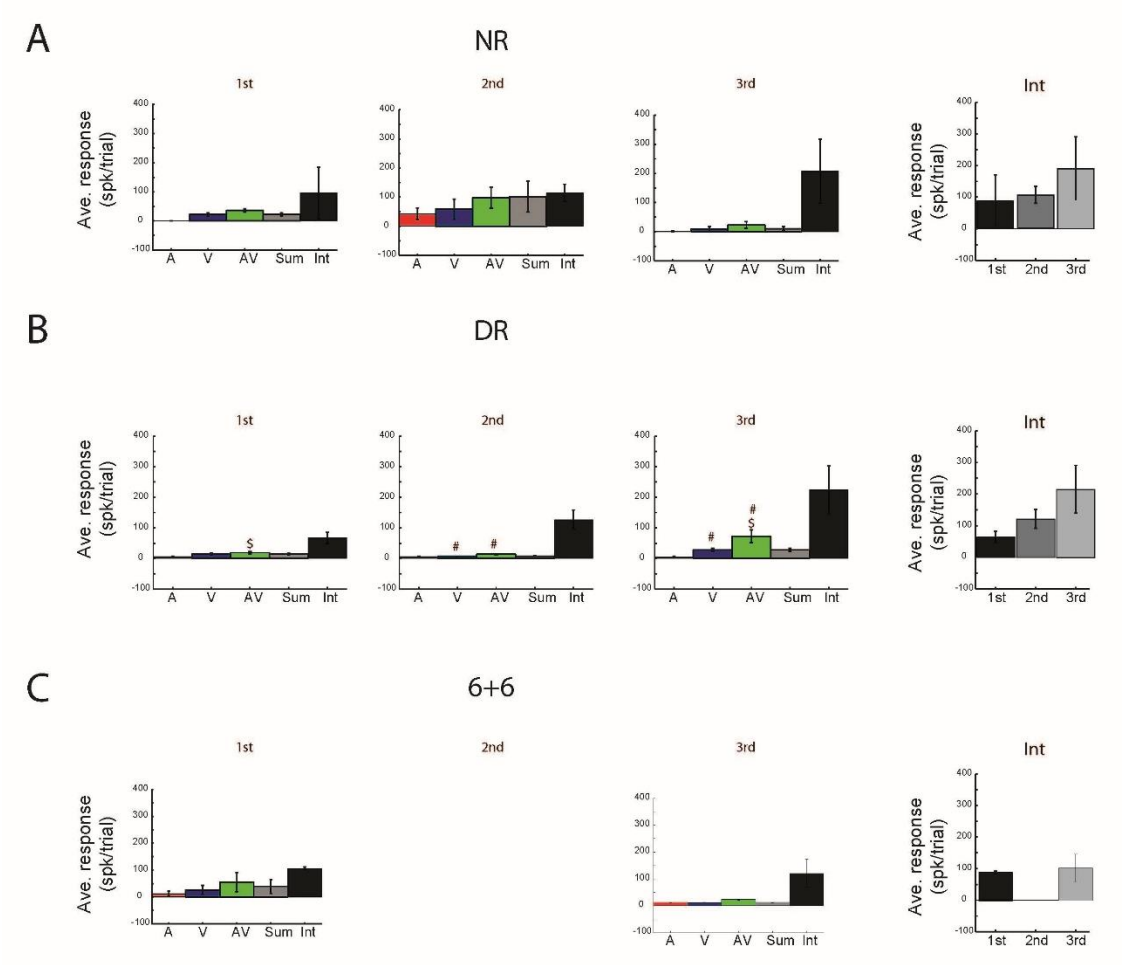


Figure 2-4. Time of recording does not affect multisensory integrative capacity in the superficial SC. Superifical SC recordings exhibiting multisensory integration (response enhancements and depressions) from NR (A), DR (B) and 6+6 (C) animals were split into thirds based on the time at which they were recorded during the study. The 1st group (left) delineates the first third of recordings from each experiment, the 2nd group (middle) the second third of recordings from each experiment, and the 3rd group (right) the last third of recordings. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001 between 1st and 2nd group recordings (*), 1st and 3rd group recordings ($), and between 2nd and 3rd group recordings (#). (A) Recordings

from NR animals show no difference in responsivity to auditory (red), visual (blue) or audiovisual (green) stimulus presentations over time, and also show no difference in integrative capacity (right graph). (B) Recordings from DR animals show an increased response to visual and audiovisual stimuli over time, but no change in integrative capacity. (C) Recordings from 6+6 animals show no difference in responsivity or integrative capacity over time.

Auditory responses increased from 26.9 ± 5.97 spikes/trial in the first set of recordings to 80.15 ± 7.31 spikes/trial in middle recordings, then decreased to 20.29 ± 2.61 spikes/trial in the last group of recordings (Table 3). Visual responses also increased, from 12.68 ± 1.85 to 25.25 ± 1.9 spikes/trial from the first to middle group of recordings (Table 3). Audiovisual responses had a similar trend, increasing from 49.37 ± 10.38 to 102.36 ± 7.32 spikes/trial, then reducing to 28.11 ± 6.26 spikes/trial in the last group of recordings (Figure 5C). Over time, ii also decreased in recordings from 6+6 animals, as well, from 259.43 ± 122.22% to 122.98 ± 30.63%, then 50.52± 17.26% over time (Figure 5C, Table 3).

Visual experience modification affects SC neural responses at the LFP level

Local field potential recordings were also performed in these studies. Examining LFP recordings in the superficial SC layers, ANOVA revealed an effect of experience group on mean magnitude (AUC) (Table 1, Supplementary Figure 2). Auditory AUC was enhanced in 6+6 and reduced in DR compared to NR recordings (Figure 6A). Visual AUC increased in 6+6 and DR recordings compared to NR. Audiovisual AUC showed the same trend, increasing in 6+6 and DR compared to NR recordings. Multisensory gain also differed between the three groups. Much like auditory AUC, there was a negative increase in gain in 6+6 compared to NR recordings and positive increase in gain when comparing DR and NR recordings. Additionally, DR AUC was greater than 6+6 (Figure 6A, Table 4). Peak LFP showed no difference between the three experience groups.

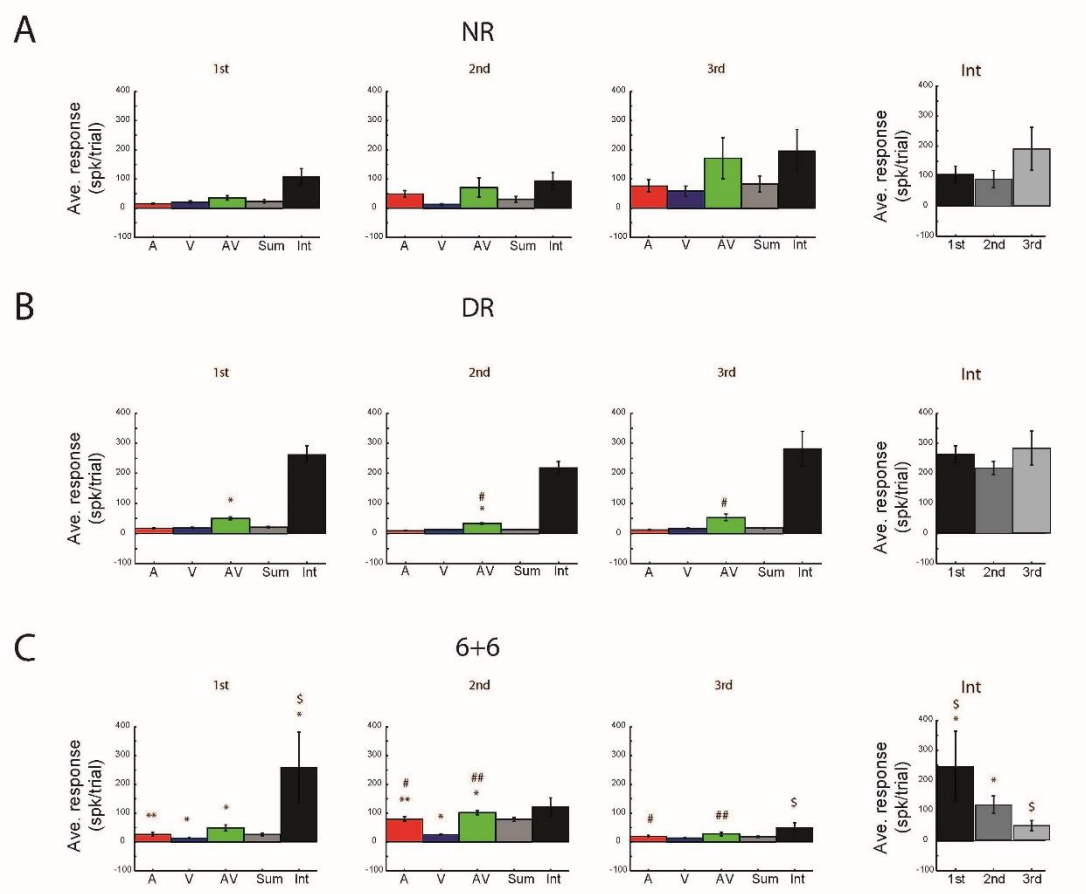


Figure 2-5. Time of recording affects multisensory capacities of 6+6, but not DR, recordings in the deep SC. Deep SC recordings exhibiting multisensory integration (response enhancements and depressions) from NR (A), DR (B) and 6+6 (C) animals were split into thirds based on the time at which they were recorded during the study. The 1st group (left) delineates the first third of recordings from each experiment, the 2nd group (middle) the second third of recordings from each experiment, and the 3rd group (right) the last third of recordings. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001 between 1st and 2nd group recordings (*), 1st and 3rd group recordings ($), and between 2nd and 3rd group recordings (#). (A) Recordings from NR animals show no difference in responsivity to auditory (red), visual (blue) or

audiovisual (green) stimulus presentations over time, and also show no difference in integrative capacity (right graph). (B) Recordings from DR animals show a decreased response to audiovisual stimuli over time, but no change in integrative capacity. (C) Recordings from 6+6 animals show an increased response to auditory (red), visual (blue) and audiovisual (green) stimulus presentations. There is also a decrease in integrative capacity over time (black; right graph).

	1st and 2nd Third	2nd and 3rd Third	1st and 3rd Third
NR Superficial MUA Recording Time Auditory Responses	p>0.05	p>0.05	p>0.05
NR Superficial MUA Recording Time Visual Responses	p>0.05	p>0.05	p>0.05
NR Superficial MUA Recording Time Audiovisual Responses	p>0.05	p>0.05	p>0.05
NR Superficial MUA Recording Time ii	p>0.05	p>0.05	p>0.05
DR Superficial MUA Recording Time Auditory Responses	p>0.05	p>0.05	p>0.05
DR Superficial MUA Recording Time Visual Responses	p>0.05	t(60)=2.35, p=0.0221	p>0.05
DR Superficial MUA Recording Time Audiovisual Responses	p>0.05	t(60)=1.55, p=0.0413	t(66)=1.69, p=0.0496
DR Superficial MUA Recording Time ii	p>0.05	p>0.05	p>0.05
6+6 Superficial MUA Recording Time Auditory Responses	p>0.05	p>0.05	p>0.05
6+6 Superficial MUA Recording Time Visual Responses	p>0.05	p>0.05	p>0.05
6+6 Superficial MUA Recording Time Audiovisual Responses	p>0.05	p>0.05	p>0.05
6+6 Superficial MUA Recording Time ii	p>0.05	p>0.05	p>0.05
NR Deep MUA Recording Time Auditory Responses	p>0.05	p>0.05	p>0.05
NR Deep MUA Recording Time Visual Responses	p>0.05	p>0.05	p>0.05
NR Deep MUA Recording Time Audiovisual Responses	p>0.05	p>0.05	p>0.05
NR Deep MUA Recording Time ii	p>0.05	p>0.05	p>0.05
DR Deep MUA Recording Time Auditory Responses	p>0.05	p>0.05	p>0.05
DR Deep MUA Recording Time Visual Responses	p>0.05	p>0.05	p>0.05
DR Deep MUA Recording Time Audiovisual Responses	t(374)=2.38, p=0.0179	t(214)=2.21, p=0.0280	p>0.05
DR Deep MUA Recording Time ii	p>0.05	p>0.05	p>0.05
6+6 Deep MUA Recording Time Auditory Responses	t(105)=2.69, p=0.00832	t(101)=2.25, p=0.0267	p>0.05
6+6 Deep MUA Recording Time Visual Responses	t(105)=1.85, p=0.037	p>0.05	p>0.05
6+6 Deep MUA Recording Time Audiovisual Responses	t(105)=2.41, p=0.0178	t(101)=2.72, p=0.00767	p>0.05
6+6 Deep MUA Recording Time ii	t(105)=1.38, p=0.0272	p>0.05	t(101)= 1.34, p=0.0398

Table 2-3. t-test results for MUA data, divided into thirds by recording time.

Focusing on the deep SC layers, ANOVA revealed an effect of experience group on AUC (Figure 6B, Table 1). Auditory AUC was greater in 6+6 compared to NR and DR AUC (Table 4). DR auditory AUC was also less than NR recordings (Figure 6B). Visual AUC showed much the same trend; 6+6 visual AUC were more negative than NR and DR visual AUC, and DR AUC was more positive than NR. Audiovisual AUC were more negative in 6+6 recordings than NR and DR recordings, and DR were less than NR recordings. Multisensory gains were also different between the three experience groups. Gains were increased in 6+6 recordings compared to NR and DR recordings, and DR recordings exhibited a greater negative gain compared to NR recordings, which showed no statistically significant gain from 0 (Figure 6B, Table 4). While there were differences between auditory, visual, and audiovisual mean peak amplitudes within each experience group, these did not differ between the three experience groups (Tables 1, 4).

To mirror the analysis performed on MUA data, we split LFP recording data from each individual animal into thirds, based on the time during the study in which they were recorded. This was performed to determine if the visual LED flash stimulus used during the recording session was sufficient visual experience to alter multisensory responsivity and processing. ANOVAs revealed an effect of time of recording in superficial SC layers for NR, DR, and 6+6 groups (Table 1, Figure 7). Audiovisual experience gained by exposure to repeated stimulus presentation had an effect LFP AUC in all three experimental groups. To highlight some of the important effects, average visual AUC increased from first to middle recordings in the study, and then reduced again in the third set of recordings (Table 5). Auditory and audiovisual AUC showed a similar trend,

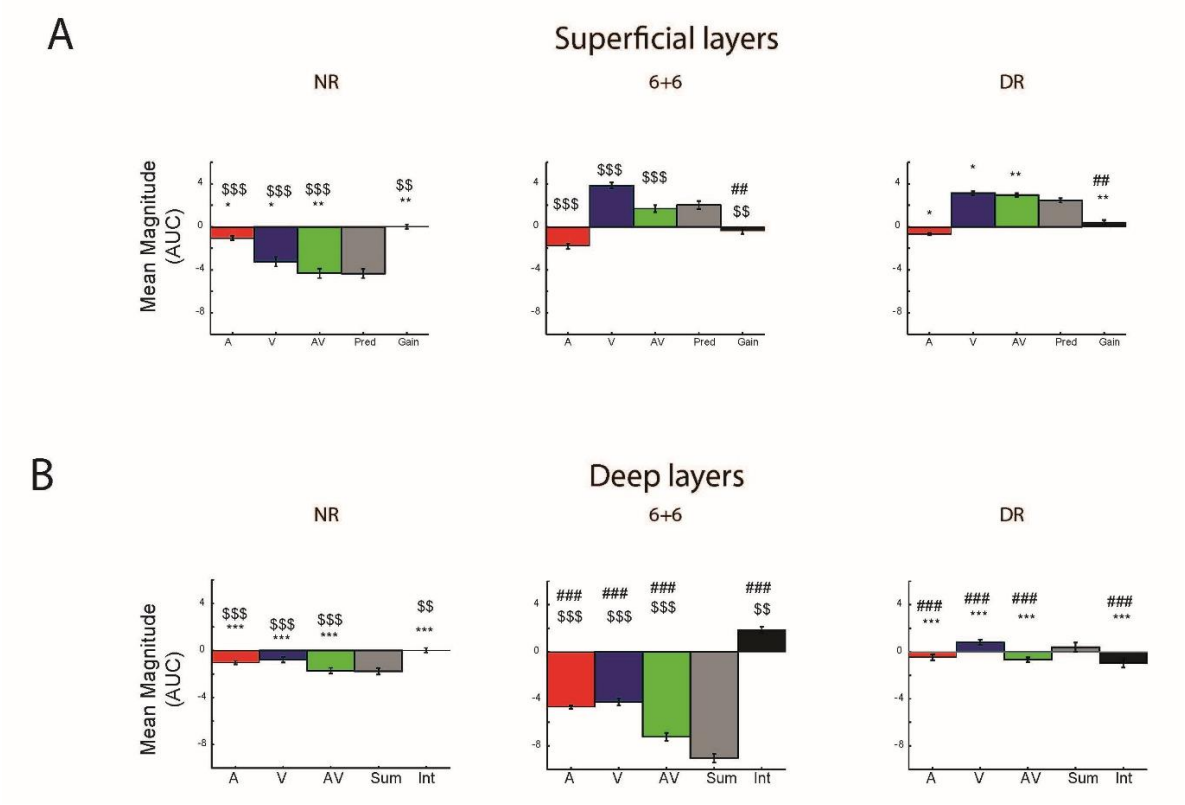


Figure 2-6. Visual experience modification affects SC neural responses at the LFP level. Local field potentials (LFP) from superficial (A) and deep (B) layer SC were recorded in NR (left), 6+6 (middle) and DR (right) animals. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001 between NR and DR recordings (*), NR and 6+6 ($), and between DR and 6+6 recordings. (A) Auditory (red), visual (blue), audiovisual (green) AUC and integration (black; right graph) differed between all three experience groups, with the 6+6 group exhibiting an increased response to visual stimuli and reduced integrative capacity within the superficial layers. (B) Greater negative AUC in the deep layer SC recordings was exhibited in the 6+6 recordings compared to NR and DR. Multisensory gains were also increased in 6+6 compared to NR and DR recordings.

	NR to DR	NR to 6+6	DR to 6+6
Superficial LFP Auditory AUC	t(627)=2.51, p=0.0167	t(267)=5.12, p<0.0001	p>0.05
Superficial LFP Visual AUC	t(627)=2.44, p=0.0197	t(267)=14.19, p<0.0001	p>0.05
Superficial LFP Audiovisual AUC	t(627)=3.13, p=0.00347	t(267)=12.73, p<0.0001	p>0.05
Superficial LFP AUC Gain	t(627)=3.85, p=0.00474	t(267)=2.83, 0.00833	t(618)=2.94, p=0.00574
Superficial LFP Auditory Peak	p>0.05	p>0.05	p>0.05
Superficial LFP Visual Peak	p>0.05	p>0.05	p>0.05
Superficial LFP Audiovisual Peak	p>0.05	p>0.05	p>0.05
Superficial LFP Peak Gain	p>0.05	p>0.05	p>0.05
Deep LFP Auditory AUC	t(2428)=21.27, p<0.0001	t(769)=7.86, p<0.0001	t(2715)=10.93, p<0.0001
Deep LFP Visual AUC	t(2428)=13.22, p<0.0001	t(769)=5.40, p<0.0001	t(2715)=6.85, p<0.0001
Deep LFP Audiovisual AUC	t(2428)=10.86, p<0.0001	t(769)=8.02, p<0.0001	t(2715)=9.26, p<0.0001
Deep LFP AUC Gain	t(2428)=21.55, p<0.0001	t(769)=2.08, p=0.0441	t(2715)=5.50, p<0.0001
Deep LFP Auditory Peak	p>0.05	p>0.05	p>0.05
Deep LFP Visual Peak	p>0.05	p>0.05	p>0.05
Deep LFP Audiovisual Peak	p>0.05	p>0.05	p>0.05
Deep LFP Peak Gain	p>0.05	p>0.05	p>0.05

Table 2-4. t-test results for LFP data, divided by experience experimental groups.

reducing over time and stimulus exposure (Figure 7A). AUC gain also reduced throughout the experiment from 5.38 ± 0.67 to 2.18 ± 0.36 and then -0.62 ± 0.54 mA across the three time frames (Figure 7A).

DR recordings also showed changes in LFP signal based on exposure to unisensory and multisensory test stimuli (Figure 7B). Overall, average AUC increased with exposure. To highlight, auditory AUC increased nearly seven-fold with exposure to auditory, visual, and audiovisual test stimuli; from 0.64 ± 0.31 mA to 7.06 ± 0.97 mA (Table 5). Visual AUC also increased by nearly three-fold with exposure, from 2.02 ± 0.53 mA to 5.38 ± 0.17 mA. Audiovisual AUC followed the same trend, increasing from 2.66 ±0.65 to 5.87 ± 0.34 mA over time and stimulus exposure (Table 5). Multisensory gain of AUC signal was enhanced from -0.69 ± 0.77 to -6.41 ± 0.98 mA with this exposure, as well (Figure 7B).

The only real trend seen in 6+6 recordings was on gain of responses (Figure 7C). In the second group of recordings, in the middle of the studies, no recordings showed any multisensory interactions, thus there is no data for the '2nd' group here. Gain of AUC increased with the additional exposure via test stimuli (Table 5).

Focusing on deep SC layers, ANOVAs revealed an effect of time of recording on NR, DR and 6+6 recording groups (Table 1, Figure 8). Exposure to test stimuli seemed to have an overall reduction effect on NR recordings (Figure 8A). No significant effect of exposure was found on visual AUC in NR recordings. Auditory AUC were reduced, from 0.47 ± 1.25 to -2.34 ± 0.33 mA (Table 5, Figure 8A). Audiovisual AUC showed a similar trend, reducing from 4.07 ± 1.4 to -0.55 ± 0.46 mA over recording time. AUC gain also

altered with exposure to stimulus presentations, decreasing over time from 2.88±1.35 mA to -0.231±0.545 mA.

Deep SC LFP recordings in DR animals showed a similar trend to test stimuli exposure as in superficial layers; exposure to auditory, visual, and audiovisual stimuli used in recordings decreased AUC (Figure 8B) LFP signals. Auditory AUC reduced from 6.4 ± 1.85 to -1.04 ± 0.26 mA across first and middle groups of recordings (Table 5).Visual AUC also decreased across recordings, from 1.91 ± 0.31 to -1.21 ± 0.92 mA. Audiovisual AUC decreased from 6.62 ± 1 to 1.72 ± 0.22 mA over recordings. Additionally, multisensory gain increased with exposure to test stimuli, increasing from -1.81 ± 2.08 to 3.85 ± 0.67 mA between the first two groups of recordings (Figure 8B).

6+6 followed the trends seen in DR deep SC recordings (Figure 8C). Visual AUC decreased from 0.76 ± 0.92 to -11.44 ± 1.85 mA with exposure to auditory, visual and audiovisual test stimuli (Table 5). Audiovisual AUC also decreased, from 1.21 ± 0.66 to -9.29 ± 1.68 mA. However, there were no changes to auditory or multisensory gain between these groups (Figure 8C).

Discussion

To our knowledge, the findings described here are the first to provide evidence of the impact of visual sensory experience during adulthood in this manner. These results have important implications for our understanding of SC multisensory function.

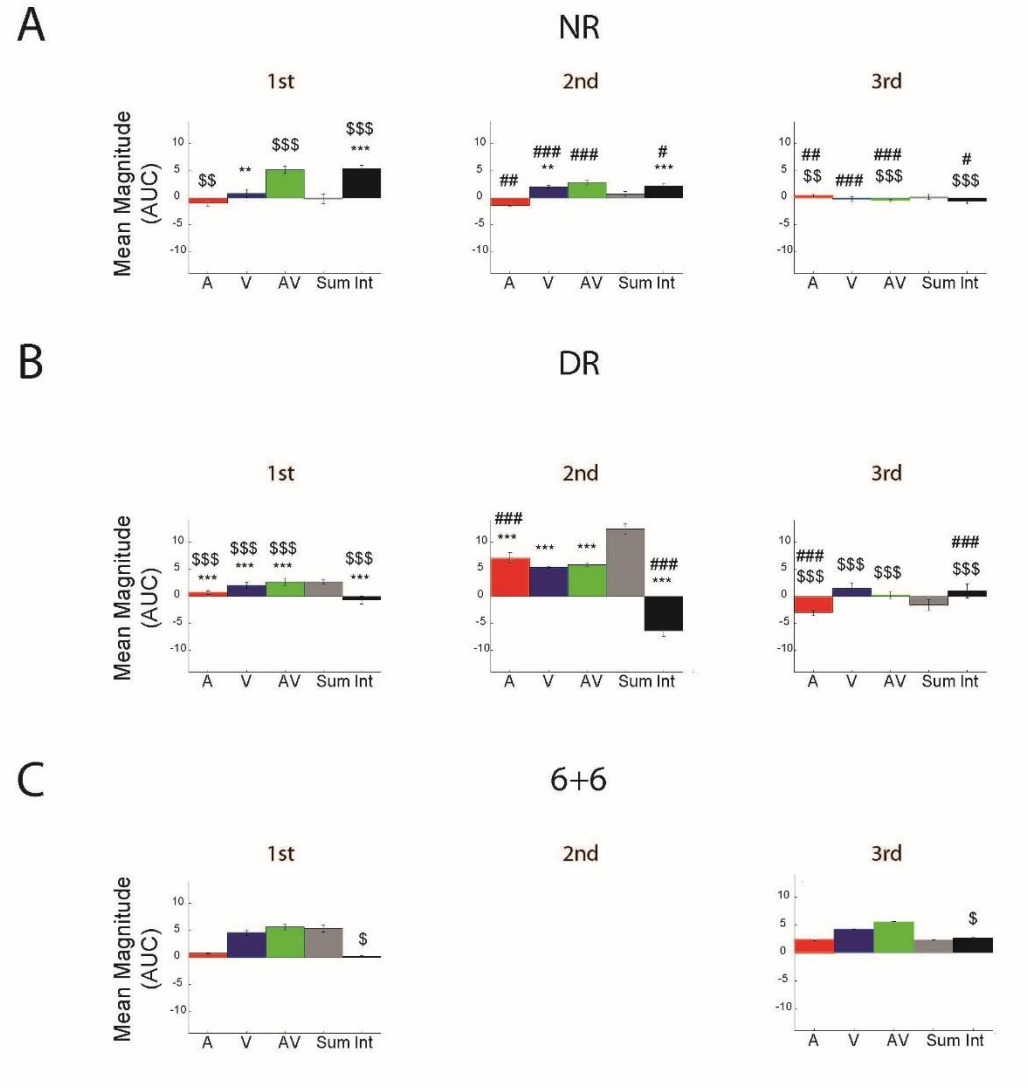


Figure 2-7. Time of recording affects multisensory capacities of 6+6, but not DR, LFP recordings in the superficial SC. Superficial SC recordings exhibiting multisensory integration (response enhancements and depressions) from NR (A), DR (B) and 6+6 (C) animals were split into thirds based on the time at which they were recorded during the study. The 1st group (left) delineates the first third of recordings from each experiment, the 2nd group (middle) the second third of recordings from each experiment, and the 3rd group (right) the last third of recordings. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001 between 1st and 2nd group

recordings (*), 1st and 3rd group recordings ($), and between 2nd and 3rd group recordings (#). (A) Mean magnitude (AUC) recorded from NR animals. AUC showed a trend of decreasing over time, including AUC for auditory (red), visual (blue), audiovisual (green), and integration (black; right graph). (B) Mean magnitude (AUC) recorded from DR animals. AUC showed an increase from 1st to 2nd thirds of recordings, then a decrease back towards baseline for the 3rd group of recordings. (C) Mean magnitude (AUC) recorded from 6+6 animals. AUC integration increased over time while peak decreased over time. There are no graphs for the 2nd grouping of recordings for 6+6 animals because, in that time, no recordings exhibited multisensory response enhancements or depressions.

	1st and 2nd Third	2nd and 3rd Third	1st and 3rd Third
NR Superficial LFP Recording Time Auditory AUC	p>0.05	t(97)=2.79, p=0.00634	t(94)=2.66, p=0.00915
NR Superficial LFP Recording Time Visual AUC	t(46)=2.75, p=0.00857	t(94)=3.89, p=0.000186	p>0.05
NR Superficial LFP Recording Time Audiovisual AUC	p>0.05	t(94)=6.76, p<0.0001	t(94)=9.297, p<0.0001
NR Superficial LFP Recording Time AUC Gain	t(46)=4.84, p<0.0001	t(94)= 2.14, p=0.0349	t(94)=5.89, p<0.0001
DR Superficial LFP Recording Time Auditory AUC	t(52)=6.13, p<0.0001	t(52)=6.29, p<0.0001	t(52)=5.29, p<0.0001
DR Superficial LFP Recording Time Visual AUC	t(52)=6.06, p<0.0001	p>0.05	t(52)=7.12, p<0.0001
DR Superficial LFP Recording Time Audiovisual AUC	t(52)=4.37, p<0.0001	p>0.05	t(52)=6.22, p<0.0001
DR Superficial LFP Recording Time AUC Gain	t(52)=4.59, p<0.0001	t(52)=5.07, p<0.0001	t(52)=4.31, p<0.0001
6+6 Superficial LFP Recording Time Auditory AUC	p>0.05	p>0.05	p>0.05
6+6 Superficial LFP Recording Time Visual AUC	p>0.05	p>0.05	p>0.05
6+6 Superficial LFP Recording Time Audiovisual AUC	p>0.05	p>0.05	p>0.05
6+6 Superficial LFP Recording Time AUC Gain	p>0.05	p>0.05	t(253)=2.32, p=0.0209
NR Deep LFP Recording Time Auditory AUC	p>0.05	t(160)=3.23, p=0.00148	p>0.05
NR Deep LFP Recording Time Visual AUC	p>0.05	p>0.05	p>0.05
NR Deep LFP Recording Time Audiovisual AUC	t(94)=1.94, p=0.049	p>0.05	t(94)=2.12, p=0.021
NR Deep LFP Recording Time AUC Gain	p>0.05	t(94)=2.55, p=0.0216	t(94)=3.67, p=0.016
DR Deep LFP Recording Time Auditory AUC	t(371)=5.11, p=0.035	p>0.05	t(371)=4.60, p<0.0001
DR Deep LFP Recording Time Visual AUC	t(371)=2.54, p=0.0116	t(371)=3.56, p=0.034	t(371)=2.48, p=0.0092
DR Deep LFP Recording Time Audiovisual AUC	t(371)=5.53, p<0.0001	t(371)=6.28, p<0.0001	t(371)=5.01, p<0.0001
DR Deep LFP Recording Time AUC Gain	t(371)=2.44, p=0.0153	p>0.05	t(371)=4.03, p<0.0001
6+6 Deep LFP Recording Time Auditory AUC	p>0.05	p>0.05	p>0.05
6+6 Deep LFP Recording Time Visual AUC	t(151)=6.89, p<0.0001	t(151)=8.08, p=0.0115	t(151)=6.07, p<0.0001
6+6 Deep LFP Recording Time Audiovisual AUC	t(151)=8.17, p=0.0351	t(151)=8.19, p=0.0164	t(151)=6.50, p<0.0001
6+6 Deep LFP Recording Time AUC Gain	p>0.05	p>0.05	p>0.05

Table 2-5. t-test results for LFP data, divided into thirds by recording time during each experiment.

Visual experience is critical for the maintenance of proper SC multisensory processing

We found a general pattern of more instances of multisensory integration in NR animals in comparison to DR animals (Figure 1). This finding is in line with previous work detailing the effects of total dark-rearing on multisensory processing (Wallace et al. 2004). We also encountered less instances of multisensory integration in 6+6 animals as compared to NR animals. In addition, multisensory response depression was seen more often than enhancement in 6+6 animals, the opposite of what we found in NR animals. This finding is intriguing, and suggests that visual experience throughout a lifetime is important in maintaining not only normal amounts of multisensory processing occurring in the SC, but also in maintaining normal ratios of response enhancement-to-depression, as well.

It was also interesting to find multisensory enhancement and depression in recordings performed in DR animals overall. This general finding is seemingly in opposition with previous work in total dark-reared animals in which no multisensory interactions were encountered (Wallace et al. 2004). This discrepancy can be partially resolved by the methods and techniques used to acquire the data for this study. This study used multiunit recordings collected through the use of a multichannel U-probe. This is different from the isolated single unit recordings performed in the SC of DR cats previously (Wallace et al. 2004). Previous work presented unisensory and multisensory stimuli 8-20 times at 8-12 s interstimulus intervals, whereas in this study we presented stimuli for at least 20 repetitions with 3-5 s interstimulus intervals. This allowed us to see that the repeated presentations of visual, auditory and audiovisual stimuli had an effect

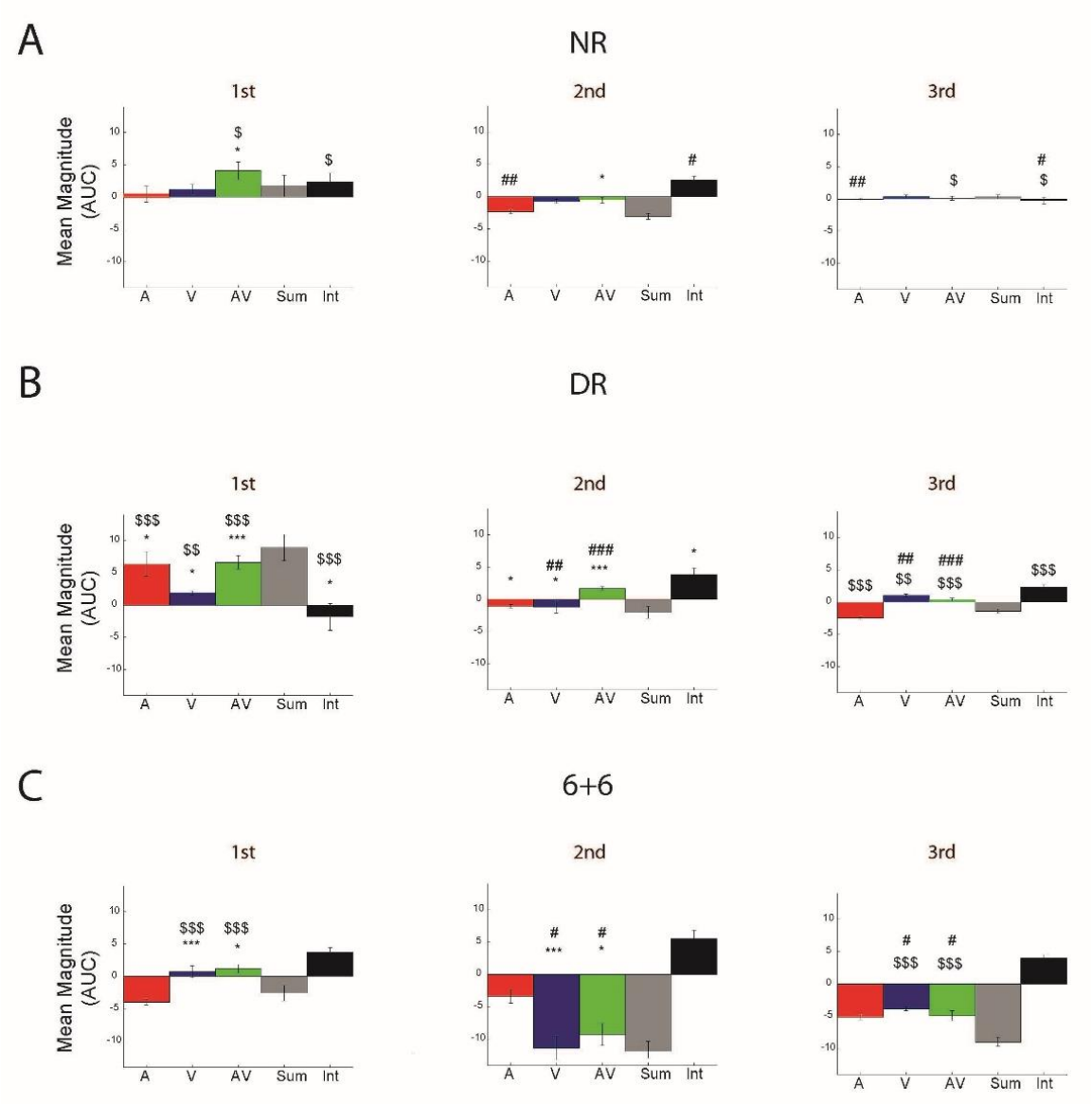


Figure 2-8. Time of recording affects multisensory capacities in the deep SC. Superficial SC recordings exhibiting multisensory integration (response enhancements and depressions) from NR (A), DR (B) and 6+6 (C) animals were split into thirds based on the time at which they were recorded during the study. The 1st group (left) delineates the first third of recordings from each experiment, the 2nd group (middle) the second third of recordings from each experiment, and the 3rd group (right) the last third of recordings. Asterisks display comparisons of significance, *p<0.05, **p<0.01,

***p<0.001 between 1st and 2nd group recordings (*), 1st and 3rd group recordings ($), and between 2nd and 3rd group recordings (#). (A) Mean magnitude (AUC) recorded from NR animals. AUC showed a trend of decreasing over time, including AUC for auditory (red), visual (blue), audiovisual (green), and integration (black; right graph). (B) AUC recorded from DR animals. AUC showed a general decrease as a result of time of recordings. (C) Mean AUC recorded from 6+6 animals. Only visual and audiovisual responses were altered, increasing from 1st to 2nd group of recordings, then decreasing in the 3rd set of recordings.

on visual and multisensory responsiveness in DR animals in the deep SC (Figures 4, 5). Over time, with more stimulus presentations, responsivity as well as integrative capacity increased in these animals. This finding is in accordance with previous studies in the cat SC, as well (Yu et al. 2012; Yu et al. 2013). These results show that multisensory neurons in deep SC of DR animals maintain the capacity to be shaped and influenced by sensory experience.

Another interesting pattern we encountered is the decrease of auditory responsiveness in the DR group compared with NR recordings, but an increase in auditory responsiveness in 6+6 recordings (Figure 3). Focusing on the pattern involving DR recordings, previous work has shown an increase in auditory-responsive neurons as well as a dramatic increase in auditory receptive field size in DR animals (Wallace et al. 2004). As these neurons did not receive the necessary sensory inputs required for normal maturation, they remain in a quasi-immature state even in adulthood. This immature state also typically means that the neurons will respond to a more broad range of stimuli (Wallace and Stein 1997). These responses to a broad range of stimuli are not specific and therefore not as robust as responses seen in adulthood once neurons have specialized and RFs have refined. This can explain the pattern of reduced auditory responsiveness in DR animals. In addition, an increased response to auditory stimuli was seen in 6+6 animals as compared to NR. This can partly be explained by the effect of stimulus presentations over time on 6+6 recordings (Figure 5). With the continued presentation of auditory, visual and audiovisual stimuli, 6+6 recordings showed an increase in auditory, visual and audiovisual responsivity. This could explain

how the average auditory response in 6+6 recordings was greater than that seen in NR animals.

A curious finding in our results is the presence of overt auditory responses in MUA superficial SC recordings across all three experience groups (Figure 2). While auditory stimuli have been shown to have an impact on visual responsivity in the superficial SC (Ghose et al. 2014), and reciprocal connections that may involve the transmittance of visual and auditory information have been found between superficial and intermediate/deep SC layers (Appell and Behan 1990; Behan and Appell 1992; Behan and Kime 1996; Doubell et al. 2003), overt responses have never been found. Multi-unit activity can be recorded from far-away neurons, and the same neuron can presumably influence recordings at multiple contacts. The auditory influence in these recordings can possibly be coming from intermediate/deep large auditory neurons that may have a reciprocal connection with superficial SC neurons. Further investigation into these reciprocal connections, including their locations and functions, is necessary in order to determine this.

Over time, with continued sensory exposure through the presentation of auditory, visual and audiovisual stimuli during recordings, deep layer responses in DR and 6+6 animals changed. Specifically focusing on recordings in 6+6 animals, both unisensory and multisensory responsivity, as well as integrative capacity, changed over time and sensory exposure (Figure 5). The increase in unisensory responsivity with continued exposure fits with previous work accomplished in total DR animals (Yu et al. 2012; Yu et al. 2013). With additional experience, neurons increase their responsivity. This suggests that the neurons in 6+6 animals as similar to those in DR animals in that they maintain

the capacity to be influenced by sensory experiences even in adulthood, even after the revocation of normal sensory experience after development. The decrease in multisensory integration is accompanied by an increase in unisensory responsivity, in alignment with the principle of inverse effectiveness (Meredith and Stein 1986; Meredith and Stein 1996; Perrault et al. 2003).

LFP recordings coordinated with our MUA recordings in this study, providing further evidence that visual experience is necessary for normal multisensory processing. Overall, recordings from DR animals exhibited a decrease in magnitude as compared to recordings from NR, and often even 6+6, animals, while still maintaining an evoked response to visual stimulus presentations. When examining how these signals changed over time and exposure to visual stimuli during recording procedures in the superficial SC layers, DR recordings seem to be more malleable as compared to NR and 6+6. DR recordings showed an increase in magnitude over time, whereas there was little, if any change in signals over time in 6+6 and NR animals. This change was not seen so specifically within MUA recordings, though it is unsurprising as LFP recordings are thought to be more sensitive to minute changes (Ghose et al. 2012). Additionally, when exmining deep SC layers, we find a change in both 6+6 and DR recordings, in accordance with trends in the MUA data. Previous research has shown that visual influences can appear to take place both at early and late periods of evoked LFP (Ghose et al. 2012), which is why we focused on mean magnitude (AUC) here.

Overall, this work suggests that multisensory SC neurons, especially those found in the deep SC layers, maintain their ability to be influenced by sensory experiences even in adulthood. This has been shown in two ways and over two time periods. This

has been shown over a longer time period by the altered patterns of sensory and multisensory responsivity and multisensory integrative capacities in 6+6 recordings as compared to NR recordings, suggesting that elimination of visual experience even after normal development can have an effect on sensory processing in the SC. The malleability has also been shown on a shorter time scale, showing that sensory and multisensory stimulus presentations, for the purposes of recording responses, even under anesthesia, have the ability to alter neuronal responses. These findings support the idea that experience plays a role in shaping sensory and multisensory responses throughout a lifetime. This work is important in order to understand the capacities of SC neurons across the entire lifespan.

Caveats of this work

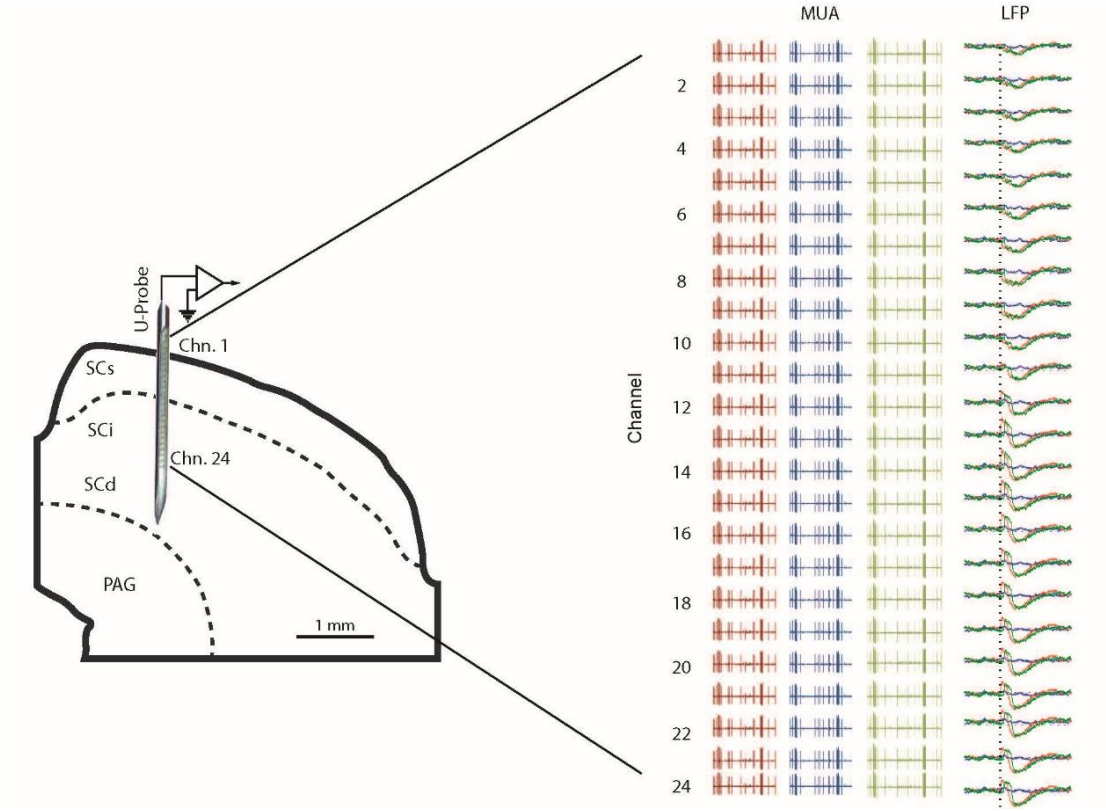
One important caveat of this work is the potential effect of anesthesia. While we acknowledge this concern, we argue that anesthesia is necessary for this experimental set up in that implantation and craniotomy surgery and recording sessions occurred all at once. Previous work has shown marked commonalities in multisensory response characteristics between anesthetized and awake recordings in the SC (Wallace et al. 1998), so the use of anesthesia has precedence and should not invalidate the current findings.

Another possible concern is unintended light exposure of DR and 6+6 animals. A multitude of precautions were taken to ensure that these animals were not exposed to light before recording procedures occurred. Animal care staff members and lab personnel were specially trained in the use of night vision goggles for daily care tasks

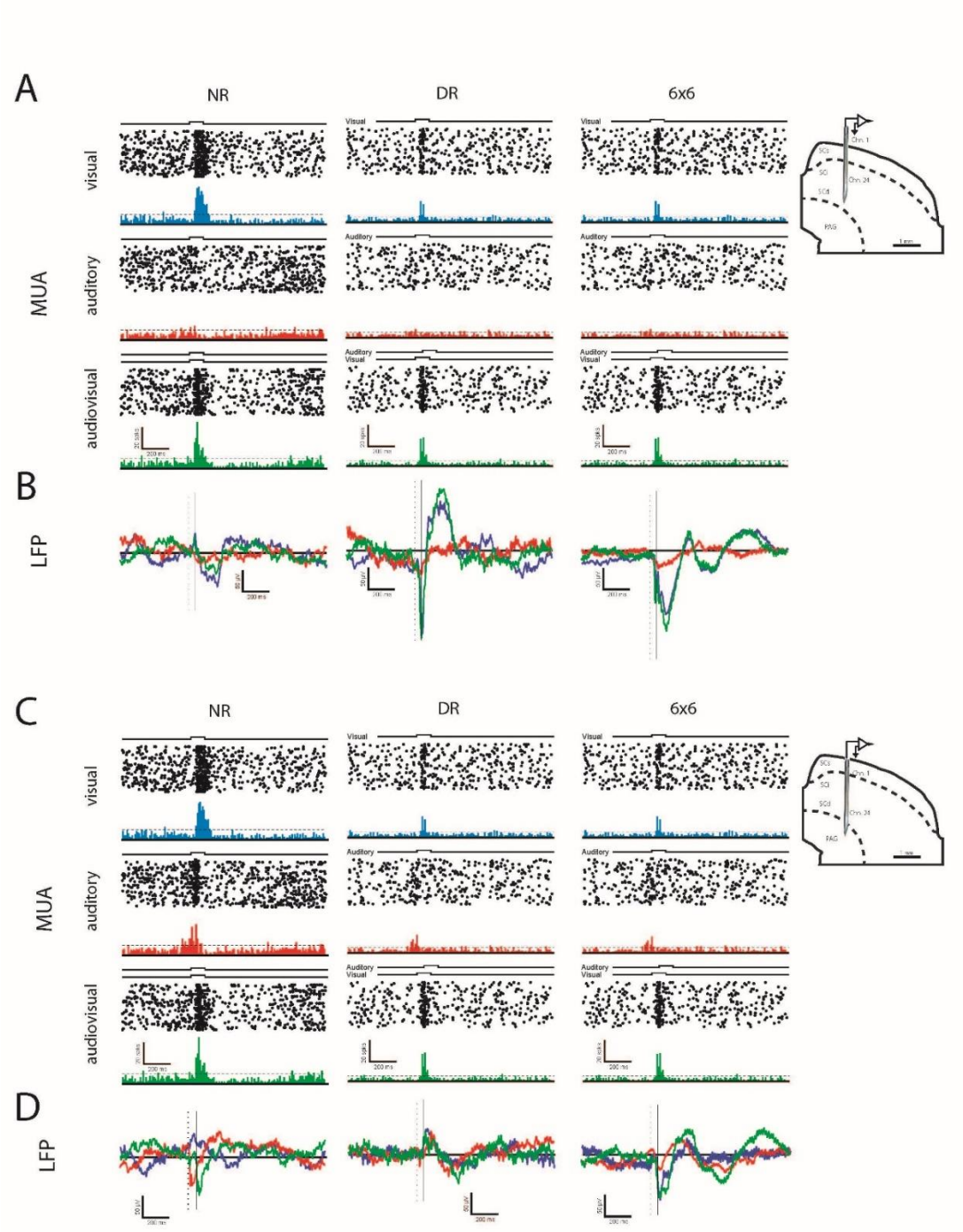
as well as sedation procedures for anesthesia induction that also took place in dark room facilities. Before moving to procedure rooms, animals' eyes were covered using light-deprivation masks. They were placed in covered, blacked-out transfer cages for relocation to a dark surgical room. Animals' eyes continued to be covered throughout craniotomy and implantation procedures, and minimal, localized light was used throughout these procedures. Minimal light was used to map the SC before recording procedures, as well. While it is possible that these animals were exposed to light unintentionally, a multitude of precautions were taken to vastly minimize these events.

Future directions

While this study helped to improve our understanding of the importance of visual sensory experience throughout a lifetime, future experiments are required for us to fully understand this phenomenon. One such future direction should involve understanding just how little visual deprivation is needed in order to significantly alter multisensory processing in the SC. The current study described, along with previous work, has shown that deprivation during development and during adulthood is sufficient to change integrative capacities in SC neurons. However, the amount of visual deprivation in these studies was vast, depriving the animals used in the study for many months at a time. Shortening that amount of deprivation into discrete periods to understand how small amounts of visual deprivation would affect the multisensory circuit in the SC would help to give us a better understanding of the mechanisms on which the sensory experience is acting. For example, if a longer period of deprivation is required for multisensory integration to be affected, perhaps slower, more modulatory mechanisms involving neuromodulators is influenced by visual experience. Understanding how visual sensory



Supplementary Figure 2-1. Experimental recording procedure. Multi-unit activity (MUA) and local field potential (LFP) recordings to visual (red), auditory (blue) and audiovisual (green) stimulus presentations were performed using a Plexon 24 multi-channel U-probe (125 μm inter-channel spacing). Recordings were initiated when visual responses characteristic of superficial SC layers were seen in more than half but not in at least two of the uppermost electrode channels. 3-4 azimuths and 2-3 elevations were used to sample responsiveness and multisensory integration of these units. The electrode was advanced 2000 μm for a second set of recordings, then another 2000 μm for a third set of recordings to ensure capture of the entirety of SC.



Supplementary Figure 2-2. Example MUA and LFP recordings. Example multi-unit activity (MUA; A, C) and local field potential (LFP; B, D) recordings to visual (red), auditory (blue) and audiovisual (green) stimulus presentations in superficial (A, B) and deep (C, D) SC layers from NR (left), DR (middle) and 6+6 (right) animals.

experience affects multisensory processing in the SC is important for us to understand how this system works and, in turn, how SC-mediated behaviors are influenced.

References Cited

Appell, P. P. and Behan, M. (1990). Sources of subcortical GABAergic projections to the superior colliculus in the cat. *J Comp Neurol* **302,** 143-158.

Behan, M. and Appell, P. P. (1992). Intrinsic circuitry in the cat superior colliculus: projections from the superficial layers. *J Comp Neurol* **315**, 230-243.

Behan, M. and Kime, N. M. (1996). Intrinsic circuitry in the deep layers of the cat superior colliculus. *Vis Neurosci* **13**, 1031-1042.

Bell, A. H., Corneil, B. D., Meredith, M. A. and Munoz, D. P. (2001). The influence of stimulus properties on multisensory processing in the awake primate superior colliculus. *Can J Exp Psychol* **55**,: 123-132.

Burton, H., Snyder, A. Z., Diamond, J. B. and Raichle, M. E. (2002). Adaptive changes in early and late blind: a fMRI study of Braille reading. *J Neurophysiol* **87**, 589-607.

Calvert, G. A., Campbell, R. and Brammer, M. J. (2000). Evidence from functional magnetic resonance imaging of crossmodal binding in the human heteromodal cortex. *Curr Biol* **10**, 649-657.

Carriere, B. N., Royal, D. W., Perrault, T. J., Morrison, S. P., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2007). Visual deprivation alters the development of cortical multisensory integration. *J Neurophysiol* **98**, 2858-2867.

Champoux, F., Collignon, O., Bacon, B. A., Lepore, F., Zatorre, R.J. and Theoret, H. (2011). Early- and late-onset blindness both curb audiotactile integration on the parchment-skin illusion. *Psychol Sci* **22**, 19-25.

Collignon, O., Charbonneau, G., Lassonde, M. and Lepore, F. (2009). Early visual deprivation alters multisensory processing in peripersonal space. *Neuropsychologia* **47**, 3236-3243.

Collignon, O., Dormal, G., Albouy, G., Vandewalle, G., Voss, P., Phillips, C. and Lepore, F. (2013). Impact of blindness onset on the functional organization and the connectivity of the occipital cortex. *Brain* **136**, 2769-2783.

Doubell, T.P., Skaliora, I., Baron, J. and King, A. J. (2003). Functional connectivity between superficial and deeper layers of the superior colliculus: an anatomical substrate for sensorimotor integration. *J Neuroscience* **23**, 6596-6607.

Eimer, M. (2004). Multisensory integration: how visual experience shapes spatial perception. *Curr Biol* **14**, R115-117.

Focker, J., Holig, C., Best, A. and Roder, B. (2015). Neural plasticity of voice processing: Evidence from event-related potentials in late-onset blind and sighted individuals. *Restor Neurol Neurosci* **33**, 15-30.

Frens, M. A. and Van Opstal, A. J. (1998). Visual-auditory interactions modulate saccade-related activity in monkey superior colliculus. *Brain Res Bull* **46**, 211-224.

Ghose, D., Barnett, Z.P. and Wallace, M.T. (2012). Impact of response duration on multisensory integration. *J Neurophysiol* **108**, 2534-2544.

Ghose, D., Maier, A., Nidiffer, A. and Wallace, M. T. (2014). Multisensory response modulation in the superficial layers of the superior colliculus. *J Neurosci* **34**, 4332-4344.

Guerreiro, M. J., Putzar, L. and Roder, B. (2015). The effect of early visual deprivation on the neural bases of multisensory processing. *Brain* **138**, 1499-1504.

Hauthal, N., Debener, S., Rach, S., Sandmann, P. and Thorne, J. D. (2015). Visuo-tactile interactions in the congenitally deaf: a behavioral and event-related potential study. *Front Integr Neurosci* **8**.

Hotting, K. and Roder, B. (2009). Auditory and auditory-tactile processing in congenitally blind humans. *Hear Res* **258**, 165-174.

Kujala, T., Alho, K., Huotilainen, M., Ilmoniemi, R. J., Lehtokoski, A., Leinonen, A., Rinne, T., Salonen, O., Sinkkonen, J., Standertskjold-Nordenstam, C. G. and Naatanen, R. (1997). Electrophysiological evidence for cross-modal plasticity in humans with early- and late-onset blindness. *Psychophysiology* **34**, 213-216.

Leo, F., Bertini, C., di Pellegrino, G. and Ladavas, E. (2008). Multisensory integration for orienting responses in humans requires the activation of the superior colliculus. *Exp Brain Res* **186**, 67-77.

Leo, F., Bolognini, N., Passamonti, C., Stein, B. E. and Ladavas, E. (2008). Cross-modal localization in hemianopia: new insights on multisensory integration. *Brain* **131**, 855-865.

Meredith, M., Nemitz, J. W. and Stein, B. E. (1987). Determinants of multisensory integration in superior colliculus neurons. I. Temporal factors. *The Journal of Neuroscience* **7**, 3215-3229.

Meredith, M. A. and Stein, B. E. (1983). Interactions among converging sensory inputs in the superior colliculus. *Science* **221**, 389-391.

Meredith, M. A. and Stein, B. E. (1986). Spatial factors determine the activity of multisensory neurons in cat superior colliculus. *Brain Res* **365**, 350-354.

Meredith, M. A. and Stein, B. E. (1986). Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. *J Neurophysiol* **56**, 640-662.

Meredith, M. A. and Stein, B. E. (1996). Spatial determinants of multisensory integration in cat superior colliculus neurons. *Journal of Neurophysiology* **75**, 1843-1857.

Munoz, D. P. and Guitton, D. (1985). Tectospinal neurons in the cat have discharges coding gaze position error. *Brain Res* **341**, 184-188.

Munoz, D. P. and Guitton, D. (1989). Fixation and orientation control by the tecto-reticulo-spinal system in the cat whose head is unrestrained. *Rev Neurol* **145**, 567-579.

Occelli, V., Spence, C. and Zampini, M. (2013). Auditory, tactile, and audiotactile information processing following visual deprivation. *Psychol Bull* **139**, 189-212.

Perrault, T. J., Jr., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2005). Superior colliculus neurons use distinct operational modes in the integration of multisensory stimuli. *J Neurophysiol* **93**, 2575-2586.

Perrault, T. J., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2003). Neuron-Specific Response Characteristics Predict the Magnitude of Multisensory Integration. *Journal of Neurophysiology* **90**, 4022-4026.

Putzar, L., Goerendt, I., Lange, K., Rosler, F. and Roder, B. (2007). Early visual deprivation impairs multisensory interactions in humans. *Nat Neurosci* **10**, 1243-1245.

Putzar, L., Gondan, M. and Roder, B. (2012). Basic multisensory functions can be acquired after congenital visual pattern deprivation in humans. *Dev Neuropsychol* **37**, 697-711.

Roder, B., Rosler, F. and Spence, C. (2004). Early vision impairs tactile perception in the blind. *Curr Biol* **14**, 121-124.

Royal, D. W., Krueger, J., Fister, M. C. and Wallace, M. T. (2010). Adult plasticity of spatiotemporal receptive fields of multisensory superior colliculus neurons following early visual deprivation. *Restor Neurol Neurosci* **28**, 259-270.

Stein, B. E. (2012). The new handbook of multisensory processing, MIT Press.

Stein, B. E., Scott Huneycutt, W. S. and Meredith, M. A. (1988). Neurons and behavior: the same rules of multisensory integration apply. *Brain Research* **448**, 355-358.

Stein. (1986). Visual, Auditory, and Somatosensory Convergence on Cells in Superior Colliculus Results in Multisensory Integration. *J Neurophysiology* **56**, 640-662.

Tao, Q., Chan, C. C., Luo, Y. J., Li, J. J., Ting, K. H., Wang, J. and Lee, T. M. (2015). How does experience modulate auditory spatial processing in individuals with blindness? *Brain Topogr* **28**, 506-519.

Voss, P., Lassonde, M., Gougoux, F., Fortin, M. Guillemot, J. P. and Lepore, F. (2004). Early- and late-onset blind individuals show supra-normal auditory abilities in far-space. *Curr Biol* **14**, 1734-1738.

Wallace, M. T. and Stein, B. E. (2007). Early experience determines how the senses will interact. *J Neurophysiol* **97**, 921-926.

Xu, J., Yu, L., Rowland, B. A., Stanford, T. R. and Stein, B. E. (2014). Noise-rearing disrupts the maturation of multisensory integration. *Eur J Neurosci* **39**, 602-613.

Yu, L., Rowland, B. A. and Stein, B. E. (2010). Initiating the Development of Multisensory Integration by Manipulating Sensory Experience. *The Journal of Neuroscience* **30**, 4904-4913.

Yu, L., Rowland, B.A., Xu, J. and Stein, B.E. (2013). Multisensory plasticity in adulthood: cross-modal experience enhances neuronal excitability and exposes silent inputs. *J Neurophysiol* **109**, 464-474.

Chapter III

Serotonergic modulation of sensory and multisensory processing in superior colliculus

This chapter is accepted for publication in *Multisensory Research* as: Kurela, L. R. and Wallace, M. T. (2017). Serotonergic modulation of sensory and multisensory processing in superior colliculus. *Multisensory Research*.

Introduction

The ability to properly orient to and interact with our surrounding world is a central facet of human behavior. This capacity is predicated on the proper combination of sensory information. Integrating multisensory information improves various aspects of behavioral performance, including target detection (Lovelace et al. 2003), response times (Hughes et al. 1994; Frens et al. 1995; Murray et al. 2001; Corneil et al. 2002; Molholm et al. 2002; Amlot et al. 2003; Diederich et al. 2003), orientation (Stein et al. 1988), and localization (Wilkinson et al. 1996; Hughes et al. 1998). Although multisensory convergence takes place at a variety of levels of the neuraxis (Murray and Wallace 2011; Stein 2012), one of the best-studied structures for elucidating the combinatorial operations that underpin multisensory function is the midbrain superior colliculus (SC). In the SC intermediate and deep layers (i.e., below stratum opticum), many individual neurons receive and integrate information from two or more sensory modalities (Meredith and Stein 1983; Munoz and Guitton 1985; Alex Meredith and Stein 1986; Stein 1986; Meredith et al. 1987; Munoz and Guitton 1989; Meredith and Stein 1996; Perrault et al. 2003; Perrault et al. 2005). While much is known regarding the principles by which multisensory neurons encode and transform their various sensory

inputs, less is understood about the mechanistic processes that underlie the integrative operations of these neurons. Although several studies have begun to build our knowledge of the neurotransmitters that are important in multisensory function, such as gamma-amino-butyric-acid (GABA) (Allman et al. 2008; Fuentes-Santamaria et al. 2008; Gogolla et al. 2014), our view into the role of these transmitters in multisensory function remains fairly rudimentary.

The molecule 5-hydroxytryptamine (5-HT; serotonin) is the most widely distributed neurotransmitter in the brain (Dahlstrom and Fuxe 1964; Steinbusch 1981; Hay-Schmidt 2000). Substantial serotonergic innervation of the SC originates from the dorsal raphe nuclei, and is essential for proper development of sensory representations within the SC (Lauder et al. 1982; Mize and Horner 1989; Rhoades et al. 1990). More generally, 5-HT has been shown to play an important role in the development of sensory and motor representations across the brain (Ueda et al. 1985; Villar et al. 1988; Rhoades et al. 1990; Arce et al. 1992; Huang et al. 1993; Gu and Singer 1995; Janusonis et al. 1999; Ke et al. 1999; Foehring et al. 2002; Hurley et al. 2002; Xiang and Prince 2003; Hurley et al. 2004; Lottem et al. 2016). In addition to playing a central part in neurodevelopment, 5-HT is also important for the maintenance of sensory representations, having been shown to be integral in shaping the firing patterns and tuning functions of the neurons that make up these representations. Previous work has illustrated the importance of 5-HT for the response profiles of neurons in the auditory (Hurley and Pollak 1999; Hurley and Pollak 2001; Hurley 2006), visual (Waterhouse et al. 1986) and somatosensory (Jitsuki et al. 2011) systems. While this evidence

illustrates the importance of 5-HT for the development and maintenance of unisensory function (Ke et al. 1999), its role in multisensory processing remains unknown.

The striking role that 5-HT has been demonstrated to play in modulating sensory function, coupled with the presence of substantial serotonergic inputs to the multisensory layers of the SC, was the motivation for the current study. The work set out to explore the impact of manipulating serotonergic signaling via pharmacological methods on auditory, visual and combined audiovisual information processing in the SC. One important facet of these analyses sought to tease out the relative impact of 5-HT function on unisensory (i.e., auditory alone, visual alone) responses as compared to multisensory (i.e., combined audiovisual) responses, thus revealing the specificity of 5-HT neuromodulation on the multisensory filtering and integrative capacity of these neurons. The previously established role of the 5-HT system in sensory processing and sensory representations (Ueda et al. 1985; Villar et al. 1988; Rhoades et al. 1990; Arce et al. 1992; Huang et al. 1993; Gu and Singer 1995; Janusonis et al. 1999; Ke et al. 1999; Foehring et al. 2002; Hurley et al. 2002; Xiang and Prince 2003; Hurley et al. 2004; Lottem et al. 2016) led us to expect changes in sensory function during manipulation of serotonergic signaling in the SC. In addition, given the extent of 5-HT inputs into multisensory layers of the SC as well as the impact of the 5-HT system on both visual and auditory processing, we also anticipated changes to multisensory filters and firing patterns that extended beyond those predicted by simple linear summation of effects on unisensory responses, namely alterations in the gain seen under multisensory conditions. We found marked heterogeneity of 5-HT influences on the firing patterns of multisensory SC neurons, and many of these effects were not readily

predictable based on the unisensory effects. Although the effects of manipulations of 5-HT signaling on multisensory integration were quite evident, the most selective effects appeared to be on visual responses, with a preferential bias of these influences for the more superficial aspects of the deeper layers (i.e., intermediate layers). To our knowledge, these results are the first to support the importance of the 5-HT system for mediating facets of unisensory and multisensory signaling within the deeper layers of the SC.

Materials and Methods

General procedures

Experiments were conducted in adult male cats (n=3) raised under standard housing conditions. All experiments were completed in an anesthetized and paralyzed semichronic preparation and consisted of single unit and local field potential (LFP) extracellular recordings from the midbrain SC as described in previous studies (Royal et al. 2010; Ghose et al. 2012; Ghose et al. 2014; Ghose and Wallace 2014). Experiments were run on a weekly basis for each animal. All surgical and recording procedures were performed in compliance with the Guide for the Care and Use of Laboratory Animals at Vanderbilt University Medical Center, which is accredited by the American Association for Accreditation of Laboratory Animal Care.

Implantation and recording procedures

For surgical implantation procedures, animals were initially induced with ketamine hydrochloride (20 mg/kg, administered intramuscularly (IM)) and acepromazine maleate (0.04 mg/kg IM). Animals were transported to a central surgical

suite, intubated and artificially respired. A stable plane of anesthesia was achieved prior to the start of the procedure using inhalation of isoflurane (0.5-2%). Body temperature, expiratory CO2, blood pressure and heart rate were continually monitored (VSM7, Vetspecs/SCIL), recorded and maintained within ranges consistent with a deep and stable plane of anesthesia. A craniotomy was made in order to allow access to the SC. A head holder was attached to the skull using stainless steel screws and orthopedic cement. This was used to hold the animal in a comfortable and stable recumbent position during recording sessions without obstructing the face, ears, or access to the SC. Postoperative care including antibiotics and analgesics were administered in close consultation with the Vanderbilt veterinary care staff.

For neurophysiological recordings, animals were initially anesthetized with ketamine hydrochloride (20 mg/kg IM) and acepromazine maleate (0.04 mg/kg IM). Animals were intubated and artificially respired, and a stable plane of anesthesia and paralysis was maintained using a constant rate of infusion of ketamine hydrochloride (5mg/kg/hr administered intravenously (IV)) and rocuronium bromide (2.2-2.5 mg/kg/hr IV) delivered through a cannula placed in a saphenous vein for the remainder of the procedure. The head-holder hardware was used to place the animal in a comfortable and stable recumbent position. Animals were given 100-200 mL of Lactated Ringer Solution subcutaneously throughout the procedure. A contact lens was used in order to focus the eye on the plane in which stimuli were delivered. Once a neuron was isolated (signal-to-noise ratio > 3:1), single unit neural activity (SUA) and LFPs were recorded, amplified and routed to an oscilloscope, audio monitor and computer for preforming online and offline analyses.

Stimulus presentation, receptive field mapping and search strategy

Parylene-insulated tungsten electrodes (initial impedance at 1 kHz = 4-5 MΩ) were used for initial procedures in order to map the location of the SC. Once the SC was mapped, further neuronal isolation and recordings were performed using glass electrodes with embedded ejection ports (Carbostar, Kations). The electrode was advanced into the SC using an electronically controlled mechanical microdrive. The top of the SC was determined by its characteristic fast visual responses. After determining the top of the SC, the electrode was moved at least 1000 µm, into the intermediate/deep layers, and a single unit was isolated. Visual fields and RF boundaries were determined using a handheld Keeler pantoscope with moving spots of light.

Visual stimuli consisted of illumination of stationary light emitting diodes (LEDs; 100 ms duration; 104 cd/m^2 luminance) and auditory stimuli consisted of broadband noise bursts (20 Hz-20 KHz; 100 ms duration; 67 dB SPL). LEDs and speakers were concurrently mounted on a hoop placed 60 cm in front of the animal at azimuthal locations ranging from 0° to 90° on either side of the midline in 10° increments. The hoop's rotation abilities allowed the sampling of multiple locations within and outside the RFs of the recorded neurons. Electrophysiological criteria were implemented in order to ensure the restriction of recordings to deep SC layers, including the presence of larger visual RFs (when compared with the superficial layers) and visual response latencies of greater than 50 ms (Ghose et al. 2014).

In most cases, a total of 3-4 elevations with 4-5 azimuths per elevation were chosen to fully encompass central and peripheral RF boundaries for recordings from

each single unit. Visual (V), auditory (A) and audiovisual (AV) stimuli were presented in a pseudorandomized interleaved manner at multiple azimuthal locations along a single elevation at a time. Multisensory presentations always consisted of visual and auditory stimuli presented in the spatial synchrony with a temporal offset where the visual stimulus preceded the auditory stimulus by 50 ms. This interval was chosen in order to improve the chances for multisensory interactions in the SC neurons, as it accounts for the temporal difference in input latencies for auditory and visual stimuli to the SC (Meredith et al. 1987; Stein et al. 1993; Ghose et al. 2012). A minimum of 60 trials were collected for each location (20 visual, 20 auditory, 20 multisensory stimulus presentations). Consecutive stimulus presentations were separated by at least 3 s (jittered) to avoid response habituation.

Agonist injection

Following initial recordings, 5HT2a receptor agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-amino-propane (DOI; 30µM concentration, 300 nL) was pressure-injected through back-filled ejection ports on the recording electrode into the surrounding extracellular space of the single unit. DOI was chosen for its reliable action and high selectivity for 5HT2 receptor types, which are abundant in the SC and have been implicated in gating sensory function and the excitation of neurons. This particular amount of DOI was used based on modeling of the spread and diffusion of the injected compound to fully encompass the single neuron recorded from, but no so large so as to result in global effects on the SC (Egan et al. 2000; Lyon et al. 1987; McKenna and Peroutka 1989; Wright et al. 1990; Marek and Aghajanian 1994; Hurley 2006; Riga et al. 2016). Recordings at the same spatial locations and parameters were then completed

approximately 10 minutes following injection (peri), in order to allow for diffusion and action of the agonist. These recordings were performed a third time, 3-4 hours following the initial recordings (post) in order to allow comparison of pre, peri and post-DOI responsiveness of the multisensory neuron to unisensory and multisensory stimuli at multiple spatial locations.

Data acquisition and analysis

A custom-built, PC-based real-time data acquisition system controlled the structure of the trials and timing of the stimuli using custom scripts written in LabView (National Instruments). The analog waveforms of the extracellular voltage fluctuations picked up by the electrode were transferred to a Plexon MAP system (Plexon) and digitized, high-pass filtered at 40 kHz for spikes and low-pass filtered at 1 kHz for LFPs. SUA was thresholded and sorted online using SortClient software and stored for further analysis. Neuronal responses were detailed through the construction of peristimulus time histograms (PSTHs) for each stimulus condition (V, A, AV) for each location (elevation and azimuth) tested. Baseline for each PSTH was calculated as mean firing rate during 500 ms immediately preceding the stimulus onset for each of the 3 conditions. Response threshold was set at 3 SD above this baseline in order to delineate a stimulus-evoked response. Response onset was defined as the time at which the PSTH crossed and remained above the 3 SD line for at least 30 ms. Response offset was measured as the time at which the PSTH fell below the 3 SD line and stayed below the line for $\geq$ 30 ms. Response duration was determined as the time interval between response onset and response offset. Mean stimulus evoked response was defined as the average number of spikes elicited per trial during the demarcated

response duration interval. Mean spontaneous firing rate was subtracted from the response. Responses peri- and post- DOI are presented here in relation to pre-DOI responsivity; average best unisensory pre-DOI response was divided from peri- and post-DOI responsivity to determine relative response. This was done to normalize responses from all single units in order to compare groups of neurons. 3 X 2 analysis of variance (ANOVA) with Tukey's multiple comparisons tests were utilized for all experiments. ANOVA were performed in a 3x2 structure with neuronal type (i.e. auditory, visual, audiovisual neuron) and DOI (i.e before and after DOI addition) as factors. ANOVAs were run separately on unisensory and multisensory neuronal responses, with unisensory responses encompassing the best unisensory response of the neuron (i.e. auditory or visual response) and multisensory responses encompassing the response to audiovisual stimulus presentations. Separation of ANOVAs for unisensory and multisensory responses was done to be able to fully capture the effects of DOI on all responsivity of the neurons, to eliminate the possibility of its effect on one type of response (i.e. multisensory responses) driving the overshadowing of another. Standard error of the mean is presented. Neuronal responses after administration of DOI (peri) and following cessation of DOI action (post) were normalized to pre-DOI responses for population measures and compared using Student's t-tests. Latency of response was defined as the time between stimulus onset and response onset. Fano factor (FF), a measure of response reliability, was measured as the ratio of variance (σ^2) to the mean (μ) spike count across trials (Fano 1947; Sarko et al. 2013). We calculated the change in FF values (ΔFF) between the maximum unisensory response (U_{ff}) and the multisensory response (M_{ff}) by

$$\Delta FF = U_{ff} - M_{ff}$$

where a positive ΔFF indicates that unisensory responses are more variable than multisensory responses and a negative ΔFF reveals a multisensory response to be more variable than the best unisensory response (Sarko et al. 2013).

Spatial receptive fields (SRF) were determined for a subset of neurons recorded. SRF plots were created as previously described (Ghose and Wallace 2014). Briefly, mean stimulus-evoked responses were normalized to the highest stimulus-evoked response recorded from all tested stimulus conditions and locations. These response values ranged from 0 to 1 and were subsequently used to create pseudocolor SRF plots in order to show relative activity as a function of stimulus location. SRF plots were created for each unisensory condition as well as the multisensory condition pre-, peri- and post- DOI application in order to determine the action of the agonist on SRF structure. In addition, a predicted SRF plot was created by summing the unisensory (V and A) SRFs, which was then subtracted from the multisensory SRF plot in order to generate a contrast plot. Warmer colors indicate superadditive interactions while cooler colors represent subadditive interactions (Carriere et al. 2008). For visualization purposes, the SRF structure was interpolated using a 2D gaussian filter (filter size = 100 deg, resize factor = 100) (Ghose and Wallace 2014).

Quantification of multisensory integration

Interactive index (ii) was used to quantify multisensory integration. ii measures how the multisensory response differs from the best unisensory response of a neuron. The magnitude of this change was calculated as

$$[(CM - SMmax)/(SMmax)] \times 100 = \% \text{ interaction}$$

where CM is the mean response evoked by the combined modality (i.e., multisensory) stimulus and SMmax is the mean response evoked by the most effective single modality stimulus (Meredith and Stein 1983; Stein 1986). Statistical comparisons between the mean stimulus evoked responses of the multisensory and best unisensory conditions and the additive prediction were done using a Wilcoxon Rank Test.

LFP analysis

LFPs were sampled at 1 kHz and converted into voltage as a function of time. LFP amplitudes were compared pre- and post- stimulus onset for pre-, peri- and post-DOI addition conditions. The mean voltage within a window of 150 ms pre-stimulus onset was used as a baseline and compared to peak voltage change within a 300 ms post-stimulus timeframe. These were compared between V, A and AV stimulus conditions within each spatial location. T-tests were used to determine if LFP amplitude differed between stimulus and DOI conditions. Area under the curve (AUC) for the averaged evoked LFPs were computed for each of the stimulus conditions (V, A, AV) and DOI conditions (pre-, peri-, post- DOI injection).

Results

Sensory and multisensory responsiveness in intermediate and deep SC neurons are affected by the administration of the serotonin receptor agonist DOI

Data from a total of 54 single neurons within the intermediate and deep layers of the SC (i.e. those below stratum opticum) were collected and analyzed. No differences

between animals in the presence or types of multisensory neurons encountered, as well as the characteristics of those multisensory neurons (responsivity, interactive index, etc.), were found in the neurons recorded. Neurons were categorized based on their overt responses to sensory stimuli. Visual neurons (11/54) were overtly responsive to only visual and audiovisual stimuli (Figure 1A, Supplementary Figure 1A); auditory neurons (25/54) were overtly responsive to only auditory and audiovisual stimuli (Figure 1B, Supplementary Figure 1B); audiovisual neurons (18/54) were overtly responsive to visual, auditory and audiovisual stimuli (Figure 1C, Supplementary Figure 1C).

The addition of the serotonin 5HT2a receptor agonist 1-[2,5-dimethoxy-4-iodophenyl]-2-amino-propane (DOI) had a general tendency to increase responses to both unisensory (i.e., visual alone, auditory alone) and multisensory stimuli (Figure 2A). However, these effects appeared to be specific for certain neuronal types, in that whereas DOI generally increased responses in visual and audiovisual neurons, it failed to show a similar effect in auditory neurons (Figure 1). Two separate 3x2 ANOVAs were performed in order to compare main effects of neuron type (e.g. visual, auditory, multisensory) and DOI (e.g. pre- and peri- DOI administration) on unisensory and multisensory neuronal responses. The analysis performed on unisensory responses revealed no significant main effect of neuronal type, a main effect of DOI injection and an interaction effect (Table 1). The analysis performed on multisensory responses revealed a main effect of neuron type and DOI injection, but no interaction effect. Visual neurons showed an increase in relative response to audiovisual stimuli by 14.1$\pm$29.4% ($t(31)= 19.91$, $p=0.0112$) with the administration of DOI (Figure 2A). In contrast, DOI administration resulted in no significant change in the relative responses of these

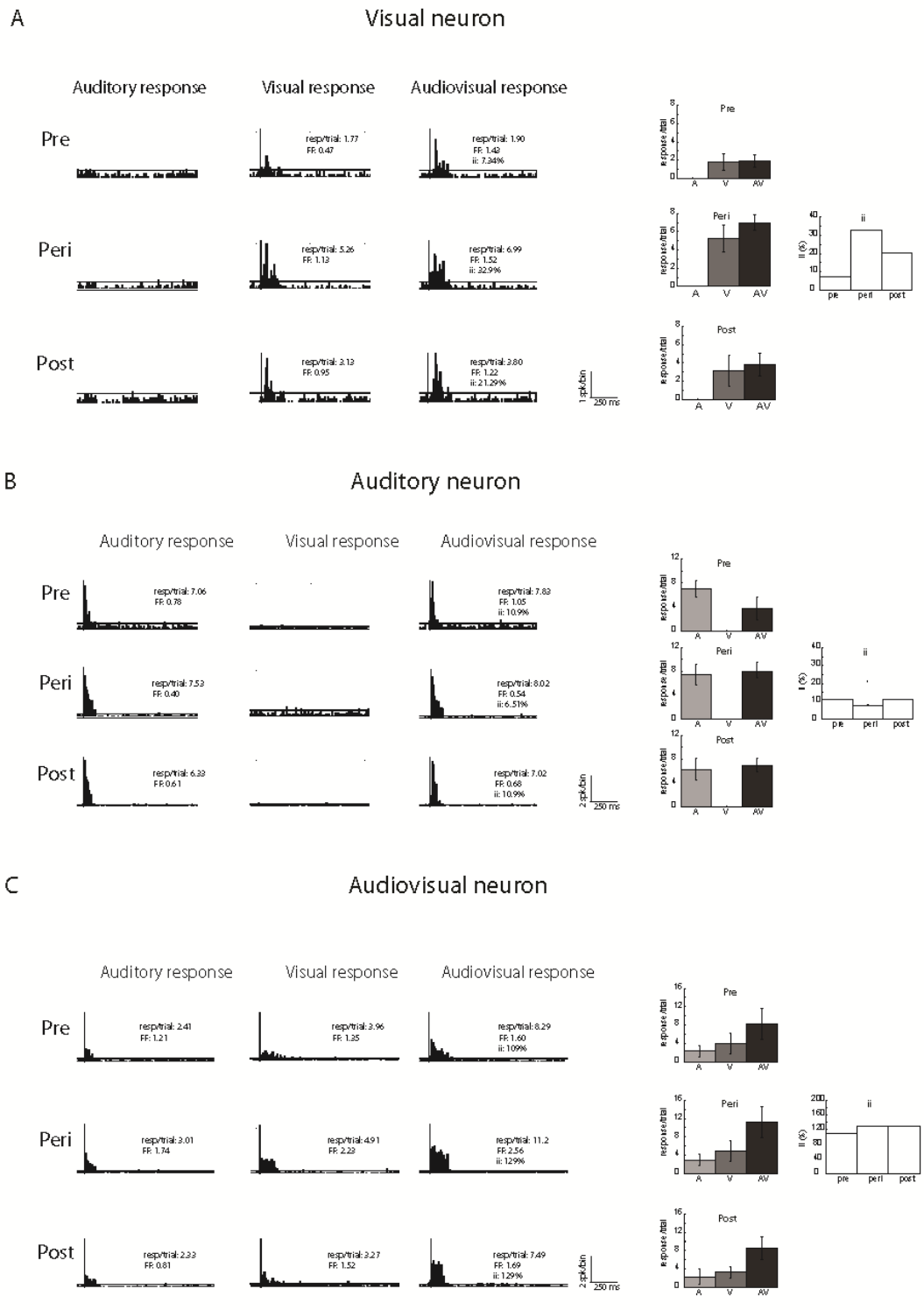


Figure 3-1. Example responses of visual, auditory and audiovisual SC multisensory neurons. Peristimulus-time histograms (PSTHs) from an example visual

(A), auditory (B) and audiovisual (C) multisensory neuron showing responsiveness to sensory stimulus presentations before (pre), 10 minutes following (peri) and 3.5 hours following (post) DOI injection. Bar graphs to the right are plotting response per trial (resp/trial) and integrative index (ii), quantifications of the example PTSH shown to the left. The administration of DOI induces an increase in both resp/trial and ii in all three neuronal types.

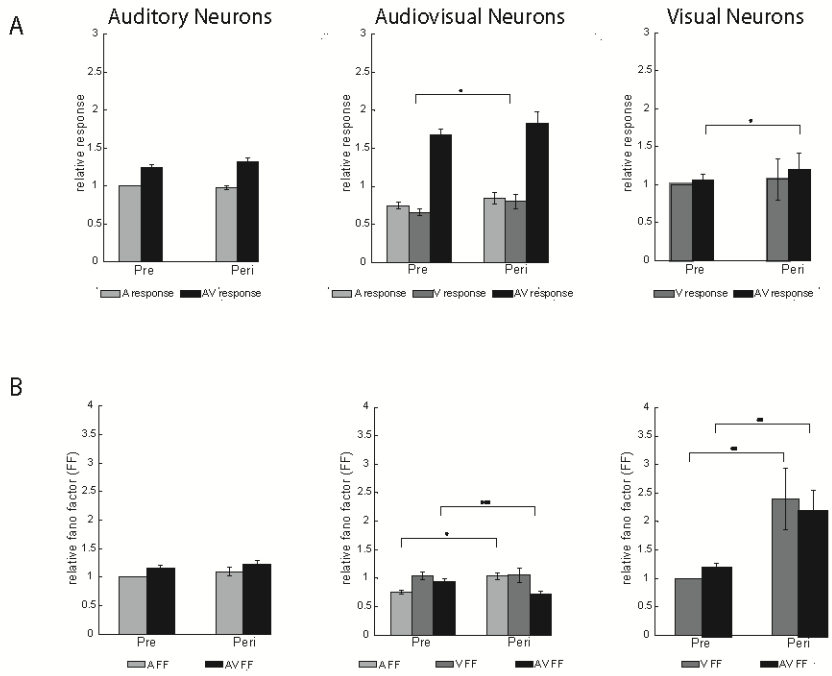


Figure 3-2. DOI affects unisensory and multisensory responsiveness in deep SC neurons. Average relative responses (A) and fano factor (B) of auditory (left), visual (right), and audiovisual (center) SC neurons to unisensory and multisensory stimulus presentations before (pre) and 10 minutes following (peri) DOI injection. Responses after administration of DOI (peri) were normalized to unisensory pre-DOI responses to compare populations of neurons. Significance was determined by comparing conditions following DOI addition to the 'pre' condition. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***p<0.001. (A) Addition of DOI significantly increased neuronal responses to unisensory and multisensory stimulus presentations. Addition of DOI increased visual neurons' responses to multisensory stimuli as well as audiovisual neurons' responses to visual stimulus presentations. (B) Addition of DOI increased fano factor (FF) of visual neurons' visual and audiovisual responses as well as audiovisual neurons' auditory responses, while decreasing FF of audiovisual neurons' multisensory responses.

	Neuron Type	DOI Injection	Interaction
Unisensory Neuronal Response	F(1,236)=4.74, p=0.0591	F(1,236)=3.67, p=0.0460	p=0.000120
Multisensory Neuronal Response	F(1,236)=2.63, p<0.0001	F(1,236)=6.57, p=0.0110	p=0.227
Unisensory Fano Factor	F(2,236)=1.65, p<0.0001	F(2,236)=6.43, p=0.0110	p=0.000100
Multisensory Fano Factor	F(2,236)=13.8, p<0.0001	F(2,236)=8.72, p=0.00300	p=0.0571
Interactive Index	F(1,236)=2.35, p<0.0001	F(1,236)=2.41, p=0.0101	p<0.0001
Central RF Location Unisensory Response	F(1,134)=0.771, p=0.465	F(1,134)=2.32, p<0.0001	p=0.0682
Central RF Location Multisensory Response	F(1,134)=1.854, p=0.161	F(1,134)=7.71, p=0.000110	p=0.0617
Central RF Location Fano Factor	F(1,134)=1.62, p=0.00291	F(1,134)=9.011, p=0.0723	p=0.000273
Central RF Location Interactive Index	F(1,134)=8.33, p=0.0785	F(1,134)=1.86, p=0.000224	p=0.00116
Peripheral RF Location Unisensory Response	F(1,101)=3.38, p=0.0381	F(1,101)=6.28, p<0.0001	p=0.0854
Peripheral RF Location Multisensory Response	F(1,101)=2.78, p<0.0001	F(1,101)=7.22, p=0.00815	p=0.0380
Peripheral RF Location Fano Factor	F(1,101)=1.69, p=0.00448	F(1,101)=1.854, p=0.0611	p=0.000126
Peripheral RF Location Interactive Index	F(1,101)=2.49, p=0.262	F(1,101)=2.49, p<0.0001	p=0.0634
Peak LFP	F(1,234)=8.73, p<0.0001	F(1,234)=8.25, p=0.00422	p=0.128

Table 3-1. Results of analysis of variance (ANOVA) tests. ANOVA were performed in a 3X2 structure with neuronal type and DOI as factors.

neurons to visual stimuli. The addition of DOI did not significantly change either the auditory or multisensory responses of auditory neurons. In audiovisual neurons, administration of DOI increased the relative responses to visual stimuli by 14.2±2.27% ($t(80)=21.05$, $p=0.0310$) (Figure 2A). In contrast, no significant changes were seen in the responses of multisensory neurons to auditory or audiovisual stimuli. These significant changes in visual and multisensory responses generally returned to baseline values following a post-injection recovery interval of approximately 3.5 hours. In contrast to the DOI condition, no changes in visual, auditory or audiovisual responses were observed with injections of artificial cerebral spinal fluid (aCSF) (Figure 9A). Additionally, DOI did not have any significant effects on relative response latencies ($p>0.0500$), response durations ($p>0.0500$), or baseline firing rates ($p>0.0500$) for any of the neuronal types.

DOI administration results in altered sensory and multisensory response reliability

Fano factor (FF), the ratio of variance (σ^2) to the mean (μ) spike count across trials, was used to determine the changes in variability (reliability) of the responses induced by DOI administration. In the same manner as for the analyses of neuronal responses (see above), the data was subjected to two separate 3 X 2 ANOVAs (Table 1). For the analyses of unisensory responses, the ANOVA revealed a main effect of neuron type, DOI administration and an interaction. For the analyses of multisensory responses, the ANOVA revealed a main effect of neuron type and DOI administration, but no interaction. Follow-up analyses revealed that upon DOI administration, visual neurons showed an increase in FF for responses to visual stimuli by 139±53.0%

(t(31)=4.524, p=0.00710) and an increase in FF for responses to audiovisual stimuli by 98.9±44.3% (t(31)=14.59, p=0.00410) (Figure 2B). Auditory neurons showed no significant change in FF with addition of DOI, reinforcing the absence of effects on auditory responses in general. In audiovisual neurons, while there was no significant change in FF for responses to visual stimuli, addition of DOI increased FF for responses to auditory stimuli by 28.04±6.22% (t(80)= 10.49, p=0.0450) and decreased FF for responses to audiovisual stimuli by 21.2±8.17% (t(80)=16.05, p=0.000250) (Figure 2B). No significant changes in FF were observed during and after aCSF injections (Figure 9B).

Administration of DOI results in increased multisensory gain

The addition of DOI also frequently altered multisensory gain as measured via the interactive index (ii), which represents how the multisensory response differs from the largest unisensory response (Figure 3A). A change in multisensory gain was seen in the majority of the neurons recorded for each of the neuronal categories (V neurons = 10/11; A neurons = 21/25; AV neurons = 12/18). A 3 X 2 ANOVA revealed a main effect of neuron type and DOI administration, as well as an interaction effect (Table 1). Interactive index increased in visual and auditory neurons with the administration of DOI by 290±66.8% (t(31)=2.451, p=0.00950) and 187±66.4% (t(123)=7.53, p=0.0120, respectively (Figure 3A). In contrast, no significant change in ii was observed in audiovisual neurons. In most instances, upon recovery from DOI (i.e., 3.5-4 hours post-DOI administration), ii returned to baseline values. These results suggest a selectivity of DOI action on ii for unisensory (i.e., visual, auditory) neurons when compared with audiovisual neurons (see discussion).

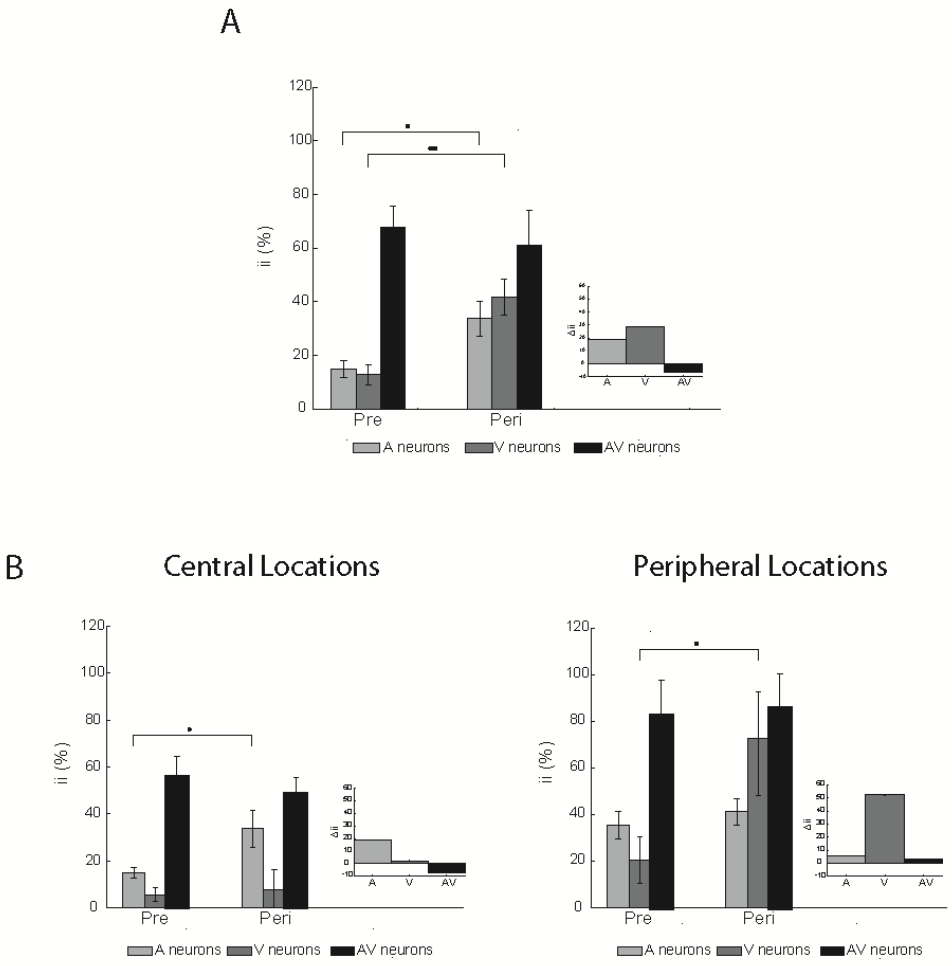


Figure 3-3. DOI affects interactive index of multisensory deep SC neurons. (A) interactive index (ii) of auditory, visual and audiovisual neurons with DOI administration. ii of auditory and visual neurons increased with the addition of DOI. *Inset*: change in ii (Δii) between pre- and peri-DOI recordings. The greatest change in ii with DOI addition was observed in visual neurons. (B) ii divided between neuronal types as well as RF location. At central RF locations (left), auditory neuron ii was significantly increased with the addition of DOI, whereas at peripheral locations both auditory and visual neuron ii increased with DOI addition. Insets show Δii between pre- and peri- recordings; the greatest change in ii was observed in visual neurons at peripheral RF locations.

DOI administration has differential influences on neuronal responses at central vs. peripheral receptive field locations

Due to the wealth of information implicating the 5-HT system's involvement in modulation of receptive fields (RF) (Waterhouse et al. 1986; Gu and Singer 1995; Hurley et al. 2004; Jitsuki et al. 2011), as well as evidence that this modulation differs across the RF (Hurley and Pollak 2001), we examined the action of DOI on RFs in intermediate and deep SC neurons. In order to carry out a first-level analysis of potential differential effects of 5-HT signaling on receptive fields, data was divided to examine the impact of DOI on central versus peripheral RF locations. Central RF locations were defined as the 2-3 azimuth/elevation locations most central in the neuron's receptive field. Peripheral locations were defined as the 2-3 azimuth/elevation locations at the borders of each neuron's receptive field, at which stimulus presentations still elicited a neuronal response.

As performed above, 3 X 2 ANOVAs revealed a main effect of DOI administration by neuron type at central RF locations for both unisensory and multisensory responses, but no interaction effects (Table 1). Follow up analyses revealed that these effects were driven largely by changes in visual neurons. Thus, at central RF locations, only the responses of visual neurons were altered by DOI administration, with these neurons increasing their responses to visual stimuli by 41.3$\pm$23.8% ($t(15)=4.36$, $p=0.0310$) and increasing their responses to audiovisual stimuli by 31.9$\pm$27.3% ($t(15)=43.94$, $p=0.0232$) (Figure 4A *top*). In contrast, responses of auditory and audiovisual neurons at central RF locations remained unchanged with DOI administration ($p>0.05$).

At peripheral RF locations, a different pattern of effects was observed following DOI administration. A 3 X 2 ANOVA revealed a main effect of neuronal type and DOI administration on unisensory responses at peripheral locations, but no interaction effect (Table 1). An additional 3 X 2 ANOVA revealed a main effect of neuronal type, DOI administration and an interaction effect on multisensory responses at peripheral RF locations (Figure 4B *top*). For these peripheral locations, DOI increased responses of visual neurons to visual stimuli by 77.5±49.3% ($t(15)=3.456$, $p=0.0171$), but had little effect on the audiovisual responses of these neurons. Administration of DOI did not significantly alter the responses of auditory neurons to auditory or multisensory stimulus presentations at peripheral locations. Administration of DOI increased the responses of audiovisual neurons to visual (33.7±19.3% ($t(31)=8.58$, $p=0.0110$) and audiovisual (48.2±25.4% ($t(31)=11.88$, $p=0.00562$) stimuli, and decreased the responses of these neurons to auditory stimuli (60.6±11.6% ($t(31)=6.61$, $p=0.0128$) (Figure 4B *top*).

Changes in multisensory interactions as indexed via the interactive index (ii) were also found to differ depending upon central vs. peripheral locations (Figure 3B). A 3 X 2 ANOVA revealed a main effect of DOI administration and an interactive effect at central locations, but no effect of neuron type. ANOVA also revealed a main effect of DOI administration at peripheral RF locations, but no effect of neuron type or interaction effect (Table 1). Whereas central RF locations only showed a significant increase in ii for auditory neurons (18.7±8.07% ($t(69)=4.95$, $p=0.0134$) , peripheral RF locations only showed an increase in ii for visual neurons (70.6±13.6% ($t(15)=2.13$, $p=0.0117$)) (Figure 3B). The increase in ii was greater in visual neurons at peripheral locations compared to ii increase in auditory neurons at central locations (Figure 3B *insets*).

In addition to these effects on responsivity and interactive index, differential changes in Fano Factor were seen based on central versus peripheral locations. ANOVA did not reveal a main effect of DOI but did reveal a main effect of neuron type and an interaction of neuron type and DOI administration at central locations. Separate ANOVA revealed similar patterns; no main effect of DOI administration alone, but a main effect of neuron type and an interaction of neuron type and DOI administration at peripheral locations (Table 1). At central locations in visual neurons, DOI increased the FF of visual responses by 97.0$\pm$33.2% ($t(15)=2.131$, $p=0.00506$) and increased the FF of audiovisual responses by 100$\pm$48.2% ($t(15)=11.35$, $p=0.0251$) (Figure 4A *bottom*). The administration of DOI also altered FF in visual neurons at peripheral locations, increasing the FF in response to visual stimuli by 185$\pm$100% ($t(15)=2.743$, $p=0.0472$) and to audiovisual stimuli by 98.8$\pm$62.4% ($t(15)=10.82$, $p=0.039$) (Figure 4B *bottom*). In an additional analysis, DOI was also found to change the ΔFF of these visual neurons. DOI increased ΔFF of visual neurons from -0.0581 to 0.894, indicating that the visual responses of these neurons became more variable when compared with the audiovisual responses of these neurons at peripheral locations with the administration of DOI ($t(15)=7.11$, $p=0.00130$). The same was not seen at central locations; the addition of DOI did not significantly change ΔFF of any neuronal types at central locations ($p>0.05$).

Audiovisual neurons showed an opposite effect on FF as that seen in visual neurons. For central RF locations, DOI administration decreased FF in response to audiovisual stimuli by 27.0$\pm$7.00% ($t(48)= 11.39$, $p=0.00251$) (Figure 4A *bottom*). A similar trend was seen at peripheral locations, with the administration of DOI decreasing the FF for responses to audiovisual stimuli by 11.5$\pm$7.48% ($t(31)=11.77$, $p=0.0177$)

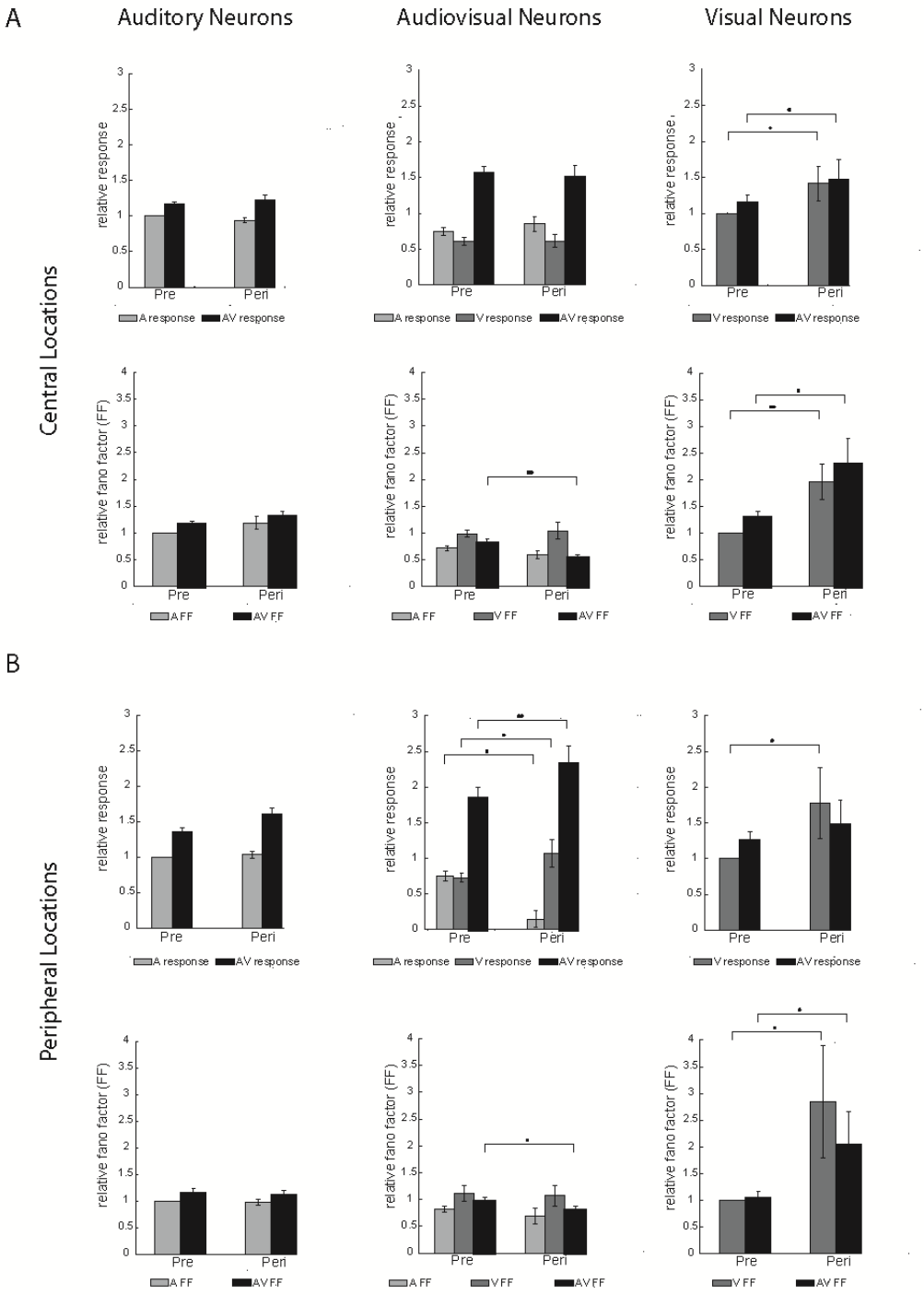


Figure 3-4. DOI effects are more dramatic at peripheral receptive field locations. (A) (*top*) Relative responses of auditory (left), visual (right), and audiovisual (center) SC neurons from central RF locations. Relative responses of visual neurons to unisensory stimuli increased with DOI addition. (*bottom*) Fano factor (FF) of auditory (left), visual

(right), and audiovisual (center) neurons at central RF locations. FF increased at central locations with DOI addition for visual neurons, whereas FF of audiovisual neurons decreased with the addition of DOI. (B) (*top*) Relative responses to visual stimuli increased with addition of DOI for both visual and audiovisual neurons at peripheral locations. Audiovisual neurons also exhibited an increase in response to audiovisual stimuli, and a decrease in response to auditory stimuli with addition of DOI at peripheral locations. (*bottom*) Relative FF at peripheral locations increased for visual neurons with DOI addition while decreasing for audiovisual neurons (right). Significance was determined by comparing conditions following DOI addition to the 'pre' condition. Responses after administration of DOI (peri) were normalized to pre-DOI responses to compare populations of neurons. Asterisks display comparisons of significance, *p<0.05, **p<0.01, ***<p,0.001.

(Figure 4B *bottom*). No change was observed for FF of auditory or visual responses of these neurons ($p>0.05$). Fano Factor was not significantly altered by DOI addition in auditory cells at either central or peripheral locations ($p>0.05$).

The administration of DOI induces heterogeneous effects on spatial receptive fields

Further investigation into location-specific effects of DOI on the responses of intermediate and deep SC neurons led to the construction of spatial receptive fields (SRFs) for a subset of neurons (see methods section, [Ghose and Wallace 2014] for details). These SRFs highlight the large size and complexity of RFs for SC neurons, and also illustrate a striking heterogeneity of DOI effects. SRFs were similar in size between deep SC neurons in the same neuronal category (i.e. visual, auditory or audiovisual multisensory neurons), and of the SRFs constructed, visual RFs tended to be smaller than auditory or audiovisual RFs. The large majority of the subset of neurons studied systematically in this manner (10/12) showed changes in RF architecture upon DOI administration, and these changes were often not symmetrical across the entire RF or comparable between unisensory and audiovisual RFs. Figure 5 depicts several examples that illustrate the heterogeneity of DOI effects. For the visual neuron shown in Figure 5A, the administration of DOI expanded both the visual and audiovisual RFs of this neuron. The changes induced by DOI administration can be best seen in the contrast plots shown in Figure 5B and which compare the pre-, peri- and post-DOI RFs. This expansion of the RFs trends back toward baseline after the cessation of agonist action (Figure 5A and B, *bottom*). In contrast, the administration of DOI to the auditory

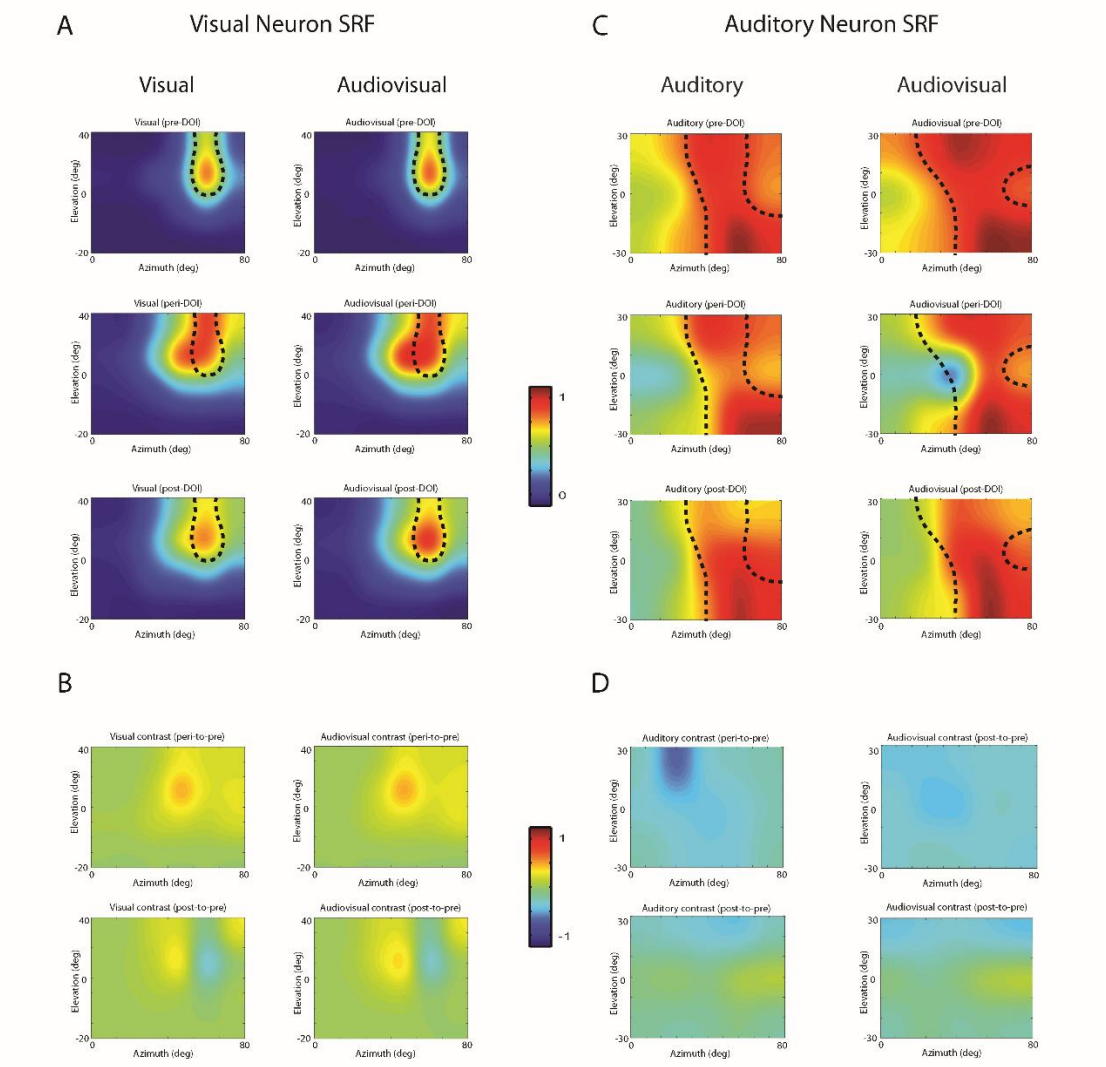


Figure 3-5. DOI changes SRF of two deep SC multisensory neurons. (A) Pseudo color spatial receptive field (SRF) plots for a visual neuron representing the unisensory (visual, left) and multisensory (right) evoked responses normalized across all locations and all conditions to produce a range from 0 to 1, where 0 represents no response and 1 represents maximum response. Greater responses are depicted as warmer colors and smaller responses are depicted as cooler colors. Dotted outlines on each plot indicate the RF shape pre-DOI (*top*). (*middle*) Addition of DOI (peri) increases both overall SRF size and relative magnitude of response in both the visual (left) and audiovisual (right) stimulus conditions. (*bottom*) The SRF trends back to baseline once

DOI action has ceased (post). (B) Contrast plots showing the difference between peri and pre-DOI application (*top*) and post and pre-DOI application (*bottom*) for both stimulus conditions (visual only, audiovisual). Warmer colors represent more response in the peri or post-DOI condition compared to the baseline condition. From the plots, there was more and wider responsivity in the peri-DOI condition, which trended to return back to baseline in the post-DOI condition. (C) SRF plots for an auditory neuron. Color representations and scales are the same as in (A). (*middle*) Addition of DOI (peri) decreases both overall SRF size and relative magnitude of response in both the auditory (left) and audiovisual (right) stimulus conditions. (*bottom*) The SRF trends to return back to baseline once DOI action has ceased (post). (D) Contrast plots showing the difference between peri and pre-DOI application (*top*) and post and pre-DOI application (*bottom*) for both stimulus conditions (auditory only, audiovisual). Color representations and scales are the same as in (B).

neuron shown in Figure 5C resulted in a narrowing of the RFs, which was most evident for the audiovisual conditions. Again, upon recovery, the RF structure returned to resemble the pre-administration condition (Figure 5C and D, *bottom*). Further illustrating the heterogeneity of DOI effects, the example audiovisual neuron shown in Figure 6 shows a differential action of DOI on visual vs. audiovisual RFs (Figure 6A). In this neuron, while the administration of DOI expands the visual RF, it narrows the multisensory RF. Both unisensory and multisensory RFs return to pre-DOI-like structure upon cessation of DOI action (Figure 6 A and B, *bottom*).

Injections of DOI had little effect on local field potentials

While DOI had significant effects on the responses of intermediate and deep SC neurons as measured at the level of action potentials, there were only small changes observable at the level of the local field potential (LFP) (Figure 7). A 3 X 2 ANOVA revealed no main effect of neuronal type or DOI administration on evoked LFP or area under the curve (AUC). A separate ANOVA did reveal a main effect of neuronal type and DOI injection on peak LFP, but no interaction effect (Table 1). Significant increases in peak LFP were seen in visual neurons during DOI administration at both central and peripheral locations. For these neurons, at central RF locations, LFP peak responses to visual stimuli increased with DOI administration by 0.325 ± 0.168 μV ($t(15)=3.39$, $p=0.0374$) (Figure 7B). However, these increases did not return to baseline 3.5-4 hours following DOI administration. For these same neurons, at peripheral RF locations, LFP peak responses to visual stimuli increased by 0.310 ± 0.152 μV ($t(15)=4.51$, $p=0.0135$) (Figure 7C). Again, these effects on peak LFP failed to return to baseline in post-DOI

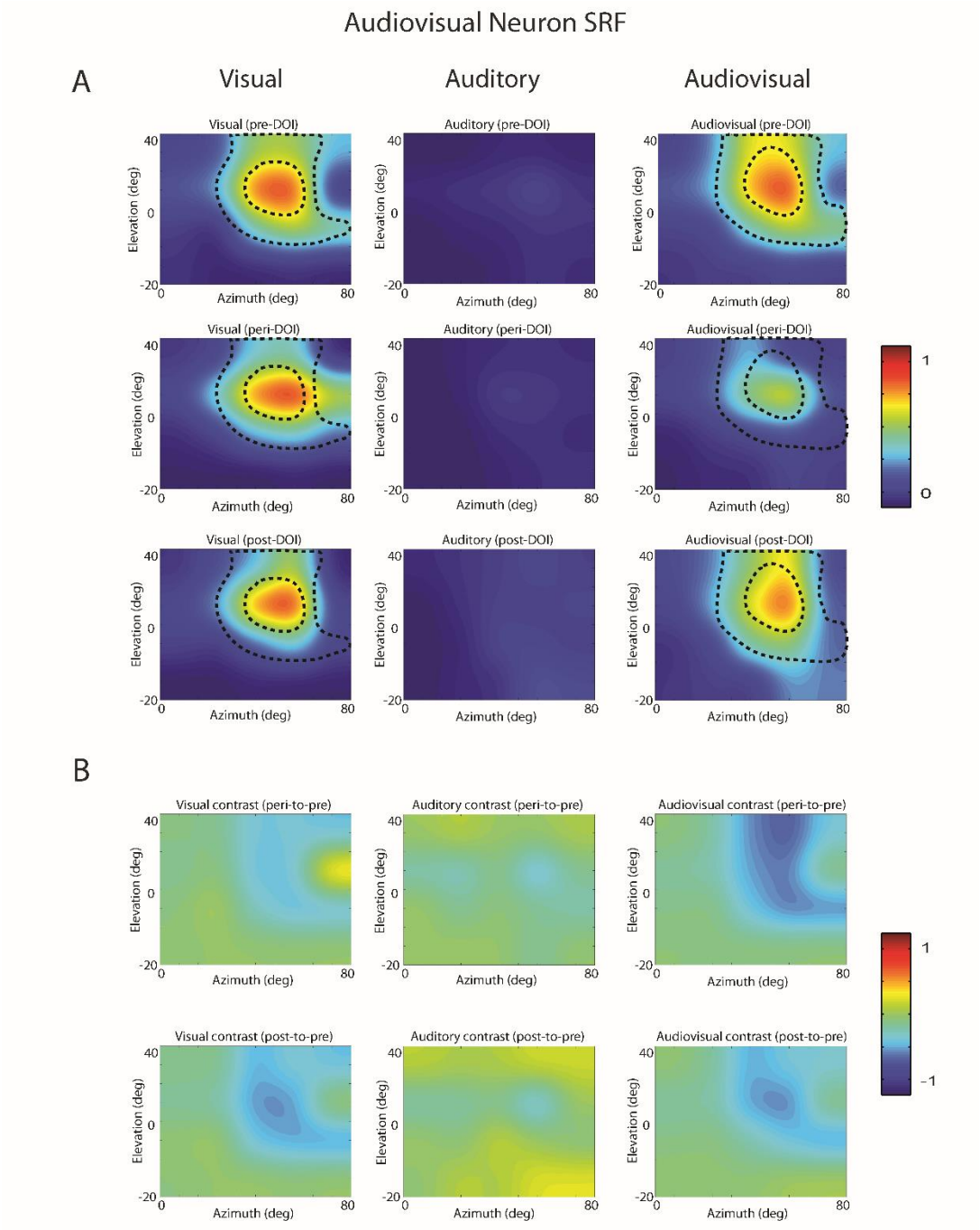


Figure 3-6. DOI changes SRFs of a deep SC audiovisual multisensory neuron. (A) Pseudo color spatial receptive field (SRF) plots representing the unisensory (visual, left; auditory, middle) and multisensory (right) evoked responses of an audiovisual neuron normalized across all locations and all conditions to produce a range from 0 to 1, where 0 represents no response and 1 represents maximum response. This audiovisual

neuron showed greater responses to visual as opposed to auditory unisensory stimulus presentations, hence the cooler colors in the auditory SRF plots. Greater responses are depicted as warmer colors and lesser responses are depicted as cooler colors. Dotted outlines on each plot indicate the RF shape pre-DOI. (*middle*) Addition of DOI (peri) increases both overall SRF size and relative magnitude of response in the unisensory stimulus conditions, but a decrease in overall SRF size and response magnitude in the audiovisual (right) stimulus condition. (*bottom*) The SRF trends back to baseline once DOI action has ceased (post). (B) Contrast plots showing the difference between peri and pre-DOI application (top) and post and pre-DOI application (bottom) for both stimulus conditions (visual only, audiovisual). Warmer colors represent more response in the peri or post-DOI condition compared to baseline pre conditions. These contrasts indicate less responsivity in the peri-DOI condition, which did not quite trend to return back to baseline in the post-DOI condition.

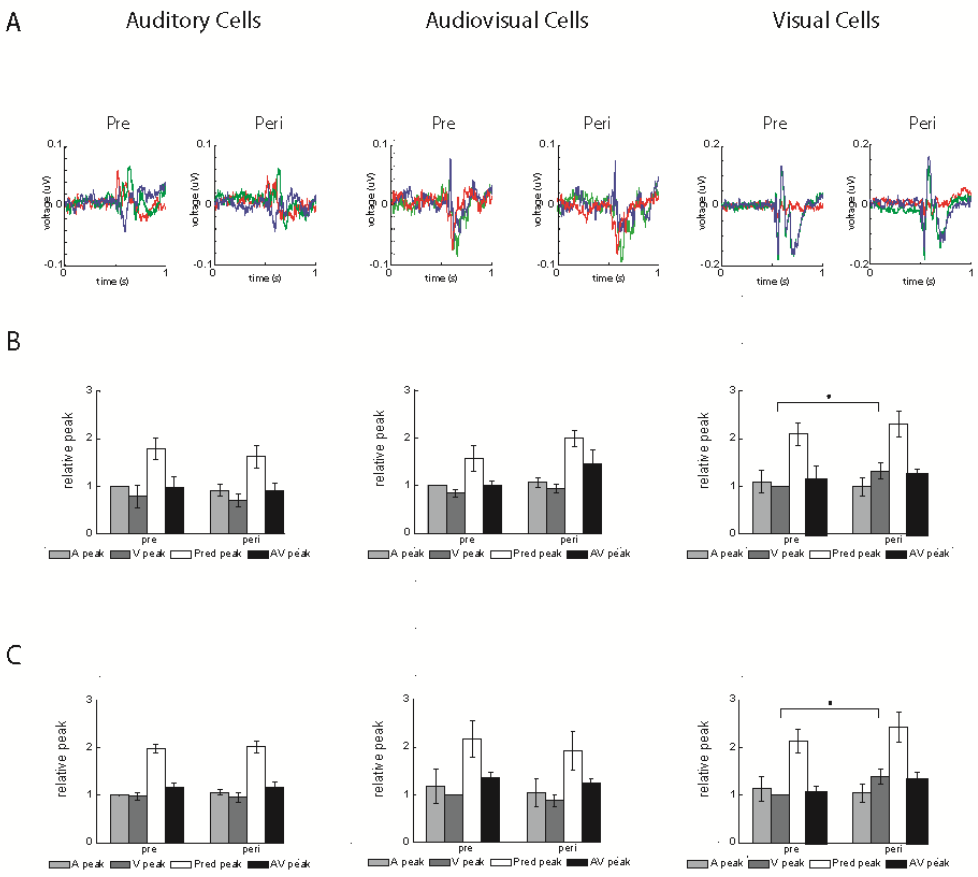


Figure 3-7. DOI application evokes minimal changes on local field potential signals. (A) LFP traces from an example visual, audiovisual and auditory neuron showing signal responses to auditory (red), visual (blue) and audiovisual (green) stimulus presentations. (B) Relative LFP peak responses of auditory (left), audiovisual (middle), and visual (right) SC neurons to unisensory and multisensory stimulus presentations before (pre) and 10 minutes following (peri) DOI administration. White bars indicate the predicted peak LFP as determined by the sum of the unisensory peaks (pred peak). Relative local field potential (LFP) peak for auditory (left), audiovisual (middle) and visual (right) neuronal types at central RF locations. Addition of DOI increased visual peaks in visual neurons. (C) At peripheral RF locations, visual peaks were increased in visual neurons.

measurements. Additionally, control experiments injecting aCSF resulted in no significant change in LFP signal.

DOI administration affects multisensory integration in a laminar specific manner

In a final analysis, the impact of DOI administration was evaluated based on the depth of the recorded neurons in an effort to see whether serotonergic influences on sensory and multisensory function may have a laminar specificity. When plotting neuronal depth relative to the top of the SC, neurons located more superficially (i.e. likely within the intermediate layers) appeared much more likely to exhibit an increase in unisensory (Figure 8A) and audiovisual (Figure 8B) responses with DOI administration. Additionally, neurons located more superficially were more likely to show an effect of DOI administration on multisensory integration as indexed by the interactive index (Figure 8C). Neurons that exhibited an increase in responsivity and/or ii with the administration of DOI (*filled circles*) were more often encountered in the intermediate layers as compared to cells that exhibited a decrease in ii (*open circles*) or no change in ii (*grey circles*) with DOI administration (Figure 8).

Discussion

To our knowledge, the findings described here are the first to provide evidence of serotonergic system involvement in visual, auditory and audiovisual processing occurring within the intermediate and deep layers of the SC. These results have important functional implications for our understanding of SC function.

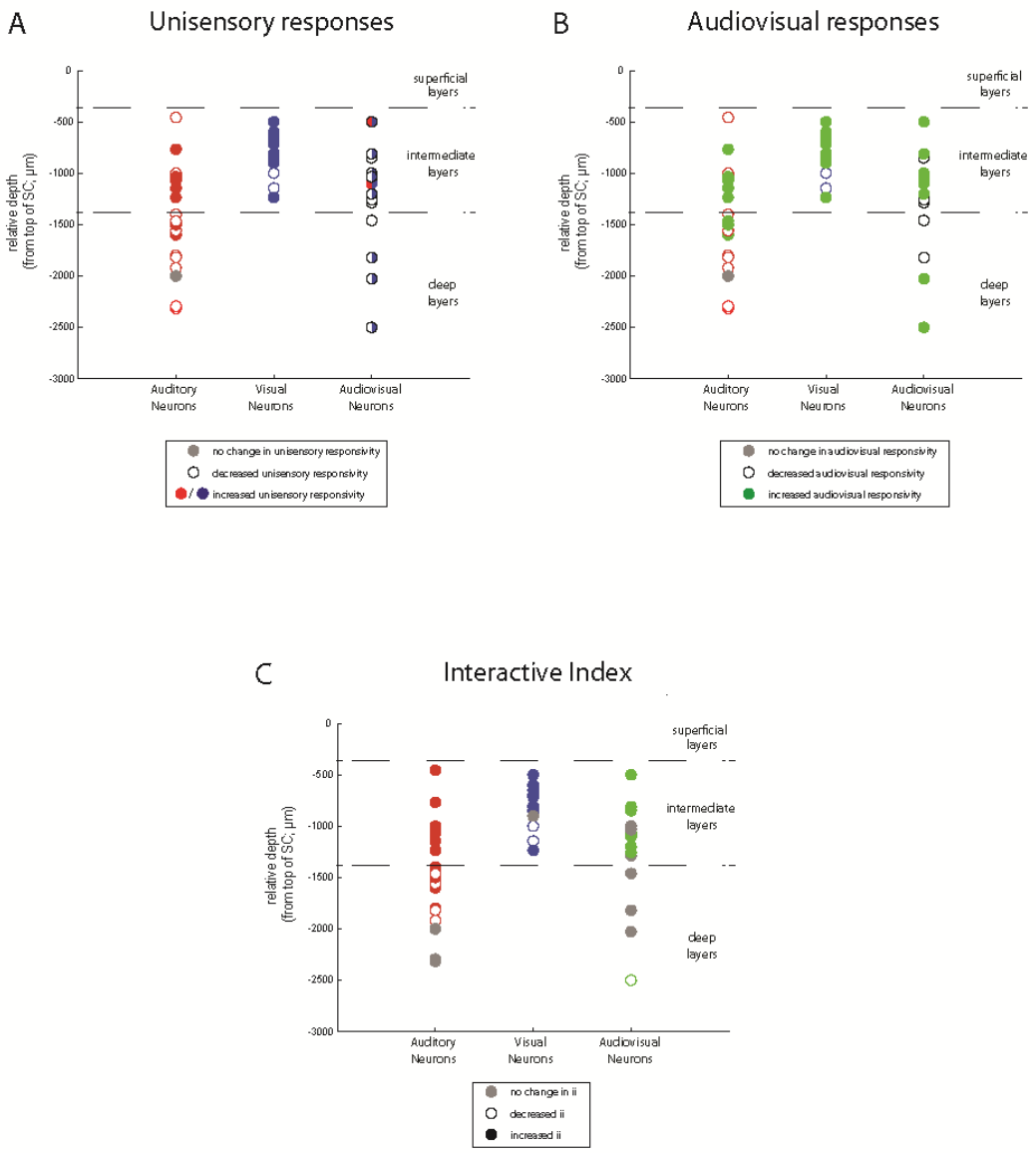


Figure 3-8. Depths of single unit recordings of SC multisensory neurons relative to the top of the SC. Auditory (left), visual (middle) and audiovisual (right) multisensory SC neurons were recorded throughout the intermediate and deep SC layers. (A) Auditory (red) and visual (blue) unisensory responses of each neuron. Filled circles indicate an increase in unisensory responsivity with the addition of DOI, and open circles indicate a decrease of unisensory responsivity. In the case of some audiovisual neurons, responsivity to one modality increased with DOI while the other did not. This is

shown with a half-filled circle, the color of which corresponds to the modality increased by DOI addition. Neurons located more superficially (i.e. likely in the intermediate SC layers) were more likely to show increases in unisensory responsivity with DOI administration. (B) Multisensory responsivity of the neurons in relation to depth of recording. Filled (green) circles indicate an increase in audiovisual responses with DOI addition while open circles indicate a decrease or no change in responses. Neurons located within more intermediate, as opposed to deep, SC layers more often showed increases in audiovisual response with DOI addition. (C) Multisensory gain of response (ii) for each neuron recorded. Grey circles indicate the locations of neurons which did not exhibit a change in ii with the addition of DOI. Filled circles indicate neurons which exhibited a response enhancement and open circles a response depression with DOI injection. Neurons located more superficially more often showed an increase in ii as compared to neurons located in more deep SC layers.

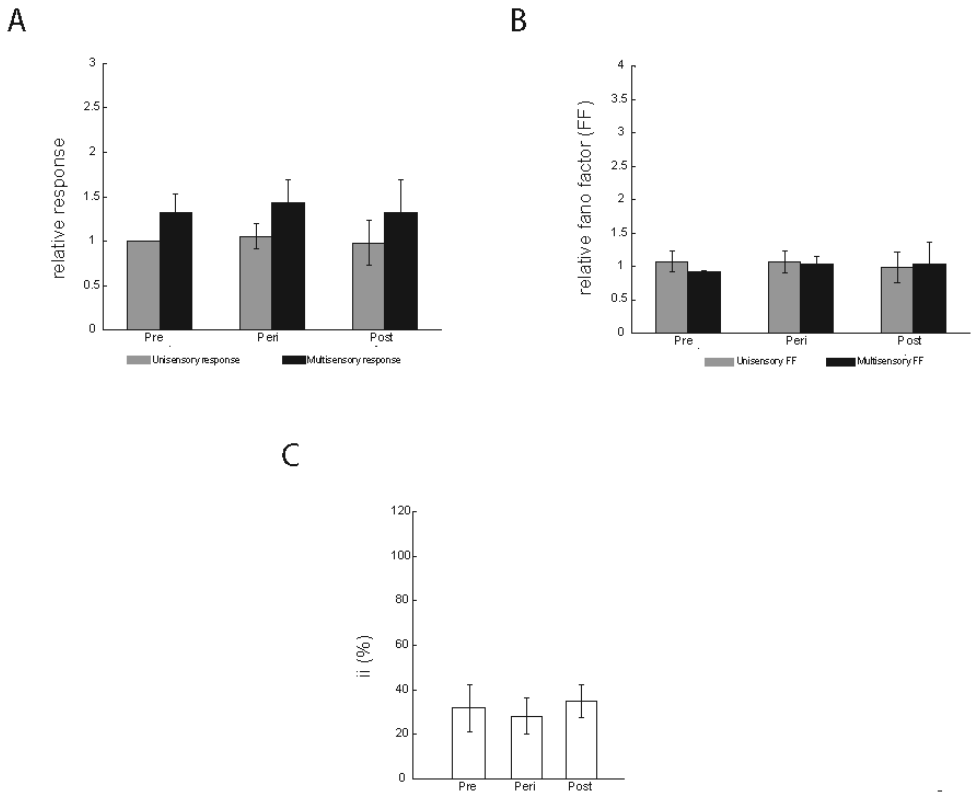


Figure 3-9. Injection of aCSF does not alter responses of multisensory SC neurons. Relative responses (A), fano factor (B) and interactive index (C) of auditory, visual and audiovisual SC neurons before (pre), 10 minutes following (peri) and 3.5 hours following (post) aCSF injection. Neuronal subtypes are collapsed as there was no effect of neuron type on responses to aCSF injection. Responses were normalized to pre-aCSF responses to compare populations of neurons. (A) Addition of aCSF did not significantly change responses to unisensory (gray) or multisensory (black) stimulus presentations. (B) Addition of aCSF did not alter fano factor (FF) of neuronal responses or (C) interactive index.

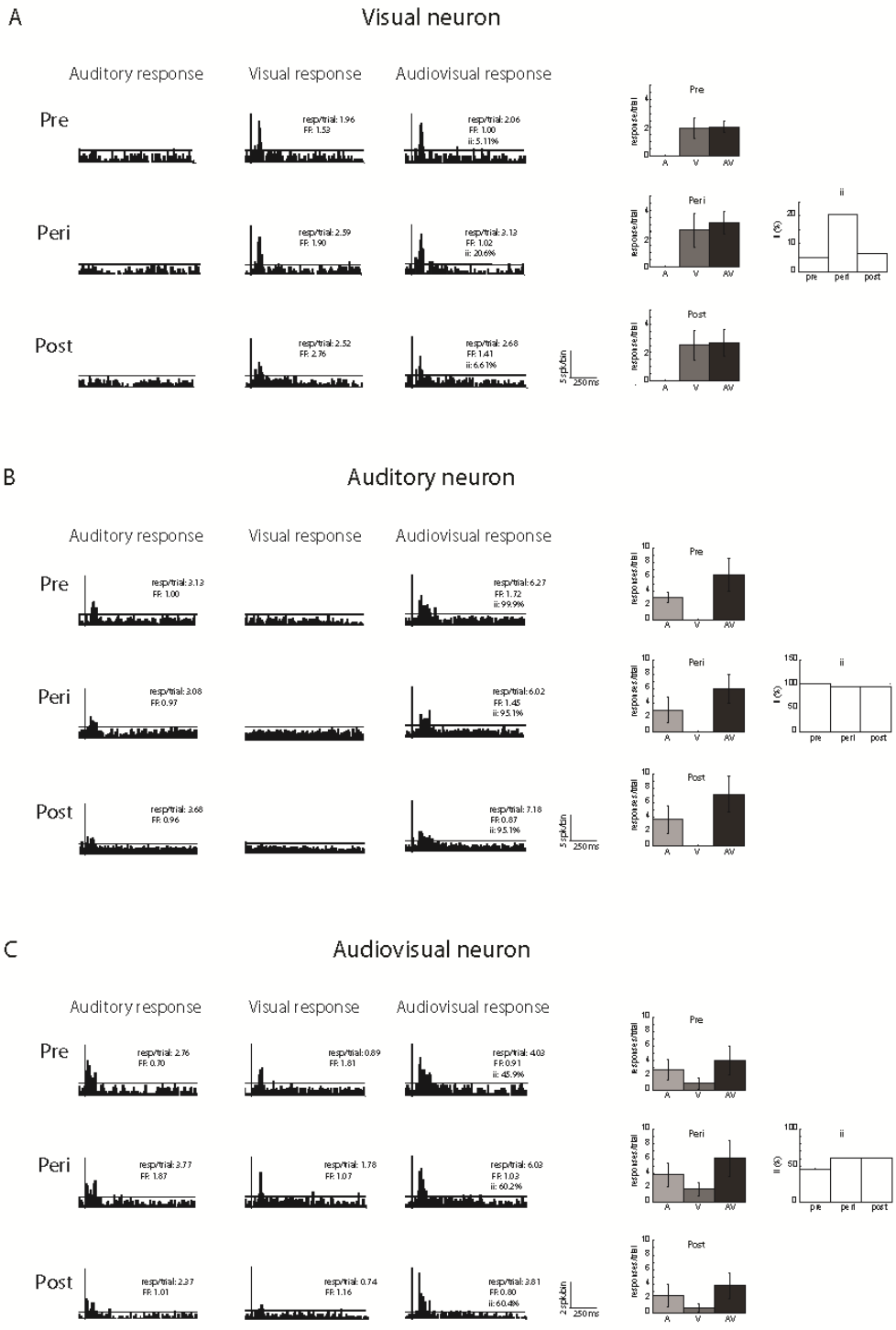


Figure 3-S1. Example responses of visual, auditory and audiovisual SC multisensory neurons. Peristimulus-time histograms (PSTHs) from an example visual

(A), auditory (B) and audiovisual (C) multisensory neuron showing responsiveness to sensory stimulus presentations before (pre), 10 minutes following (peri) and 3.5 hours following (post) DOI injection. Bar graphs to the right are plotting response per trial (resp/trial) and integrative index (ii). The administration of DOI induces an increase in both resp/trial and ii in all three neuronal types.

Neuronal responses and multisensory interactions tend to be enhanced during 5-HT2a receptor agonism

We found a general pattern of increases in response to unisensory and multisensory stimuli with the addition of the 5HT2a receptor agonist DOI. This evidence is in line with previous studies in other brain regions which have shown substantial effects of DOI administration on sensory responses (Wright et al. 1990; Hurley 2006) as well as a general serotonergic modulation of sensory responsiveness (Dahlstrom and Fuxe 1964; Ueda et al. 1985; Waterhouse et al. 1986; Villar et al. 1988; Rhoades et al. 1990; Arce et al. 1992; Huang et al. 1993; Janusonis et al. 1999; Lottem et al. 2016). In most instances, the changes of responsiveness seen following DOI administration were no longer observed 3-4 hours after agonist injection. Nonetheless, in a number of instances, recovery was incomplete. The absence of complete recovery in some neurons may be a result of the lingering action of DOI, a result consistent with prior work in other brain regions including the frontal cortex (Wright et al. 1990).

Along with a generalized increase in sensory responsiveness, DOI administration often also resulted in an increase in multisensory gain. This is a paradoxical finding in light of the principle of inverse effectiveness, which predicts an inverse relationship between strength of unisensory responses and magnitude of multisensory interactions (Meredith and Stein 1986; Stein et al. 1993; Meredith and Stein 1996; Perrault et al. 2003; Stanford et al. 2005). The fact that multisensory interactions tended to grow larger under circumstances of increased unisensory responses suggests an active and selective role for 5-HT in gating the multisensory responses of these neurons. Although future work is needed in order to elucidate the specific mechanisms of such effects, one

possibility is that the neuromodulatory inputs provided by 5-HT change the temporal filters within which SC neurons can combine their different sensory inputs, thus expanding the integrative capacity of these neurons. Indeed, as described below, the evidence for 5-HT's impact on the spatial filters (i.e., receptive fields) of these neurons is concordant with the possibility of a generalized effect on neuronal filter functions and selectivity. Consistent with a temporally-based mechanism of effect, prior work has shown that activation of the 5-HT system via agonism of 5HT1a receptors alters the temporal characteristics of auditory neurons in the inferior colliculus (Hurley and Pollak 2005a, 2005b; Hurley 2006, 2007), and other work has suggested that temporal filters can be changed through the manipulation of the norepinephrine (Kossl and Vater 1989) and GABA (Park and Pollak 1993) systems. Future work in which the temporal structure of multisensory inputs are manipulated should provide important insight into this possibility and the potential mechanism by which 5-HT modulates multisensory function in these neurons. Finally, the paradoxical increase in multisensory gain with increases in responsivity was selective for neurons characterized as unisensory in the current context, as they lacked overt responses to both sensory modalities. Such a result is intriguing and suggests that the 5HT2a system may exert preferential effects on the integrative capacity of neurons with a driving input from one sensory modality and a modulatory input from another, perhaps because the 5HT2a receptor system is more targeted to the modulatory input as opposed to the driving input.

A serotonergic selectivity for visual responses in the SC?

In general, visual cells and visual responses were more sensitive to DOI administration. Such a finding is consistent with previous anatomical work highlighting

the heaviest serotonergic innervation to the SC terminating in the superficial and intermediate layers (Ueda et al. 1985; Mize and Horner 1989). Indeed, as much of the visual input to the SC targets the superficial (Graybiel 1975; May 2006) and intermediate (Baleydier et al. 1983; Huerta and Harting 1983) layers, with very little direct input into the deep layers (Beckstead and Frankfurter 1983), it is not surprising that visually-responsive neurons were biased to more superficial locations in the current recordings. In contrast, auditory inputs from areas such as the inferior colliculus and nucleus of the lateral lemniscus tend to target regions deeper than these visual inputs (Moore and Goldberg 1966; Kudo and Niimi 1980; Kudo 1981; Henkel 1983). Hence, the bias toward visual neurons and visual responses in the current study may reflect a preferential role for 5-HT (or more selectively the 5-HT2a system) in modulating visual responses. Other 5-HT receptor systems found in the intermediate and deep SC (e.g., 5-HT2c) may play a role in gating the responses to other modalities (e.g., audition). Future work is needed to better elucidate the full spectrum of serotonergic receptor systems in the deeper layers of the SC.

Response reliability generally declines under DOI action

We found that DOI injections generally increased fano factor (FF) – a measure of response reliability or variability. Firing rate plays a critical role in the calculation of FF, thus it is no surprise that the increase in sensory responsiveness we encountered is accompanied by an increase in FF. It has previously been shown that at higher firing rates, responses are usually less reliable (Tolhurst et al. 1983; Softky and Koch 1993; Holt et al. 1996; Kara et al. 2000; Carandini 2004; Gur and Snodderly 2006; Sarko et al. 2013). This was supported in our results, as well; the addition of DOI generally

increased neuronal responses to sensory stimuli and also increased FF. However, and perhaps more importantly, changes in FF (i.e., ΔFF) were seen during addition of DOI that altered the FF of visual and audiovisual cells from negative to positive values, indicating a selective shift in response reliability during serotonin receptor agonism. Such a result is quite intriguing, as it suggests that 5-HT changes the relative weighting of sensory evidence. This finding has important implications in the context of the growing body of work examining cue reliability under multisensory conditions, and which has shown that less reliable sensory cues are weighted less heavily in perceptual estimates that combine information across the different senses (Morgan et al. 2008; Ohshiro et al. 2011; Fetsch et al. 2013). Collectively, these results suggest that the 5-HT system may play a highly dynamic role in the weighing of sensory inputs and evidence within the SC.

While the general pattern followed that of decreased response reliability with the addition of DOI, in a few instances, particularly involving audiovisual neurons, we saw the opposite pattern. With the addition of DOI, audiovisual FF decreased in audiovisual neurons. This pattern remained when examining central and peripheral RF locations separately. This finding is intriguing, as it contradicts the more prominent pattern in our results. This may possibly indicate the presence and activity of different receptor subtypes in the case of audiovisual neurons' response to audiovisual stimulus presentations. It is important to note that the neurons defined as audiovisual neurons in this study are those that are overtly responsive to both auditory and visual stimuli. This is in contrast to so-called 'modulatory' multisensory neurons, which are overtly responsive to only a single sensory modality, but whose responses are modulated by

the second modality. It is very plausible that the mechanisms underlying the multisensory responses (including the influences of 5-HT signaling) of these two neuronal types differ. The signaling cascade activated by 5-HT2a receptor stimulation results in changes in protein kinase C activity and intracellular calcium release (Barnes and Sharp 1999). These molecules are widely utilized to stimulate, suppress and modulate a variety of receptor subtypes in the brain. The complete molecular makeup of these multisensory neurons is unknown, as is the differences in receptor types between different types of these neurons (i.e. visual, auditory, audiovisual multisensory neurons). It is possible that the result of decreased FF in audiovisual neurons to audiovisual stimuli with DOI administration is due to the activation of a different receptor subtype via effects downstream of 5-HT 2a receptors. Detailed studies to elucidate the receptors found on these cells, and their activations, are essential in order to understand this phenomenon.

The serotonergic system modulates the size and shape of SC sensory RFs

The current work suggests that serotonin receptor agonist-induced changes in sensory and multisensory responsiveness, FF and multisensory integration (ii) appear to be more impactful at peripheral regions of neuron's receptive fields. The spatial-specificity of these effects is in line with previous work implicating the 5-HT system in sensory receptive field and sensory map development (Lauder et al. 1982; Mize and Horner 1989; Rhoades et al. 1990; Ke et al. 1999). Within the SC, manipulations of the serotonergic system elevate 5-HT levels and reduce postnatal refinement of receptive fields during development. This lack of refinement is primarily limited to changes seen at peripheral locations of the RFs. These effects seen at the peripheral RF locations could

be a result of changes in local circuits involved in RF maintenance and/or alterations in inputs to the SC. Indeed, retinotectal inputs to the superficial layers of the SC have been shown to be imperative for proper RF maturity, and alterations of the 5-HT system during development prevent this refinement process (Ke et al. 1999). Removal of one eye either after birth or in adulthood results in a marked reorganization of serotonergic innervation of the SC (Rhoades et al. 1990), which may change RF architecture. While the receptor makeup of each type of SC neuron remains unknown, DOI administration as applied in this study may be affecting both local circuitries within the deep SC while also influencing the inputs to these local circuits. Future studies exploring the molecular configuration of the SC and its specific inputs are required in order to delineate these possibilities.

Although the effects of DOI administration were, on average, greater for peripheral versus central RF locations, it must be emphasized that these effects were highly heterogeneous from neuron-to-neuron and for unisensory compared to multisensory RF representation, suggesting a complex mechanism through which 5-HT shapes RF architecture. In the context of development, it has been proposed that alterations in RF architecture may be related to the attenuation of neuronal activity caused by long-term elevation of 5-HT levels which prevents refinement of RFs (Ke et al. 1999). In adulthood, the 5-HT system has been shown to maintain this ability to modulate cell tuning and RF architecture in the inferior colliculus (Hurley and Pollak 1999, 2001; Hurley 2006). Our current results add to the body of evidence that show an important role for 5-HT signaling in the maintenance of RF structure. Indeed, one important role for 5-HT suggested by the current work is in the dynamic modulation of

RF structure, and in which the serotonergic system can provide on-line adjustments to the spatial filters and selectivity of individual SC neurons.

Introduction of DOI does not drastically alter LFP signals

Previous work has shown that the neural signatures of multisensory interactions exhibited at the spiking level in the SC are also evident in the LFP signals (Ghose et al. 2014). Surprisingly, in the current study, little changes in the LFP signal were evident under DOI conditions. Several factors could be responsible for this dissociation between spiking effects and LFP effects. First, in contrast to the extracellularly-recorded spikes that come from the neuron under study, the LFP signal reflects the synaptic activity coming from a large population of neurons (Mitzdorf 1985; Kayser and Logothetis 2007), with estimates that elements several mm from the electrode tip contribute to the summed signal (Kajikawa and Schroeder 2011; Sarko et al. 2013). Indeed, prior work has shown that spikes and LFPs, although often well correlated, can be decoupled from one another because of differences in the nature of the two signals (Maier et al. 2008; Rasch et al. 2008). Thus, one plausible possibility for the current work is that DOI influences were sufficiently local to impact spiking and not the largest component of the LFP signals. A second possibility for the observed differences between spikes and LFPs is the cell-type specificity of the DOI influences. Thus, in our current analysis, which focused on the modality identity of the neurons under study (i.e., visual alone, auditory alone, visual-auditory), we saw differential effects on spiking of DOI administration based on modality, with visual neurons being most impacted. Such cell-type specificity to the effects would dilute the overall impact of DOI when measured at larger scales of analysis (i.e. LFPs). Extending this further, it is reasonable to speculate

that the various sensory neuron-types could have different complements of 5-HT receptors. While, to our knowledge, work has not examined 5-HT receptor distribution in the SC at the level of individual neurons, studies have shown that 5-HT1a, 5-HT1b (Binns 1999), 5-HT2a and 5-HT2c (Pompeiano et al. 1994) receptors are present in the intermediate and deep SC layers. Additionally, whereas 5-HT 1a and 1b receptors have been implicated in auditory processing (Hurley 2007; Hurley et al. 2008; Ramsey et al. 2010), 5-HT2a and 2c receptors tend to be found in areas relating to visual processing (Li et al. 2004). It is possible that this receptor-type specificity for sensory processes is retained in the SC, with 1-type receptors acting on auditory processes while 2-type receptors acting on visual processing (see discussion above). These modality differences could relate to the differing modes of action of the various receptor types; whereas 5-HT1 receptor activation leads to a signaling cascade resulting in a decrease in intracellular calcium and hyperpolarization of neurons, 5-HT2 receptor activation results in neuronal depolarization (Barnes and Sharp 1999). These mechanisms of action may be related to the specific developmental and refinement processes of auditory and visual neuron receptive field and tuning. Future work is necessary in order to more thoroughly examine this possibility.

Caveats of the current study

One important caveat of this work is the potential effect of anesthesia. While we acknowledge this concern, we argue that anesthetized recordings are the first step in establishing a role of the serotonergic system in multisensory processing. Anesthesia is necessary here because of the need to record from single units over many hours and conditions (pre, peri, post-DOI injection) in order to get a first order view into

serotonergic influences. Previous work has shown marked commonalities in multisensory response characteristics between anesthetized and awake recordings in the SC (Wallace et al. 1998), thus the use of anesthesia should not invalidate the current findings.

Another possible concern is the use of pressure-injection for the delivery of DOI. While a relatively coarse technique, this approach represents an effective first step in understanding the role of the serotonin system in SC multisensory processing. This method has been used in similar experimental designs and has yielded stable and consistent results (Deschênes and Hu 1990; Malpeli 1999; Jen et al. 2001; Chen et al. 2012). Control experiments injecting aCSF, which resulted in little changes in cell responsivity or integrative capacity (Figure 9), argue against a major concern regarding this method. Nonetheless, future work should employ methods that allow for more spatial and temporal control of the injections.

Future directions and implications for these study findings

Multisensory integration is an essential process for proper orientation and interaction with the world. The SC is an important brain area for this process (Meredith and Stein 1983, 1986, 1996; Munoz and Guitton 1985; Stein 1986; Meredith et al. 1987; Munoz and Guitton 1989; Perrault et al. 2003; Perrault et al. 2005). Understanding the molecular makeup of the neurons involved in, as well as the molecular systems that interact with and influence this process is essential in determining the details of how multisensory integration normally occurs. The finding that the 5-HT system modulates multisensory processing in the SC may pave the way for future studies examining the

behavioral consequences of perturbing serotonergic signaling. This is also a first step that may lead to the determination of the types of receptors likely found on these multisensory neurons, and if/how the receptor types on multisensory neurons differ from those found on unisensory cells. This study examines only one receptor type of one neuromodulatory system and its effects on sensory processing. To solidify the involvement of this receptor type in multisensory processing, studies utilizing a selective 5-HT 2a receptor antagonist, such as M100907, would be necessary (Marek et al. 1994). To get a more complete picture of the molecular mechanisms that support multisensory integration, studies examining interactions between the serotonin and other modulatory systems present in the SC, such as the noradrenergic (Binns 1999; Tan et al. 1999; Bezdudnaya and Castro-Alamancos 2014), GABAergic (Gogolla et al. 2014), and cholinergic (Binns 1999; Wang et al. 2009; Stubblefield et al. 2015) systems, is also necessary. For example, it has previously been shown that early pharmacological manipulation of the GABA neurotransmitter system can rescue a multisensory integration deficit found in a mouse model of idiopathic autism spectrum disorder (Gogolla et al 2014). To expand upon this work as well as the current study, investigation into the interactions between GABA and 5-HT systems and their influence on multisensory processing will help to paint a clearer picture of this system under normal circumstances. It is only then that we may begin to understand how this system can be perturbed in instances of neurological disorder such as autism spectrum disorder (Foss-Feig et al. 2010; Russo et al. 2010; Azmitia et al. 2011; Kwakye et al. 2011; Hammock et al. 2012; Ruggeri et al. 2013).

References Cited

Allman, B. L., Bittencourt-Navarrete, R.E., Keniston, L.P., Medina, A.E., Wang, M.Y. and Meredith, M.A. (2008). Do cross-modal projections always result in multisensory integration? *Cereb Cortex* **18**, 2066-2076.

Amlot, R., Walker, R., Driver, J. and Spence, C. (2003). Multimodal visual-somatosensory integration in saccade generation. *Neuropsychologia* **41,** 1-15.

Arce, E. A., Bennett-Clarke, C.A., Mooney, R.D. and Rhoades, R.W. (1992). Synaptic organization of the serotoninergic input to the superficial gray layer of the hamster's superior colliculus. *Synapse* **11,** 67-75.

Azmitia, E. C., Singh, J. S. and Whitaker-Azmitia, P. M. (2011). Increased serotonin axons (immunoreactive 5-HT transporter) in postmortem brains from young autism donors. *Neuropharmacology* **60**, 1347-1354.

Baleydier, C., Kahungu, M. and Mauguiere, F. (1983). A crossed corticotectal projection from the lateral suprasylvian area in the cat. *J Comp Neurol* **214,** 344-351.

Barnes, N. M. and Sharp, T. (1999). A review of central 5-HT receptors and their function. *Neuropharmacology* **38**, 1083-1152.

Beckstead, R. M., Edwards, S. B. and Frankfurter, A. (1981). A comparison of the intranigral distribution of nigrotectal neurons labeled with horseradish peroxidase in the monkey, cat, and rat. *J Neurosci* **1**, 121-125.

Bezdudnaya, T. and Castro-Alamancos, M. A. (2014). Neuromodulation of whisking related neural activity in superior colliculus. *J Neurosci* **34**, 7683-7695.

Binns, K. E. (1999). The synaptic pharmacology underlying sensory processing in the superior colliculus. *Progress in Neurobiology* **59**, 129-159.

Carandini, M. (2004). Amplification for trial-to-trial response variability by neurons in visual cortex. *PloS Biol* **2**, 24.

Carriere, B. N., Royal, D. W. and Wallace, M. T. (2008). Spatial Heterogeneity of Cortical Receptive Fields and Its Impact on Multisensory Interactions. *Journal of Neurophysiology* **99**, 2357-2368.

Chen, X., Doug, J. and Jiang, Z. Y. (2012). Nesfatin-1 influences the excitability of glocosensing neurons in the hypothalamic nuclei and inhibits the food intake. *Regul Pept* **177**, 21-26.

Corneil, B. D., Van Wanrooij, M., Munoz, D. P. and Van Opstal, A. J. (2002). Auditory-Visual Interactions Subserving Goal-Directed Saccades in a Complex Scene. *Journal of Neurophysiology* **88**, 438-454.

Dahlstrom, A. and Fuxe, K. (1964). Localization of monoamines in the lower brain stem. *Experientia* **20**, 398-399.

Dechenes, M. and Hu, B. (1990). Electrophysiology and pharmacology of the corticothalamic input to lateral thalamic nuclei: an intracellular study in the cat. *European J Neuroscience* **2**, 140-152.

Diederich, A., Colonius, H., Bockhorst, D. and Tabeling, S. (2003). Visual-tactile spatial interaction in saccade generation. *Exp Brain Res* **148**, 328-337.

Egan, C., Grinde, E., Dupre, A., Roth, B. L., Hake, M., Teitler, M. and Herrick-Davis, K. (2000). Agonist and low affinity state ratios predict drug intrinsic activity and a revised ternary complex mechanism at serotonin 5-HT(2A) and 5-HT(2C) receptors. *Synapse* **35**, 144-150.

Fano, U. (1947). Ionization yield of radiations. II. The Fluctuations of the number of ions. *Physical Review* **72**, 26-29.

Fetsch, C. R., DeAngelis, G. C. and Angelaki, D. E. (2013). Bridging the gap between theories of sensory cue integration and the physiology of multisensory neurons. *Nat Rev Neurosci* **14**, 429-442.

Foehring, R.C., van Brederode, J. F., Kinney, G. A. and Spain, W. J. (2002). Serotonergic modulation of supragranular neurons in rat sensorimotor cortex. *J Neurosci* **22**, 8238-8250.

Foss-Feig, J. H., Kwakye, L. D., Cascio, C. J., Burnette, C. P., Kadivar, H., Stone, W. L. and Wallace, M. T. (2010). An extended multisensory temporal binding window in autism spectrum disorders. *Exp Brain Res* **203**, 381-389.

Frens, M. A., Van Opstal, A. J. and Van der Willigen, R. F. (1995). Spatial and temporal factors determine auditory-visual interactions in human saccadic eye movements. *Percept Psychophys* **57**, 802-816.

Fuentes-Santamaria, V., Alvarado, J. C., Stein, B. E. and McHaffie, J. G. (2008). Cortex contacts both output neurons and nitrergic interneurons in the superior colliculus: direct and indirect routes for multisensory integration. *Cereb Cortex* **18**, 1640-1652.

Ghose, D., Barnett, Z. P. and Wallace, M. T. (2012). Impact of response duration on multisensory integration. *J Neurophysiol* **108**, 2534-2544.

Ghose, D., Maier, A., Nidiffer, A. and Wallace, M. T. (2014). Multisensory response modulation in the superficial layers of the superior colliculus. *J Neurosci* **34**, 4332-4344.

Ghose, D. and Wallace, M. T. (2014). Heterogeneity in the spatial receptive field architecture of multisensory neurons of the superior colliculus and its effects on multisensory integration. *Neuroscience* **256**, 147-162.

Gogolla, N., Takesian, A. E., Feng, G., Fogiolini, M. and Hensch, T. K. (2014). Sensory integration in mouse insular cortex reflects GABA circuit maturation. *Neuron* **83**, 894-905.

Graybiel, A. M. (1975). Anatomical organization of retinotectal afferents in the cat: an autoradiographic study. *Brain Res* **96**, 1-23.

Gu, Q. and Singer, W. (1995). Involvement of serotonin in developmental plasticity of kitten visual cortex. *Eur J Neurosci* **7**, 1146-1153.

Gur, M. and Snodderly, D. M. (2006). High response reliability of neurons in primary visual cortex (V1) of alert, trained monkeys. *Cereb Cortex* **16**, 888-895.

Hammock, E., Veenstra-VanderWeele, J., Yan, Z., Kerr, T. M., Morris, M., Anderson, G. M., Cater, C. S., Cook, E. H. and Jacob, S. (2012). Examining autism spectrum disorders by biomarkers: example from the oxytocin and serotonin systems. *J Am Acad Child Adolesc Psychiatry* **51**, 712-721.

Hay-Schmidt, A. (2000). The evolution of the serotonergic nervous system. *Proc Biol Sci* **267**, 1071-1079.

Henkel, C. K. (1983). Evidence of sub-collicular auditory projections to the medial geniculate nucleus in the cat: an autoradiographic and horseradish peroxidase study. *Brain Res* **259**, 21-30.

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

Holt, G. R., Softky, W. R., Koch, C. and Douglass, R. J. (1996). Comparison of discharge in vitro and in vivo in cat visual cortex neurons. *J Neurophysiol* **75**, 1806-1814.

Huang, X., Mooney, R. D. and Rhoades, R. W. (1993). Effects of serotonin on retinotectal-, corticotectal-, and glutamate-induced activity in the superior colliculus of the hamster. *J Neurophysiol* **70**, 723-732.

Huerta, M. F. and Harting, J. K. (1983). Sublamination within the superficial gray layer of the squirrel monkey: an analysis of the tectopulvinar projection using anterograde and retrograde transport methods. *Brain Res* **261**, 119-126.

Hughes, H. C., Nelson, M. D. and Aronchick, D. M. (1998). Spatial characteristics of visual-auditory summation in human saccades. *Vision Res* **38**, 3955-3963.

Hughes, H. C., Reuter-Lorenz, P. A., Nozawa, G. and Fendrich, R. (1994). Visual-auditory interactions in sensorimotor processing: Saccades versus manual responses. *Journal of Experimental Psychology: Human Perception and Performance* **20**, 131-153.

Hurley, L. M. (2006). Different serotonin receptor agonists have distinct effects on sound-evoked responses in inferior colliculus. *J Neurophysiol* **96**, 2177-2188.

Hurley, L. M. (2007). Activation of the serotonin 1A receptor alters the temporal characteristics of auditory responses in the inferior colliculus. *Brain Res* **21**, 21-29.

Hurley, L.M., Devibiss, D.M. and Waterhouse, B. D. (2004). A matter of focus: monoaminergic modulation of stimulus coding in mammalian sensory networks. *Curr Opin Neurobiol* **14**, 488-95.

Hurley, L. M. and Pollak, G. D. (1999). Serotonin differentially modulated responses to tones and frequency-modulated sweeps in the inferior colliculus. *J Neurosci* **19**, 8071-8082.

Hurley, L. M. and Pollak, G. D. (2001). Serotonin effects on frequency tuning of inferior colliculus neurons. *J Neurophysiol* **85**, 828-842.

Hurley, L. M. and Pollak, G. D. (2005). Serotonin modulates responses to species-specific vocalizations in the inferior colliculus. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **191**, 535-546.

Hurley, L. M. and Pollak, G. D. (2005). Serotonin shifts first-spike latencies of inferior colliculus neurons. *J Neurosci* **25**, 7876-7886.

Hurley, L.M., Thompson, A. M. and Pollak, G. D. (2002). Serotonin in the inferior colliculus. *Hear Res* **168**, 1-11.

Hurley, L. M., Tracy, J. A. and Bohorquez, A. (2008). Serotonin 1B receptor modulates frequency response curves and spectral integration in the inferior colliculus by reducing GABAergic inhibition. *J Neurophysiol* **100**, 1656-1667.

Janusonis, S., Fite, K. V. and Foote, W. (1999). Topographic organization of serotonergic dorsal raphe neurons projecting to the superior colliculus in the Mongolian gerbil (Meriones unguiculatus). *J Comp Neurol* **413**, 342-355.

Jen, H. S. P., Sun, X. and Chen, C. Q. (2001). An electrophysiological study of neural pathways for corticofugally inhibited neurons in the central nucleus of the inferior colliculus of the big brown bat, Eptesicus fuscus. *Exp Brain Res* **137**, 292-302.

Jitsuki, S., Takemoto, K. Kawasaki, T., Takahashi, A., Becamel, C., Sano, A., Yuzaki, M., Zukin, R. S., Ziff, E. B., Kessels, H. W. and Takahashi, T. (2011). Serotonin mediates cross-modal reorganization of cortical circuits. *Neuron* **69**, 780-792.

Kajikawa, Y. and Schroeder, C. E. (2011). How local is local field potential? *Neuron* **72**, 847-858.

Kara, P., Reinagel, P. and Reid, R. C. (2000). Low response variability in simultaneously recorded retinal, thalamic, and cortical neurons. *Neuron* **27**, 635-646.

Kayser, C. and Logothetis, N. K. (2007). Do early sensory cortices integrate cross-modal information? *Brain Structure and Function* **121**, 121-132.

Ke, M., Mooney, R. D. and Rhoades, R. W. (1999). Increased serotonin in the developing superior colliculus affects receptive-field size of retinotectal afferents but not that of postsynaptic neurons. *Vis Neurosci* **16**, 121-130.

Kossl, M. and Vater, M. (1989). Noradrenaline enhances temporal auditory contrast and neuronal timing precision in the cochlear nucleus of the mustached bat. *J Neurosci* **9**, 4169-4178.

Kudo, M. (1981). Projections of the nuclei of the lateral lemniscus in the cat: an autoradiographic study. *Brain Res* **221**, 57-69.

Kudo, M. and Niimi, K. (1980). Ascending projections of the inferior colliculus in the cat: an autoradiographic study. *J Comp Neurol* **191**, 545-556.

Kwakye, L. D., Foss-Feig, J. H., Cascio, C. J., Stone, W. L. and Wallace, M. T. (2011). Altered auditory and multisensory temporal processing in autism spectrum disorder. *Front Integr Neurosci* **4**, 1-29.

Lauder, J. M., Wallace, J.A., Krebs, H., Petrusz, P. and McCarthy, K. (1982). In vivo and in vitro development of serotonergic neurons. *Brain Res Bull* **9**, 605-625.

Li, Q. H., Nakadate, K., Tanaka-Nakadate, S., Nakatsuka, D., Cui, Y. and Watanabe, Y. (2004). Unique expression patterns of 5-HT2A and 5-HT2C receptors in the rat brain during postnatal development: western blot and immunohistochemical analyses. *J Comp Neurol* **469**, 128-140.

Lottem E., Lorincz, M. L. and Mainen, Z. F. (2016). Optogenetic activation of dorsal raphe serotonin neurons rapidly inhibits spontaneous but not odor-evoked activity in olfactory cortex. *J Neurosci* **36**, 7-18.

Lovelace, C. T., Stein, B. E. and Wallace, M. T. (2003). An irrelevant light enhances auditory detection in humans: a psychophysical analysis of multisensory integration in stimulus detection. *Cognitive Brain Research* **17**, 447-453.

Lyon, R. A., Davis, K. H. and Titeler, M. (1986). ^{3}H-DOB (4-Bromo-2,5-Dimethoxyphenylisopropylamine) labels a guanyl nucleotide-sensitive state of cortical 5-HT_2 receptors. *Molecular Pharmacology* **31**, 194-199.

Maier, A., Wilke, M. Aura, C., Zhu, C., Ye, F. Q. and Leopold, D. A. (2008). Divergence of fMRI and neural signals in V1 during perceptual suppression in the awake monkey. *Nat Neurosci* **11**, 1193-1200.

Malpeli, J. G. (1999). Reversible inactivation of subcortical sites by drug injection. *J Neurosci Methods* **86**, 119-128.

Marek, G. J. and Aghajanian, G. K. (1994). Excitation of interneurons in piriform cortex by 5-hydroxytryptamine: blockade by MDL 100,907, a highly selective 5-HT2A receptor antagonist. *Eur J Pharmacol* **259**, 137-141.

May, P. J. (2006). The mammalian superior colliculus: laminar structure and connections. *Prog Brain Res* **151**, 321-378.

McKenna, D. J. and Peroutka, S. J. (1989). Differentiation of 5-hydroxytryptamine2 receptor subtypes using 125I-R-(-)2,5-dimethoxy-4-iodo-phenylisopropylamine and 3H-ketanserin. *J Neurosci* **9**, 3482-3490.

Meredith, M., Nemitz, J. W. and Stein, B. E. (1987). Determinants of multisensory integration in superior colliculus neurons. I. Temporal factors. *The Journal of Neuroscience* **7**, 3215-3229.

Meredith, M. A. and Stein, B. E. (1983). Interactions among converging sensory inputs in the superior colliculus. *Science* **221**, 389-391.

Meredith, M. A. and Stein, B. E. (1986). Spatial factors determine the activity of multisensory neurons in cat superior colliculus. *Brain Res* **365**, 350-354.

Meredith, M. A. and Stein, B. E. (1986). Visual, auditory, and somatosensory convergence on cells in superior colliculus results in multisensory integration. *J Neurophysiol* **56**, 640-662.

Meredith, M. A. and Stein, B. E. (1996). Spatial determinants of multisensory integration in cat superior colliculus neurons. *Journal of Neurophysiology* **75**, 1843-1857.

Mitzdorf, U. (1985). Current source-density method and application in cat cerebral cortex: investigation of evoked potentials and EEG phenomena. *Physiol Rev* **65**, 37-100.

Mize, R. R. and Horner, L. H. (1989). Origin, distribution, and morphology of serotonergic afferents to the cat superior colliculus: a light and electron microscope immunocytochemistry study. *Exp Brain Res* **75**, 83-98.

Molholm, S., Ritter, W., Murray, M. M., Javitt, D. C., Schroeder, C. E. and Foxe, J. J. (2002). Multisensory auditory-visual interactions during early sensory processing in

humans: a high-density electrical mapping study. *Brain Res Cogn Brain Res* **14**, 115-128.

Moore, R. Y. and Goldberg, J. M. (1966). Projections of the inferior colliculus in the monkey. *Exp Neurol* **14**, 429-438.

Morgan, M. L., Deangelis, G. C. and Angelaki, D. E. (2008). Multisensory integration in macaque visual cortex depends on cue reliability. *Neuron* **59**, 662-673.

Munoz, D. P. and Guitton, D. (1985). Tectospinal neurons in the cat have discharges coding gaze position error. *Brain Res* **341**, 184-188.

Munoz, D. P. and Guitton, D. (1989). Fixation and orientation control by the tecto-reticulo—spinal system in the cat whose head is unrestrained. *Rev Neurol* **145**, 567-579.

Murray, M. M., Foxe, J. J., Higgens, B. A., Javitt, D. C. and Schroeder, C. E. (2001). Visuo-spatial neural response interactions in early cortical processing during a simple reaction time task: a high-density electrical mapping study. *Neuropsychologia* **39**, 828-844.

Murray, M. M. and Wallace, M. T. (2011). The neural bases of multisensory processes. Boa Raton, FL, CRC Press.

Ohshiro, T., Angelaki, D. E. and DeAngelis, G. C. (2011). A normalization model of multisensory integration. *Nat Neurosci* **14**, 775-782.

Park, T. J. and Pollak, G. D. (1993). GABA shapes sensitivity to interaural intensity disparities in the mustache bat's inferior colliculus: implications for encoding sound location. *J Neurosci* **13**, 2050-2067.

Perrault, T. J., Jr., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2005). Superior colliculus neurons use distinct operational modes in the integration of multisensory stimuli. *J Neurophysiol* **93**, 2575-2586.

Perrault, T. J., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2003). Neuron-Specific Response Characteristics Predict the Magnitude of Multisensory Integration. *Journal of Neurophysiology* **90**, 4022-4026.

Pompeiano, M., Palacios, J. M. and Mengod, G. (1994). Distribution of the serotonin 5-HT2 receptor family mRNAs: comparison between 5-HT2A and 5-HT2C receptors. *Brain Res Mol Brain Res* **23**, 163-178.

Ramsey, L. C., Sinha, S. R. and Hurley, L. M. (2010). 5-HT1A and 5-HT1B receptors differentially modulate rate and timing of auditory responses in the mouse inferior colliculus. *Eur J Neurosci* **32**, 368-379.

Rasch, M. J., Gretton, A., Murayama, Y., Maass, W. and Logothetis, N. K. (2008). Inferring spike trains from local field potentials. *J Neurophysiol* **99**, 1461-1476.

Rhoades, R. W., Mooney, R. D., Chiaia, N. L. and Bennett-Clarke, C. A. (1990). Development and plasticity of the serotoninergic projection to the hamster's superior colliculus. *J Comp Neurol* **299**, 151-166.

Riga, M. S., Bortolozzi, A., Campa, L., Artigas, F. and Celada, P. (2016). The serotonergic hallucinogen 5-methoxy-N,N-dimethyltryptamine disrupts cortical activity in a regionally-selective manner via 5-HT1A and 5-HT2A receptors. *Neuropharmacology* **101**, 370-378.

Royal, D. W., Krueger, J., Fister, M. C. and Wallace, M. T. (2010). Adult plasticity of spatiotemporal receptive fields of multisensory superior colliculus neurons following early visual deprivation. *Restor Neurol Neurosci* **28**, 259-270.

Ruggeri, B., Sarkans, U., Schumann, G. and Persico, A. M. (2013). Biomarkers in autism spectrum disorder: the old and the new. *Psychopharmacology* **6**.

Russo, F. M., Pizzorusso, T., Porciatti, V., Marubio, L. M., Maffei, L. and Changeux, J. P. (2010). Multisensory processing in children with autism: high-density electrical mapping of auditory-somatosensory integration. *Autism Res* **3**, 253-267.

Sarko, D. K., Ghose, D. and Wallace, M. T. (2013). Convergent approaches toward the study of multisensory perception. *Front Syst Neurosci* **7**.

Softky, W. R. and Koch, C. (1993). The highly irregular firing of cortical cells is inconsistent with temporal integration of random EPSPs. *J Neuroscience* **13**, 334-350.

Stanford, T. R., Quessy, S. and Stein, B. E. (2005). Evaluating the Operations Underlying Multisensory Integration in the Cat Superior Colliculus. *The Journal of Neuroscience* **25**, 6499-6508.

Stein, B. E. (2012). The handbook of multisensory processing. MIT Press.

Stein, B. E., Meredith, M. A. and Wallace, M. T. (1993). The visually responsive neuron and beyond: multisensory integration in cat and monkey. *Prog Brain Res* **95**, 79-90.

Stein, B. E., Scott Huneycutt, W. S. and Meredith, M. A. (1988). Neurons and behavior: the same rules of multisensory integration apply. *Brain Research* **448**, 355-358.

Stein, B. E. and Meredith, M. A. (1986). Visual, Auditory, and Somatosensory Convergence on Cells in Superior Colliculus Results in Multisensory Integration. *J Neurophysiology* **56**, 640-662.

Steinbusch, H. W. (1981). Distribution of serotonin-immunoreactivity in the central nervous system of the rat-cell bodies and terminals. *Neuroscience* **6**, 557-618.

Stubblefield, E. A., Thompson, J. A. and Felsen, G. (2015). Optogenetic cholinergic modulation of the mouse superior colliculus in vivo. *J Neurophysiol* **114**, 978-988.

Tan, H., Mooney, R. D. and Rhoades, R. W. (1999). Effects of norepinephrine upon superficial layer neurons in the superior colliculus of the hamster: in vitro studies. *Vis Neurosci* **16**, 557-570.

Tolhurst, D. J., Movshon, J. A. and Dean, A. F. (1983). The statistical reliability of signals in single neurons in cat and monkey visual cortex. *Vision Res* **23**, 775-785.

Ueda, S., Ihara, N. and Sano, Y. (1985). The organization of serotonin fibers in the mammalian superior colliculus. An immunohistochemical study. *Anat Embryol* **173**, 13-21.

Villar, M. J., Vitale, M.L., Hokfelt, T. and Verhofstad, A. A. (1988). Dorsal raphe serotoninergic branching neurons projecting both to the lateral geniculate body and

superior colliculus: a combined retrograde tracing-immunohistochemical study in the rat. *J Comp Neurol* **277**, 126-140.

Wallace, M. T., Meredith, M. A. and Stein, B. E. (1998). Multisensory integration in the superior colliculus of the alert cat. *J Neurophysiol* **80**, 1006-1010.

Wang, L., Rangarajan, K. V., Lawhn-Heath, C. A., Sarnaik, R., Wang, B. S., Liu, X. and Cang, J. (2009). Direction-specific disruption of subcortical visual behavior and receptive fields in mice lacking the beta2 subunit of nicotinic acetylcholine receptor. *J Neurosci* **29**, 12909-12918.

Waterhouse, B. D., Moises, H. C. and Woodward, D. J. (1986). Interaction of serotonin with somatosensory cortical neuronal responses to afferent synaptic inputs and putative neurotransmitters. *Brain Res Bull* **17**, 507-518.

Wilkinson, L. K., Meredith, M. A. and Stein, B. E. (1996). The role of anterior ectosylvian cortex in cross-modality orientation and approach behavior. *Exp Brain Res* **112**, 1-10.

Wright, I. K., Garratt, J. C. and Marsden, C. A. (1990). Effects of a selective 5-HT2 agonist, DOI, on 5-HT neuronal firing in the dorsal raphe nucleus and 5-HT release and metabolism in the frontal cortex. *Br J Pharmacol* **99**, 221-222.

Xiang, Z. and Prince, D. A. (2003). Heterogeneous actions of serotonin on interneurons in rat visual cortex. *J Neurophysiol* **89**, 1278-87.

Chapter IV

General Discussion

The importance of normal sensory experience for SC (multi)sensory processing

Project goal and general findings

The overall goal of the work described in Chapter II of this thesis was to expand our understanding of the SC and in particular the mechanistic bases of the multisensory integrative process. To that end, I describe the nature of SC neurons' malleability throughout a lifetime; the ability of visual experience deprivation to affect multisensory integration capacities regardless of the time at which deprivation occurs during life. As detailed in Chapter II, multisensory integration continues to occur in animals deprived of experience during adulthood, however there are less instances of integration in these animals compared to those with normal visual experience throughout a lifetime (NR). This evidence is in agreement with the idea that continual normal visual experience througout an entire lifespan is essential in maintaining normal multisensory processing within the SC.

In addition, interesting observations were made when examining electrophysiological recordings from completely dark-reared (DR) animals. We found occurrences of multisensory integration in the SC of cats completely deprived of all visual experience, a finding that seemingly contrasts with previous studies (Wallace et al. 2004). However, integration capacities as well as responsivity to sensory stimuli increased with increasing exposure to stimulus presentations used in the recording process; this is also consistant with previous findings (Yu et al. 2010). In effect, the presentation of visual stimuli during the recording procedures was enough visual

experience to alter sensory responsivity and multisensory processing in the SC of totally visually deprived animals, which allows these findings to fit in well with previous studies conducted in the visually deprived cat SC. Overall, this work shows that even after visual deprivation, multisensory neurons in the SC maintain the capacity to be shaped and influenced by sensory experience.

Implications of project findings

The work presented in Chapter II suggests that multisensory SC neurons maintain their ability to be influenced by sensory experience throughout an entire lifetime, even in adulthood. This has been described in two different instances of visual deprivation; multisensory integrative capacities were found in completely dark-reared animals (DR) as well as those that were only deprived during adulthood (6+6). In addition, this was found on two different timescales; alterations in sensory responsivity and multisensory processing were found between different amounts of visual deprivation (DR vs. 6+6 vs. NR) as well as across different amounts of exposure to visual stimuli during recording procedures. These support the idea that experience plays a role in shaping sensory and multisensory responses throughout a lifetime. This work is important in order to understand the capacities of SC neurons across the entire lifespan.

Project expansion upon previous work

This work expands on earlier studies originally implicating sensory deprivation in altering multisensory integrative abilities. It has been previously found that total visual deprivation throughout a lifetime abolished any integrative capacities (Wallace et al.

2004) and restoration of visual experience in adulthood after developmental visual deprivation did not restore normal multisensoy processing (Carriere et al. 2007). The current work detailed in Chapter II expands on this foundation by showing that deprivation in adulthood following normal developmental visual experience (6+6) also alters multisensory capacities in the SC, suggesting that sensory deprivation at any point throughout a lifetime can alter integrative capacities of SC neurons.

The work described in Chapter II speaks to prior deprivation studies with regards to the idea of critical periods. Previous studies have examined critical periods as time points in the development of the nervous system during which an organism is most sensitive and adaptive to sensory deprivation (Hubel and Weisel 1963; Berardi et al. 2000; Hensch 2005). Sensory deprivation outside of such a critical period would have less or no effect on functionality in comparison. Bilateral visual input of spatial and temporal congruence during critical periods in early postnatal life is important for normal visual system development (Daw 2006; Niechwiej-Szwedo et al. 2016). Similarly, developmental sensitive periods exist within the auditory system in which there is more susceptibility to changes from auditory input, or lack thereof (Takesian et al. 2009; Kral 2013; Chen and Yuan 2015). It was once thought that these critical periods during postnatal development are the only time in which alterations in sensory input can manipulate functionality of the brain (Hensch 2004, 2005). However, evidence now suggests that brain plasticity lasts throughout a lifetime. Through exposure and training, sensory representation can be changed throughout adulthood, both in the auditory and visual sensory modalities (Polley et al. 2006; Popescu and Polley 2010; Burton et al. 2002; Ruschecker 1999). The work described in Chpater II of this thesis provides

evidence to the idea that sensory deprivation during adulthood can affect multisensory processing.

This follows along with behavioral work in human populations nicely, showing that alterations in sensory experience at many different points in a lifetime alter multisensory processing at behavioral and perceptual levels (Eimer 2004; Roder et al. 2004; Putzar et al. 2007; Leo et al. 2008; Collignon et al. 2009; Hotting and Roder 2009; Putzar et al. 2012; Occelli et al. 2013; Guerreiro et al. 2015; Hauthal et al. 2015). For example, the loss of vision during development has been shown to play an important role in both tactile (Roder et al. 2004; Eimer 2004) and auditory (Leo et al. 2008; Occelli et al. 2013) perception; performance in localization of both tactile and auditory stimuli are shown to be enhanced in individuals who have been blind from early life. This improved unisensory target performance is vastly different from those involving multisensory stimuli in individuals with early visual deprivation. For example, Putzar and colleagues (2007) have shown that adult individuals who were deprived of pattern vision for the first five months of life due to binocular cataracts have reduced audiovisual interactions later in life even after visual performance is no longer impaired. However, basic multisensory functions for simple and short audiovisual stimuli are unaffected by early visual deprivation; individuals who had binocular cataracts early in life maintain the capacity to exhibit multisensory gain on simple detection tasks involving short, simple stimuli (Putzar et al. 2012). This indicates that, at least at a very simple level, brain plasticity remains past development and into adulthood.

The loss of visual input early in life functionally reorganizes visual cortical regions of the brain. Visual deprivation affects the capacity of cortical regions, for example

auditory regions, to integrate cross-modal inputs and exhibit multisensory integrative capacity (Guerreiro et al. 2015). The reorganization of visual cortical areas has been a long-standing explanation for why these alterations in sensory processing occur after visual deprivation; however there are multiple explanations for how this reorganization in the blind takes place. One justification details that these functional changes seen are a result of the unmasking of preexisting long range connections between sensory cortical areas (Occelli et al. 2013). With normal visual input, these connections are masked by typical visual processing. But, in instances of visual deprivation, they are unmasked, thus creating the functional reorganization of visual cortical areas that are observed in blind individuals. This masking and unmasking of preexisting connections can happen rather rapidly, which could explain why even a brief period of visual deprivation, in adulthood, is sufficient to induce recruitment of brain regions traditionally involved in visual perception to different sensory processing (Occelli et al. 2013; Merabet et al. 2008). For example, visual deprivation of sighted adults for five days, while undergoing tactile training on a Braille discrimination task, was sufficient to increase recruitment of occipital cortex in response to tactile stimulation for a very brief time period, as measured by BOLD signal (Merabet et al. 2008). This indicates that the functional reorganization of these areas, occipital cortex specifically, is highly reversible. Five days of deprivation was all that was necessary to increase responsivity to tactile stimulation, and this phenomenon lasted for 24 hours (Stevens et al. 2007; Merabet et al. 2008). This supports the idea of unmasking preexisting long range connections between sensory regions with visual deprivation. If visual deprivation persists, these connections can strengthen and stabilize. However, if visual deprivation ceases and

normal visual experience returns, they quickly become masked by visual processing once again. This previous work in humans, in addition to the work outlined in Chapter II of this thesis, supports the idea that the brain retains its plasticity not just throughout development, but over a lifetime.

This work also agrees with previous findings showing that repeated visual stimulus presentations during recording procedures is sufficient to alter sensory responsivity and multisensory integration capacities (Yu et al. 2010). The repeated presentation of visual stimuli for recording purposes does not completely reestablish responsivity in DR and 6+6 animals back to normality (i.e. the level seen in NR animals), but can restore some multisensory integration in these neuronal populations.

Future directions

While this study helped to improve our understanding of the importance of visual sensory experience throughout a lifetime, future studies are required for us to fully understand this phenomenon. One such future direction should involve understanding just how little visual deprivation is needed in order to significantly alter multisensory processing in the SC. The current study described, along with previous work, has shown that deprivation during development and during adulthood is sufficient to change integrative capacities in SC neurons. However, the amount of visual deprivation in these studies was vast, depriving the animals used in the study for months and years at a time. Shortening that amount of deprivation into discrete periods to understand how small amounts of visual deprivation affect the multisensory circuit in the SC would help to provide a better understanding of the mechanisms on which sensory experience is

acting. For example, if a longer period of deprivation is required for multisensory integration to be affected, perhaps slower, more modulatory mechanisms involving neuromodulators are influenced by visual experience. Understanding how visual sensory experience affects multisensory processing in the SC is important for us to understand how this system works and, in turn, how SC-mediated behaviors are influenced.

In addition to focusing only on sensory deprivation, this work can be expanded upon to determine how the alteration of sensory statistics affect development of SC multisensory neurons. Previous work has shown that when animals are raised in an alternate environment where visual and auditory stimuli are presented in temporal synchrony at spatially disparate locations, SC multisensory neurons develop an alternate version of multisensory integration in which spatailly disparate, but not coincident, audiovisual stimuli are integrated (Wallace and Stein 2007). Future work building on this study can further explore how these neuronal receptive fields can be manipulated. For example, how does the raising of animals in an environment where auditory and visual inputs are spatailly coincident but temporally disparate affect integrative capacitites of SC multisensory neurons? By comparing results of this work to those involving spatial manipulations of sensory inputs, we can aquire insight into how sensory experience is important for SC multisensory processing but also in how these neurons weight spatial vs. temporal sensory information. Adding a developmental component to these studies by first raising animals in one disparate environment and then another will add to our knowledge of the plasticity of this system, as well. In addition, transferring animals to these spatially or temporally incongruent environments

in adulthood will further our comprehension of the specific plasticity of the neurons and their receptive fields. Understanding not only how sensory experience, but the statistics of that sensory experience, affects multisensory processing in SC neurons, and then, in turn, how this affects SC-mediated behavior, is essential to determining how multisensory processing works at a fundamental level.

Another important avenue of future study relates to understanding the molecular bases of these deprivation-induced changes. Investigation into the molecular changes underlying dark-rearing-induced changes of RFs of SC neurons have shown that visual deprivation results in changes within the GABA system in the SC (Carrasco et al. 2011). Fewer GABA-immunoreactive neurons are present in the SC of dark-reared animals, therefore altering excitatory/inhibitory balance within the SC. Additionally, normal GABAergic surround inhibition of RFs is weakened, resulting in overall enlarged RFs (Carrasco et al. 2011). Future work can build upon this study by investigating the change in GABA during adult visual deprivation. How does the GABAergic innervation of the SC change with visual deprivation only during adulthood? As the alteration of the GABAergic circuit modifies excitation/inhibition balance within the SC, further studies into changes within glutamatergic synapses are also warranted. Chapters I and III of this thesis have also provided evidence that the 5-HT system is involved in RF establishment and maintenance. As previous studies have supported the idea that both visual deprivation and the GABA system are also involved in RF maintenance, it is reasonable to suspect that these interact with each other in some manner. Determining how the GABA and 5-HT systems work together in instances of visual deprivation, and how this compares to normal sensory experience circumstances, will not only add to our

knowledge of how the SC functions normally, but also how this normal function is altered in instances of sensory deprivation, providing more insight into how these SC changes play into changes seen at the behavioral level.

The role of the 5-HT system in SC-mediated multisensory integration

Project goal and general findings

The work detailed in Chapter III begins to explore the role of the serotonergic system in multisensory processing in the SC. Overall, this study shows that the 5-HT2a receptor is involved in sensory processing and multisensory integration at the level of the individual neuron. Agonism of this receptor type enhances responses to visual and audiovisual stimulus presentations, while also increasing multisensory gain of response. This effect of 5-HT2a receptor agonism was seemingly selective for visual responses as compared to auditory, which concurs with previous anatomical research conducted in the SC; 5-HT innervation of the SC is more superficial in nature, which is also where more of the visually-responsive neurons are found. The addition of DOI also altered the size and shape of sensory receptive fields (RFs) of SC multisensory neurons, highlighting that the majority of the influence of DOI is found at the periphery of RFs. This also adheres well to previous findings, in that the 5-HT system has been found to be critical in forming, shaping and developing neuronal RFs in many areas throughout the brain (Lauder et al. 1982; Mize and Horner 1989; Rhoades et al. 1990; Ueda et al. 1985; Villar et al. 1988; Arce et al. 1992; Huang et al. 1993; Gu and Singer 1995; Janusonis et al. 1999; Ke et al. 1999; Foehring et al. 2002; Hurley et al. 2002; Xiang and Prince 2003; Hurley et al. 2004; Lottem et al. 2016).

One of the most important findings from the study detailed in Chapter III is the heterogeneity in responses seen in this data set. In most cases, the addition of 5-HT2a agonist DOI increased responses to sensory stimuli, increased multisensory gain of response, and also increased Fano Factor. In some cases, however, the pattern of responses was different. Most often, this pattern was altered in audiovisual neurons, those outwardly responsive to both auditory and visual stimuli alone as well as exhibiting a change in response with an audiovisual stimulus presentation. In some instances, while the responses of the neurons increased with DOI addition, Fano Factor decreased rather than increased. This could be the result of different receptor subtypes, or different ratios of receptor subtypes, found on this certain population of neurons compared to auditory and visual multisensory neurons in the SC. The heterogeneity of responses highlights the complexity of the multisensory system in the SC, the role of the 5-HT system in multisensory processing, and requires much further investigation to fully understand.

Implications of project findings

This project is the first to provide evidence of 5-HT system involvement in multisensory processing at the level of the single neuron within the deep SC layers. The increase in 5-HT due to the injection of 5-HT receptor agonist DOI increased unisensory and multisensory interactions at the single neuron level. This suggests that the 5-HT system, specifically the 5-HT2a receptor, may be involved in gating sensory and multisensory responses of these neurons, which aligns with previous work providing evidence that 5-HT has an impact on spatial filters and temporal filters of sensory neurons (Hurley and Pollak 2005a, 2005b; Hurley 2006, 2007; Lauder et al. 1982; Mize

and Horner 1989; Rhoades et al. 1990; Ueda et al. 1985; Villar et al. 1988; Arce et al. 1992; Huang et al. 1993; Gu and Singer 1995; Janusonis et al. 1999; Ke et al. 1999; Foehring et al. 2002; Hurley et al. 2002; Xiang and Prince 2003; Hurley et al. 2004; Lottem et al. 2016). More specifically, DOI injections were shown to increase both responsivity of sensory neurons as well as multisensory gain in neurons which lacked overt responses to both auditory and visual sensory stimuli (modulatory neurons), a seemingly paradoxical finding. This suggests that 5-HT2a may have preferential effects on integrative capacities of multisensory SC neurons with a driving input from one sensory modality and a modulatory input from another, and may act more on the modulatory inputs of these neurons as opposed to the driving inputs, increasing responsivity to that modulatory input and increasing gain of response to multisensory stimuli.

The work described in Chapter III also implicates the 5-HT system in receptive field modulation in the deep SC. 5-HT agonist-induced changes in sensory and multisensory responsiveness, multisensory integration, and response reliability were greater at peripheral locations of individual neuron's RFs. In addition, sensory and multisensory RFs were altered with the addition of 5-HT2a receptor agonist DOI, sharpening or widening after agonist addition. These results show that the 5-HT system is invovled in receptive field and map refinement, even in the SC of adult animals. These results are in line with previous work showing the involvement of the 5-HT system in sensory RF and map development and refinement (Lauder et al. 1982; Mize and Horner 1989; Rhoades et al. 1990; Ke et al. 1999). This work adds to the body of

evidence that shows an important role for 5-HT signaling in RF structure refinement and maintenance.

Overall, the finding that the 5-HT system is involved in modulating mutlisensory processing in the SC is a first step to investigation of the molecular mechanisms which allow multisensory integration to occur in the SC. Understanding how the process of multisensory integration occurs includes a knowledge of the molecular makeup of the SC neurons that are part of the integrative process. This work, which shows evidence to suggest the presence of 5-HT2a receptors on these neurons, adds to this body of literature and brings us one step closer to fully understanding how multisensory processing occurs. However, the work described in Chapter III only examines one type of receptor in one neuromodulatory system and its effects on sensory and multisensory processing. Thus, many future studies are required in order to paint a clear picture of the system of multisensory processing in the SC.

Future directions

The work described in Chapter III is the first step to understanding the involvement of the 5-HT system in multisensory processing occurring in the intermediate and deep layers of the SC. However, much more research is required to fully appreciate this involvement. A first step to confirm these findings is necessary, and would involve studies utilizing a 5-HT2a receptor antagonist. Studies involving the injection of 5-HT2a antagonists to the extracellular space surrounding SC multisensory neurons to determine its effects on multisensory processing and neuronal dynamics is an essential confirmational study for the one detailed in Chapter III. This would allow for

further confirmation that this receptor subtype is found on multisensory neurons in the SC, and would pave the way for future studies focusing on other receptor subtypes. Work focusing on various 5-HT receptor subtypes to determine if they are found on multisensory SC neurons is critical in multisensory research. Studies investigating not only the presence but also the density and presence of active and functional receptors on SC neurons will allow us to determine the molecular makeup of each multisensory neuronal type within the SC, furthering our understanding of how each type of neuron responds to various sensory stimuli and how multisensory integrative outputs are formed.

Once the molecular makeup of these neurons is fully mapped, then work can be completed determining how each receptor type is involved in neuronal output. For example, studies using chemical manipulation of neuronal surrounding environments, like the current work detailed in Chapter III, to depress or facilitate different receptor subtypes with subsequent evaluation of neuronal responsivity and integrative capacity to determine the functional action of each receptor type. These studies should also have a developmental bend; research measuring the presence and functionality of each receptor type on SC multisensory neurons at various timepoints throughout life (e.g. at birth, before development of integrative capacity, after integrative maturity) will provide further insight into the development of sensory and multisensory processing within the SC.

Future work can also focus on how manipulation of the 5-HT system affects behavior. Injections of 5-HT receptor agonists and antagonists through cannulae directly into the SC preceding the action of an animal in a behavioral task would help to

understand how the 5-HT system is involved in the behavioral output of the SC. For example, one such research project could investigate the influence of 5-HT on multisensory response times. Training animals to respond to unisensory and multisensory (audiovisual) targets and comparing of response times and accuracies to targets before and after 5-HT injection to the SC could give insight into the funcionality of 5-HT within the SC. Additionally, evaluation of response times and accuracies between unisensory and multisensory targets can provide information as to whether the influence of 5-HT is specific to multisensory processing or is more widespread. Control trials should be run to determine that 5-HT is not influencing the physical act of making a response overall. These types of studies can be completed using general 5-HT receptor agonists and antagonists or, once specific subtypes of 5-HT receptors are determined to be involved, be limited to investigating the involvement of one receptor subtype on SC-mediated behaviors. Studies involving unisensory and multisensory targets, investigating response times, response accuracies and orientation behavior, would provide further information with regards to how the 5-HT system within the SC influences overall functional behavior.

Following investigation of the 5-HT system actions in multisensory processing, future work into how other modulatory systems interact and influence multisensory integration is necessary to obtain the full picture of what is happening in a normal SC multisensory system. It is only after we fully understand how the process of multisensory integration works in a normal, unperturbed system under typical circumstances that we can begin to study how this system may be dysfunctional in instances of neurological disorder, such as autism spectrum disorder (ASD). According

to the Centers for Disease Control, 1 in 68 individuals has some form of ASD, with a five-fold higher incidence of occurrence in males than females (2015). Individuals with ASD have been shown to possess atypical sensory (Iarocci and McDonald 2006) and multisensory (Wallace and Stevenson 2014; Foss-Feig et al. 2010; Stevenson et al. 2014) processing. In addition, some individuals with ASD exhibit elevated 5-HT levels in the blood (Muller et al. 2016; Schain and Freedman 1961). In order to gain a better understanding of ASD, investigation into the relationship of the 5-HT system and multisensory processing in human populations must first be completed. Studies relating 5-HT levels and performance on multisensory behavioral tasks to show a connection between the 5-HT system and multisensory processing would be an essential initial step which can lead to future work of studying ASD. Subsequent research into how this relationship between the 5-HT system and multisensory processing is perturbed would help to paint a better picture of what is occurring differently in ASD individuals. It is only after understanding how these processes work differently that we can begin to develop proper interventions.

How do sensory experience and the 5-HT system work together to maintain multisensory processing in the SC?

The work described throughout the introduction of this thesis has provided insight into how proper visual experience and 5-HT system involvement are important for the development of normal SC neurons, both sensory and multisensory. In addition, research detailed in Chapters II and III have highlighted how these are also essential for the maintenence of proper multisensory processing within the SC. Both the 5-HT system and normal visual experience are important for the maintenance of normal

integration and integrative processes within the SC, so it stands to reason that they must interact in some way, as well. Future studies are critical in order to discover this interaction. While the amount of 5-HT in the SC is known in both developing and adult animals, there is no such information in that of animals who are light deprived. Measuring not only 5-HT concentration, but also expression of specific, active 5-HT (as well as other neuromodulators such as GABA) receptors, and then comparing these measurements to that found in normally-raised animals will shed light on how sensory experience may influence the 5-HT system within the SC. Taking this even further, if it is found that 5-HT concentration in the SC is dependent on normal sensory experience, future studies may also investigate if the introduction of 5-HT can restore multisensory integration in SC neurons.

As described earlier, the 5-HT system, GABAergic system, and visual experience are critical for maintaining SC neuronal RFs. However, it is yet unknown how these interact and work together to maintain normal RFs. Investigation into these interactions is important to understanding how neurons in the SC function normally. For example, when one system is perturbed, can others overcompensate to maintain normal sensory RFs? How does the alteration of 5-HT levels in the SC change the action of GABA? Are the GABAergic and 5-HTergic systems altered when visual experience is abolished in adulthood but maintained during development? As the GABAergic and glutamatergic systems are intimately intertwined, how does the alteration of visual experience and/or 5-HT level change glutamatergic synapses in the SC? As it has been shown that NMDA receptors are incredibly important for normal multisensory processing (described in detail in Chapter I), the alterations of these systems is bound to influence multisensory

integrative capacity. For example, if visual deprivation reduces GABA inhibitory influence, glutamatergic influence may also decrease, which could impact and decrase 5-HT levels within the SC, given the interactions between the two systems in relation to brain plasticity (Takahashi et al. 2011). These interactions, while speculation for now, would be incredibly important to investigate further. Future work is essential to determine how sensory experience and modulatory neurotransmitters interact within the SC to influence not only sensory processing, but multisensory integration, and there is much work to be done.

References Cited

Arce, E. A., Bennett-Clarke, C.A., Mooney, R.D. and Rhoades, R.W. (1992). Synaptic organization of the serotoninergic input to the superficial gray layer of the hamster's superior colliculus. *Synapse* **11,** 67-75.

Berardi, N., Pizzorusso, T. and Maffei, L. (2000). Critical periods during sensory development. *Current Opinion in Neurobiology* **10**, 138-145.

Burton, H., Snyder, A.Z., Conturo, T.E., Akbudak, E., Ollinger, J.M. and Raichle, M.E. Adaptive changes in early and late blind: a fMRI study of Braille reading. *J Neurophysiology* **87**, 589-607.

Carrasco, M. M., Mao, Y. T., Balmer, T. S. and Pallas, S. L. (2011). Inhibitory plasticity underlies visual deprivation-induced loss of receptive field refinement in the adult superior colliculus. *European Journal of Neuroscience* **33**, 58-68.

Carriere, B. N., Royal, D. W., Perrault, T. J., Morrison, S. P., Vaughan, J. W., Stein, B. E. and Wallace, M. T. (2007). Visual deprivation alters the development of cortical multisensory integration. *J Neurophysiol* **98**, 2858-2867.

Chen, Z. and Yuan, W. (2015). Central plasticity and dysfunction elicited by aural deprivation in the critical period. *Front Neural Circuits* **2**.

Collignon, O., Charbonneau, G., Lassonde, M. and Lepore, F. (2009). Early visual deprivation alters multisensory processing in peripersonal space. *Neuropsychologia* **47**, 3236-3243.

Daw, N.W. (2006). Visual Development. Springer, New York, New York.

Eimer, M. (2004). Multisensory integration: how visual experience shapes spatial perception. *Curr Biol* **14**, R115-117.

Foehring, R.C., van Brederode, J. F., Kinney, G. A. and Spain, W. J. (2002). Serotonergic modulation of supragranular neurons in rat sensorimotor cortex. *J Neurosci* **22**, 8238-8250.

Foss-Feig, J.H., Kwayke, L.D., Cascio, C.J., Burnette, C.P., Kadivar, H., Stone, W.L. and Wallace, M.T. An extended multisensory temporal binding window in autism spectrum disorder. *Exp Brain Res* **203**, 381-389.

Gu, Q. and Singer, W. (1995). Involvement of serotonin in developmental plasticity of kitten visual cortex. *Eur J Neurosci* **7**, 1146-1153.

Guerreiro, M. J., Putzar, L. and Roder, B. (2015). The effect of early visual deprivation on the neural bases of multisensory processing. *Brain* **138**, 1499-1504.

Hauthal, N., Debener, S., Rach, S., Sandmann, P. and Thorne, J. D. (2015). Visuo-tactile interactions in the congenitally deaf: a behavioral and event-related potential study. *Front Integr Neurosci* **8**.

Hensch, T.K. (2004). Critical period regulation. *Annu Rev Neurosci*, **27**, 549-579.

Hensch, T.K. (2005). Critical period plasticity in local cortical circuits. *Nature Reviews Neuroscience* **6**, 877-888.

Hotting, K. and Roder, B. (2009). Auditory and auditory-tactile processing in congenitally blind humans. *Hear Res* **258**, 165-174.

Huang, X., Mooney, R. D. and Rhoades, R. W. (1993). Effects of serotonin on retinotectal-, corticotectal-, and glutamate-induced activity in the superior colliculus of the hamster. *J Neurophysiol* **70**, 723-732.

Hurley, L.M., Thompson, A. M. and Pollak, G. D. (2002). Serotonin in the inferior colliculus. *Hear Res* **168**, 1-11.

Hurley, L.M., Devibiss, D.M. and Waterhouse, B. D. (2004). A matter of focus: monoaminergic modulation of stimulus coding in mammalian sensory networks. *Curr Opin Neurobiol* **14**, 488-95.

Hurley, L. M. and Pollak, G. D. (2005). Serotonin modulates responses to species-specific vocalizations in the inferior colliculus. *J Comp Physiol A Neuroethol Sens Neural Behav Physiol* **191**, 535-546.

Hurley, L. M. and Pollak, G. D. (2005). Serotonin shifts first-spike latencies of inferior colliculus neurons. *J Neurosci* **25**, 7876-7886.

Iarocci, G. and McDonald, J. Sensory integration and the perceptual experience of persons with autism. *J Autism* **36**, 77-90.

Janusonis, S., Fite, K. V. and Foote, W. (1999). Topographic organization of serotonergic dorsal raphe neurons projecting to the superior colliculus in the Mongolian gerbil (Meriones unguiculatus). *J Comp Neurol* **413**, 342-355.

Ke, M., Mooney, R. D. and Rhoades, R. W. (1999). Increased serotonin in the developing superior colliculus affects receptive-field size of retinotectal afferents but not that of postsynaptic neurons. *Vis Neurosci* **16**, 121-130.

Kral, A. (2013). Auditory critical periods: a review from system's perspective. *Neuroscience* **5**, 117-133.

Lauder, J. M., Wallace, J.A., Krebs, H., Petrusz, P. and McCarthy, K. (1982). In vivo and in vitro development of serotonergic neurons. *Brain Res Bull* **9**, 605-625.

Leo, F., Bertini, C., di Pellegrino, G. and Ladavas, E. (2008). Multisensory integration for orienting responses in humans requires the activation of the superior colliculus. *Exp Brain Res* **186**, 67-77.

Lottem E., Lorincz, M. L. and Mainen, Z. F. (2016). Optogenetic activation of dorsal raphe serotonin neurons rapidly inhibits spontaneous but not odor-evoked activity in olfactory cortex. *J Neurosci* **36**, 7-18.

Merabet L.B., Hamilton, R., Schlaug, G., Swisher, J. D., Kiriakopoulos, E. T., Pitskei, N. B., Kauffman, T. and Pascual-Leone, A. (2008). Rapid and reversible recruitment of early visual cortex for touch. *PLoS One* **3**.

Mize, R. R. and Horner, L. H. (1989). Origin, distribution, and morphology of serotonergic afferents to the cat superior colliculus: a light and electron microscope immunocytochemistry study. *Exp Brain Res* **75**, 83-98.

Muller, C.L., Anacker, A.M. and Veenstra-VanderWeele, J. (2016). The serotonin system in autism spectrum disorder: From biomarker to animal models. *Neuroscience* **3**, 24-41.

Neichwiej-Szwedo, E., Chin, J., Wolfe, P.J., Popovich, C. and Staines, W.R. (2016). Abnormal visual experience during development alters the early stages of visual-tactile integration. *Behavioral Brain Research* **304**, 111-119.

Occelli, V., Spence, C. and Zampini, M. (2013). Auditory, tactile, and audiotactile information processing following visual deprivation. *Psychol Bull* **139**, 189-212.

Polley, D.B., Steinberg, E.E. and Merzenich, M.M. (2006). Perceptual learning directs auditory cortical map reorganization through top-down influences. *J Neuroscience* **28**, 4970-4982.

Popescu, M.V. and Polley, D.B. (2010). Monaural deprivation disrupts development of binaural selectivity in auditory midbrain and cortex. *Neuron* **65**, 718-731.

Putzar, L., Goerendt, I., Lange, K., Rosler, F. and Roder, B. (2007). Early visual deprivation impairs multisensory interactions in humans. *Nat Neurosci* **10**, 1243-1245.

Putzar, L., Gondan, M. and Roder, B. (2012). Basic multisensory functions can be acquired after congenital visual pattern deprivation in humans. *Dev Neuropsychol* **37**, 697-711.

Rauschecker, J.P (1999). Auditory cortical plasticity: a comparison with other sensory systems. *Trends Neurosci* **22**, 74-80.

Rhoades, R. W., Mooney, R. D., Chiaia, N. L. and Bennett-Clarke, C. A. (1990). Development and plasticity of the serotoninergic projection to the hamster's superior colliculus. *J Comp Neurol* **299**, 151-166.

Roder, B., Rosler, F. and Spence, C. (2004). Early vision impairs tactile perception in the blind. *Curr Biol* **14**, 121-124.

Schain, R.J. and Freedman, D.X. (1961). Studies on 5-hydroxyindole metabolism in autistic and other mentally retarded children. *J Pediatr* **58**, 315-320.

Stevens, A.A., Snodgrass, M., Schwartz, D. and Weaver, K. (2007). Preparatory activity in occipital cortex in early blind humans predicts auditory perceptual performance. *J Neurosci* **27**, 10734-10741.

Stevenson, R.A., Siemann, J.K., Woynaroski, T.G., Schneider, B.C., Eberly, H.E., Camerata, S.M. and Wallace, M.T. Evidence for diminished multisensory integration in autism spectrum disorders. *J Autism Dev Disord* **44**, 3161-3167.

Stevenson, R.A., Siemann, J.K., Schneider, B.C., Eberly, H.E., Woynaroski, T.G., Camerata, S.M. and Wallace, M.T. Multisensory temporal integration in autism spectrum disorders. *J Neurosci* **34**, 691-697.

Takahashi, T. (2011). Serotonin as a mediator of cross-modal cortical reorganization. *Commun Integr Biol* **4**, 459-461.

Takesian, A.E., Kotak, V.C. and Sanes, D.H. (2009). Developmental hearing loss disrupts synaptic inhibition: implications for auditory processing. *Future Neurology*, **4**, 331-349.

Ueda, S., Ihara, N. and Sano, Y. (1985). The organization of serotonin fibers in the mammalian superior colliculus. An immunohistochemical study. *Anat Embryol* **173**, 13-21.

Villar, M. J., Vitale, M.L., Hokfelt, T. and Verhofstad, A. A. (1988). Dorsal raphe serotoninergic branching neurons projecting both to the lateral geniculate body and superior colliculus: a combined retrograde tracing-immunohistochemical study in the rat. *J Comp Neurol* **277**, 126-140.

Wallace, M. T., Perrault, Jr., T. J., Hairston, W. D. and Stein, B. E. (2004). Visual experience is necessary for the development of multisensory integration. *J Neurosci* **24**, 9580-9584.

Wallace, M. T. and Stein, B. E. (2007). Early experience determines how the senses will interact. *J Neurophysiol* **97**, 921-926.

Wallace, M.T. and Stevenson, R.A. (2014). The construct of the multisensory temporal binding window and its dysregulation in developmental disabilities. *Neuropsychologia* **64**, 105-123.

Wiesel, T.N. and Hubel, D.H. (1963). Effects of visual deprivation on morphology and physiology of cells in the cats lateral geniculate body. *Journal of Physiology*, 978-993.

Xiang, Z. and Prince, D. A. (2003). Heterogeneous actions of serotonin on interneurons in rat visual cortex. *J Neurophysiol* **89**, 1278-87.

Yu, L., Rowland, B. A. and Stein, B. E. (2010). Initiating the Development of Multisensory Integration by Manipulating Sensory Experience. *The Journal of Neuroscience* **30**, 4904-4913.