Evaluating Multisensory Processing in the Mouse Model: Clinical and Translational Implications

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Dedicated to my parents, friends and loved ones

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CHAPTER I

Introduction

Introduction to Multisensory Processing

Multisensory integration can be described as the merging of sensory information from different modalities (Murray & Wallace, 2011). We are constantly bombarded in our daily lives by a variety of sensory stimuli, yet are able to integrate this information seamlessly in order to perceive our world. This has led to numerous studies investigating how the brain is capable of combining this sensory information into a unified percept or gestalt (G. Calvert, Spence, & Stein, 2004). To this point, investigations in both humans and animal models have shown that the combination of information from multiple senses can result in behavioral enhancements such as increased accuracies and reduced reaction times when compared to behavioral performances under unisensory (i.e. visual, auditory, or tactile alone) conditions (R. A. Stevenson, Ghose, et al., 2014). For example, human psychophysical studies have shown that the added visual information of seeing a speaker's lip movements can facilitate speech intelligibility in a noisy environment (Sumby & Pollack, 1954). In addition to this work in the behavioral and perceptual realms, much research has gone into characterizing the neural circuits and processes that underlie multisensory integration (Stein & Stanford, 2008). Collectively, this work has shown that multisensory inputs converge at a many sites in the nervous system (M. A. Meredith, 2002; Wallace, Meredith, & Stein, 1992; Wallace, Ramachandran, & Stein, 2004), and that this convergence frequently results in dramatic changes in neuronal responses when stimuli from multiple modalities are presented (M. A. Meredith & Stein, 1983). The use of animal models has been integral in determining the development (Stein, Stanford, & Rowland, 2014; Wallace &

Stein, 1997, 2001; Wallace & Stein, 2007), neural mechanisms (Jiang, Wallace, Jiang, Vaughan, & Stein, 2001; Wallace et al., 1992; Wallace & Stein, 2000) and brain systems associated with multisensory integration (Driver & Noesselt, 2008; Stein, 2012). From this, insights into the connections and neural structures critical for multisensory processing have been determined (M. A. Meredith & Stein, 1986b; Olcese, Iurilli, & Medini, 2013; Stein, Wallace, Stanford, & Jiang, 2002; Wallace & Stein, 1994). In addition to these studies, investigators have also successfully evaluated and established relationships between these underlying circuits and the resultant multisensory behavior in both large and small animal models; ranging from monkeys to rats (Cappe, Murray, Barone, & Rouiller, 2010; Hirokawa, Bosch, Sakata, Sakurai, & Yamamori, 2008; Wallace, Meredith, & Stein, 1998).

The Neurobiology of Multisensory Integration

The animal model system that has been classically studied in the context of multisensory processing is the cat (Murray & Wallace, 2011). The rationale behind this is that the cat has highly sensitive sensory systems (i.e. vision, audition, somatosensation), which allows for it to be an appropriate model to evaluate how sensory information is combined across multiple modalities (M. A. Meredith & Stein, 1983). From these initial studies, three main principles (space, time, and effectiveness) have been used to describe multisensory integration (M. A. Meredith, Nemitz, & Stein, 1987; M. A. Meredith & Stein, 1986a, 1986b). The principles of space and time illustrate that if stimuli from different modalities are presented in close spatial and/or temporal proximity that greater neural responses and behavioral benefits may be conferred under multisensory conditions (M. A. Meredith et al., 1987; M. A. Meredith & Stein, 1986a). In addition, the principle of inverse effectiveness states that as the effectiveness (i.e.

loudness, brightness) of the unisensory stimuli decreases, the resultant behavioral gain or benefit increases when these stimuli are combined compared to the gain from the individual unisensory components alone (M. A. Meredith & Stein, 1986b). Therefore, if two weak unisensory stimuli (i.e. visual and auditory) are presented within close spatial and temporal proximity, maximal neural responses and behavioral gain can be observed under these multisensory conditions (R. A. Stevenson, Ghose, et al., 2014).

Two classic ways to measure and evaluate multisensory function are with the interactive index (ii) and the mean statistical contrast (msc) (Stein & Stanford, 2008). The interactive index compares the responses to multisensory stimuli to the greatest or most effective unisensory response. The equation for ii states: $[(CM - SM_{max})/ SM_{max}] \times 100$, where CM represents the responses under combined modalities and SM_{max} represents the responses under the single most effective modality (M. A. Meredith & Stein, 1983, 1986a, 1986b). Based on this calculation, both neural and behavioral responses can result in response enhancement or depression. Enhancements may occur if the responses to multisensory stimuli are greater than the single most effective unisensory response (i.e. visual, auditory, tactile), while response depression can occur then if the responses under these multisensory conditions are smaller than the greatest individual unisensory response (Stein & Meredith, 1993). Multisensory processing can then be further evaluated by the mean statistical contrast. The msc compares the responses to multisensory stimuli to the combined responses from the individual sensory modalities. The equation for msc states: $\Sigma [(AV - (A + V)] / n$, where AV is the number of responses under multisensory conditions, A is the number of responses under auditory alone conditions, V is the number of responses under visual alone conditions and n is the number of trials (Perrault, Vaughan, Stein, & Wallace, 2003, 2005; Stanford, Quessy, & Stein, 2005). Three types of responses (i.e.

subadditive, additive or superadditive) can be observed as a result of this evaluation. Subadditivity occurs when responses under multisensory conditions are less than the summed responses under unisensory conditions, additivity results when the multisensory and the summed unisensory responses are equivalent and lastly superadditivity can be observed when the multisensory responses exceed the sum the of the unisensory responses. Therefore, superadditive responses typically result in multisensory facilitation and these responses are most likely to occur when multiple sensory stimuli are presented under the three principles of multisensory integration (R. A. Stevenson, Ghose, et al., 2014). For example, maximal multisensory gain and superadditivity may be observed when sensory stimuli are presented within close temporal and spatial proximity and the unisensory components are fairly weak (i.e. dim or quiet), in contrast, subadditive responses are more likely to occur when sensory stimuli are presented far apart both in space and in time and the unisensory stimulus components are relatively strong (i.e. bright or loud) (Stanford et al., 2005). The study of multisensory function has now been evaluated in humans and numerous animal models utilizing a variety of techniques (G. Calvert et al., 2004; Murray & Wallace, 2011; Stein & Stanford, 2008), but investigations first began with single cell electrophysiological recordings in a classic subcortical structure in the cat model system (Stein & Meredith, 1993).

The most studied brain structure in the multisensory literature is the superior colliculus (SC) (Stein, 2012) with a variety of classic animal studies demonstrating that this subcortical structure is a major hub for multisensory integration (Burnett, Stein, Perrault, & Wallace, 2007; M. A. Meredith & Stein, 1986b; Stein, 1998; Wallace & Stein, 1996). The SC is a multilayered structure that is typically divided into superficial and deep layers and is responsible for orienting behaviors (Stein & Meredith, 1993). Neurons in the superficial layers typically receive inputs

from mainly visual structures whereas the deep layers of SC receive inputs from multiple sensory modalities (Casagrande, Harting, Hall, Diamond, & Martin, 1972; Edwards, Ginsburgh, Henkel, & Stein, 1979; Huerta & Harting, 1984; May, 2006; Wallace, Meredith, & Stein, 1993) with the neurons in these deep layers classically being shown to respond to multisensory stimuli (M. A. Meredith et al., 1987; M. A. Meredith & Stein, 1983, 1985, 1986a, 1986b; Wallace & Stein, 1997, 2001). The integrative nature of the SC is possible because this neural structure is composed of overlapping sensory maps, (i.e. visual, auditory and somatosensory) which are in spatial register with one another (Stein et al., 2014). Thus, if auditory and visual stimuli are presented in close spatial proximity (i.e. within each other's respective receptive fields) there is a high likelihood that response enhancement and superadditivity will be observed in a single SC neuron under these multisensory conditions (M. A. Meredith & Stein, 1986a). In addition to the spatial principle, numerous studies have investigated the principles of time (M. A. Meredith et al., 1987) and effectiveness (M. A. Meredith & Stein, 1986b), as described above, demonstrating similar neural enhancements under multisensory conditions. Based on these neural responses, and the known connections from the SC to the brainstem and spinal cord (M. Meredith, Wallace, & Stein, 1992; M. A. Meredith & Stein, 1985) investigators were also interested in the role of the SC on multisensory behavior. In awake behaving animals, it was shown that these neural enhancements directly result in behavioral gains (Wallace et al., 1998), such as an increase in accuracies and orienting behaviors under multisensory conditions (Stein, 1998; Stein, Huneycutt, & Meredith, 1988; Stein, Meredith, Huneycutt, & McDade, 1989). Further investigations then demonstrated that the selective disruption of the SC results in a dramatic decrease in behavioral performance for orienting to multisensory stimuli with even small lesions in the SC resulting in these behavioral impairments (Burnett, Henkel, Stein, & Wallace, 2002; Burnett, Stein,

Chaponis, & Wallace, 2004). Overall, the SC has been shown to be critical for both neural responses and the resultant behavior under multisensory conditions, however investigators were also interested in evaluating the role of the neural structures that project to the superior collicus.

The SC receives both ascending input from unisensory subcortical structures (Edwards et al., 1979; Huerta & Harting, 1984; May, 2006; Sparks & Hartwich-Young, 1989) and descending input from cortical brain regions (Jiang, Jiang, & Stein, 2002; Stein & Meredith, 1993; Wallace & Stein, 1994, 2000). It has been shown in the cat model that the deep layers of the SC receive sensory inputs from multisensory cortical structures such as the anterior ectosylvian sulcus (AES) and rostro-lateral suprasylvian sulcus (rLS) (Murray & Wallace, 2011; Stein et al., 2014; Stein et al., 2002). Both neural and behavioral investigations were designed to evaluate and potentially establish a subcortical-cortical circuit between SC and AES in the cat model (Jiang et al., 2002; Jiang, Jiang, & Stein, 2006; Jiang et al., 2001; Wallace et al., 1992; Wallace et al., 1993; Wallace & Stein, 2000; Wilkinson, Meredith, & Stein, 1996). In these studies investigators utilized single cell electrophysiological recordings from multisensory neurons in the SC while simultaneously deactivating this cortical region. It was shown that by selectively cooling these cortical regions with cryoblockades it is possible to deactivate multisensory neurons in the superior colliculus (Jiang et al., 2001; Wallace & Stein, 1994, 2000). Prior to silencing AES, neurons in the SC demonstrated superadditive responses under multisensory conditions, however once AES was deactivated these superadditive properties were lost resulting in subadditive or additive neural responses. Therefore, when AES was deactivated these SC neural responses were either equivalent or smaller under multisensory compared to the combined unisensory conditions, however once AES was then reactivated these superadditive neural responses to multisensory stimuli returned in the SC (Stein et al., 2002). In addition to these neural findings, it

has been shown that the deactivation of AES can disrupt the resultant behavior under multisensory conditions (Jiang et al., 2002; Wilkinson et al., 1996). For example, once this cortical region was silenced, animals were no longer more accurate under multisensory compared to unisensory conditions, however similar to the neural studies, greater behavioral accuracies under multisensory conditions returned once AES was reactivated. Therefore, it is thought that the ascending sensory projections from subcortical sensory structures allows for neurons in the SC to respond to multiple sensory stimuli, resulting in additive responses (M. A. Meredith, 2002; Stein & Meredith, 1993). However, it is believed that it is these descending inputs from cortical structures such as AES that are modulating multisensory integration in the SC and thus allowing for multisensory facilitation via superadditive responses (Stein & Stanford, 2008; Stein et al., 2014; Wallace et al., 1993; Wallace & Stein, 1994, 2000). Thus, this cortical to subcortical circuit occurs by AES modulation of the multisensory responsive SC neurons and therefore is critical for both the neural and behavioral enhancements observed under multisensory conditions (Jiang et al., 2002, 2006; Jiang et al., 2001; Wallace et al., 1992; Wallace & Stein, 1994, 2000; Wilkinson et al., 1996). The AES is composed of higher order sensory structures that respond to visual (AEV), auditory (FAES) and somatosensory (SIV) stimuli (Stein & Stanford, 2008; Stein & Wallace, 1996; Wallace, Carriere, Perrault, Vaughan, & Stein, 2006). Interestingly, while it has been shown that the AES itself contains multisensory neurons, it is the neurons from the unisensory association cortices that project down to the SC resulting in neural enhancements under multisensory conditions (Wallace et al., 1993; Wallace & Stein, 1994). In order to determine the role of these cortical multisensory neurons specifically in the context of behavior further investigation still need to be pursued. Overall, both subcortical and cortical structures have been extensively studied along with the circuit that allows for

behavioral gains to be conferred under multiple sensory stimuli in the cat model system. In addition to these critical findings, investigators were also interested in how these response properties developed in multisensory neurons.

The cat was the initial animal model utilized and the development of multisensory processing has now been extensively evaluated in various species (Hillock, Powers, & Wallace, 2011; Ross et al., 2011; Stein, Meredith, & Wallace, 1994; Wallace, 2004; Wallace & Stein, 2001). The initial question was are these multisensory responsive neurons present at birth or is it the environment/experience that is integral for these multisensory responses? It was demonstrated that neurons in the SC respond to different unisensory modalities during development with somatosensory responses developing first then audition and then finally responses to vision developing last (Wallace & Stein, 1997). In addition, it was shown that the number of multisensory neurons is limited early in life and increases throughout development, peaking around adulthood (Wallace, 2004; Wallace & Stein, 2001). Also, early in development the limited number of neurons in the deep layers of the SC that do respond to multisensory stimuli demonstrate immature responses such as demonstrating additive but not superadditive responses to multiple sensory stimuli (characteristic of adult multisensory neurons) (Stein & Rowland, 2011; Stein et al., 2014). Interestingly, it was shown cortically that both the response properties and the number of multisensory neurons in the AES develop in a similar developmental time period compared to the superior colliculus (Wallace et al., 2006). To this point, once the connections between the SC and AES fully develop, these SC neurons are now capable of producing superadditive responses under multisensory conditions (Rowland, Jiang, & Stein, 2014; Stein et al., 2002; Wallace et al., 1993; Wallace & Stein, 1994, 2000). Therefore, it appears it is the development of these cortical projections in AES that is critical for these

multisensory response properties in SC neurons. Based on these developmental findings, investigators were also interested in determining how the sensory environment could potentially modulate these multisensory responses. To this point, various studies have demonstrated that specific environmental conditions can dramatically alter neural responses to multisensory stimuli (Xu, Yu, Rowland, Stanford, & Stein, 2012; Yu, Xu, Rowland, & Stein, 2013). For example, it has been shown that rearing animals in the absence of typical sensory stimulation (i.e. darkness or masking noise) can result in a dramatic decrease in the neural responses (i.e. subadditive or additive) to multisensory stimuli that is present in and can persist throughout adulthood (Carriere et al., 2007; Wallace, Perrault, Hairston, & Stein, 2004; Xu, Yu, Rowland, Stanford, & Stein, 2014). In addition, it has been shown that by changing the sensory environment an animal develops in, it is possible to alter the neural responses observed under the classic principles of multisensory integration (Wallace & Stein, 2007). For example in Wallace et al, cats were raised in a sensory environment where an auditory noise burst and a visual flash were presented spatially apart and outside of each others receptive fields, which typically results in multisensory depression in adult animals. Surprisingly when these animals, now as adults, were presented with spatially congruent sensory stimuli response depression was observed and surprisingly superadditive responses and response enhancements were only observed when the stimuli were presented spatially apart, similar to the environments in which they were raised. Thus this demonstrated that by altering the sensory environment, one could also change how SC neurons respond to multisensory stimuli (Wallace & Stein, 2007). Therefore, these studies demonstrated that multisensory response properties do develop over time, need cortical inputs to develop properly and are highly sensitive to the environment in which animals develop.

In addition to these classical animal model studies, investigators have also evaluated multisensory processing on the neural and behavioral level in humans (Driver & Noesselt, 2008; Murray & Wallace, 2011). Numerous human psychophysical studies have demonstrated that, like in animal models, behavioral benefits can be conferred such as increases in accuracies and reductions in reaction times under multisensory compared to unisensory conditions (G. A. Calvert & Thesen, 2004; R. A. Stevenson, Ghose, et al., 2014). It has been shown that these multisensory processes appear during childhood, develop and mature continuously throughout life peaking in adulthood (Hillock et al., 2011; Ross et al., 2011; R. A. Stevenson et al., 2015). Various brain regions have been studied however one neural structure that has been consistently implicated in multisensory processing is the superior temporal sulcus (STS) (Beauchamp, Argall, Bodurka, Duyn, & Martin, 2004; Noesselt et al., 2007; R. A. Stevenson, VanDerKlok, Pisoni, & James, 2011). It has been shown that the STS responds under simple and complex audiovisual conditions (Beauchamp, Lee, Argall, & Martin, 2004; A. R. Powers, 3rd, Hevey, & Wallace, 2012; R. A. Stevenson & James, 2009) along with audiovisual illusions (Beauchamp, Nath, & Pasalar, 2010), with the amount activity in the STS correlating with the degree of behavioral perception under these multisensory conditions (Audrey R Nath & Beauchamp, 2012; A. R. Nath, Fava, & Beauchamp, 2011). In addition to typical development, studies are now evaluating multisensory processing on both the behavioral and neural level in the context of neurodevelopmental disorders in human clinical populations (Wallace & Stevenson, 2014). Disorders such as autism and schizophrenia have been consistently associated with atypical responses to (multi)sensory stimuli (Behrendt & Young, 2004; Collignon et al., 2013; de Gelder, Vroomen, Annen, Masthof, & Hodiamont, 2003; de Gelder et al., 2005; Foss-Feig et al., 2010; Foxe et al., 2013; Kwakye, Foss-Feig, Cascio, Stone, & Wallace, 2011; Martin, Giersch, Huron,

& van Wassenhove, 2013; N. Russo et al., 2010; R. Stevenson et al., 2013) with intriguing evidence suggesting that sensory dysfunction may have cascading effects into higher order domains such as social communication (R. A. Stevenson, Siemann, Schneider, et al., 2014), which naturally utilize multiple sensory stimuli. Therefore the study of (multi)sensory processing in the context of these disorders would allow for a better understanding of the atypical underlying mechanisms along with the resultant behaviors, with the ultimate goal of potentially developing more effective remediation tools for these individuals in the future.

Most of the classical animal work that evaluated the underlying neurobiology and development of multisensory processing was done in the cat and monkey model systems (Murray & Wallace, 2011; Stein & Stanford, 2008; Stein et al., 2014). In addition, numerous human psychophysical studies have characterized multisensory integration behaviorally and determined the underlying neural structures that may be implicated (Stein, 2012; R. A. Stevenson, Ghose, et al., 2014). Emerging studies have now focused on evaluating multisensory processing as it relates to clinical disorders/diseases where sensory processing is known to be impacted (Baum, Stevenson, & Wallace, 2015a; Wallace & Stevenson, 2014). Critically, one key use of animal models is the ability to then use these relevant clinical findings to ask important questions focused on how these (multi)sensory systems may be atypical. While classical studies in larger animal models have been instrumental in our understanding of the neural and behavioral bases of multisensory processing, they are somewhat limited in the context of modeling and studying disease or disorder. This is based currently on the limited knowledge and ability for generating relevant genetic models. However, the mouse and more recently the rat are two animal models where the genetics are well established and genetic models associated with disease/disorder are continuously being generated (Nestler & Hyman, 2010). While rodents have

not been classically studied in the context of multisensory processing, a variety of emerging studies are now utilizing these animals to evaluate multisensory function and have demonstrated similar neural and behavioral enhancements under multisensory conditions to those found in larger animal species (Gleiss & Kayser, 2012; Hirokawa et al., 2008). Therefore, rodents may offer an exciting and emerging translational bridge to evaluate the underlying neural mechanisms and behaviors for multisensory function in the context of genetic models associated with disease/disorder.

Emerging Multisensory Studies in Rodents

More recently investigators have begun to use rodent models to evaluate multisensory processing (Carandini & Churchland, 2013). The rat has been utilized based on its well-characterized neural structures, physiology and ability to learn and perform complex behavioral tasks. The mouse is now emerging as a unique animal model for multisensory studies because of the relative costs, number of cohorts generated and most importantly the ability to design and study various genetic models associated with disease or disorder. However, recent advances with CRISPR-Cas systems and technology will most likely make rats more tractable for generating novel genetic models in the future (Cong et al., 2013). Numerous neural and behavioral studies have evaluated basic sensory processing in rodents with most of these investigations focusing on the detection of sensory stimuli from individual sensory modalities (Busse et al., 2011; Cohen, Rothschild, & Mizrahi, 2011; Gaspar, Cases, & Maroteaux, 2003; Radziwon et al., 2009; Sanganahalli, Bailey, Herman, & Hyder, 2009; M. Yang & Crawley, 2009). However, investigations are now beginning to evaluate both behavioral and neural responses under

multiple sensory conditions in rodent models (Hirokawa et al., 2008; Olcese et al., 2013; Raposo, Sheppard, Schrater, & Churchland, 2012; M. T. Wallace et al., 2004).

In most multisensory behavioral studies, rats have been commonly used to assess multisensory function based on their ability to complete complex operant tasks (Gleiss & Kayser, 2012; Sakata, Yamamori, & Sakurai, 2004; Sheppard, Raposo, & Churchland, 2013). In these paradigms, rats are placed in operant chambers and over a series of training sessions, learn to respond to individual sensory stimuli alone (i.e. visual lights or auditory tones) and paired multisensory stimuli for either a liquid or food reward. Typically in these paradigms, rats are required to hold a specific position until a sensory stimulus is presented and then need to respond as quickly and as accurately as possible in order to receive a reward. Based on the relative ease of stimulus presentation, the design of most operant chambers and the relevance for the human population, audiovisual stimuli have been frequently used to assess multisensory function behaviorally (Carandini & Churchland, 2013; Sakata et al., 2004) despite the fact that rodents tend to have fairly poor vision and have more specialized somatosensory and olfactory systems (Ihara, Yoshikawa, & Touhara, 2013; Petersen, 2014). Overall, in these studies a variety of different stimuli, length of stimulus durations and specific multisensory tasks have been used to evaluate multisensory processing in rats (Carandini & Churchland, 2013; Gleiss & Kayser, 2012; Hirokawa et al., 2008; Raposo et al., 2012; Sakata et al., 2004).

While cortical and subcortical brain regions responsible for multisensory processing have been previously identified in larger animal species (Stein & Meredith, 1993), these underlying neural mechanisms have been more recently evaluated in rodent models. Studies have identified neural structures in rats (Barth, Goldberg, Brett, & Di, 1995; Brett-Green, Fifkova, Larue, Winer, & Barth, 2003; Hirokawa et al., 2008; Menzel & Barth, 2005; Sieben, Roder, & Hanganu-Opatz,

2013; Tees, 1999) including specific border regions or zones between primary sensory cortices (visual, auditory, somatosensory) that contain a large number of multisensory neurons and demonstrate similar firing patterns to those found in larger animal models (M. T. Wallace et al., 2004). In addition to these cortical structures, studies have also investigated the role of the superior colliculus (SC) in rats in multisensory function on both the neural and behavioral level (Hirokawa et al., 2011; May, 2006; Sparks & Hartwich-Young, 1989). Intriguingly, these subcortical and cortical findings are similar to those that have been observed in larger animal models, suggesting that these systems may be conserved across species (May, 2006; Wallace & Stein, 1996). Prior animal studies have used a variety of techniques to further understand the underlying mechanisms needed for multisensory processing with many of these methods either permanently or transiently disrupting these neural systems in order to determine their role on the resultant multisensory behaviors (Stein et al., 2002).

Classically, studies have utilized lesions, pharmacology or cooling blockades to disrupt and evaluate multisensory function in animal models (Burnett et al., 2002; Burnett et al., 2007; Jiang et al., 2006; Jiang et al., 2001; Wilkinson et al., 1996). One study in particular, Hirokawa et al, used pharmacology to transiently disrupt and identify a cortical region that may be critical for multisensory processing in the awake behaving rat. In this study, animals completed a multisensory behavioral task and it was found that the greatest neural activity occurred at a border region between primary visual and auditory cortices known as the lateral part of V2 or V2L. In a new cohort of animals, cannula were placed bilaterally targeting V2L, and muscimol, a GABA A agonist, was injected into the cannula and behavioral performance was assessed under unisensory and multisensory conditions. It was observed that when V2L was silenced with muscimol, rats performed slower under multisensory conditions compared to animals that

received control injections of saline into V2L, however this deactivation did not affect behavioral reaction times under unisensory conditions (i.e. visual or auditory alone). Therefore, this study demonstrated that V2L is a cortical structure necessary for behavioral enhancements under multisensory conditions in the rat model system (Hirokawa et al., 2008). Interestingly, there have been recent studies showing anatomical connections between A1 and V2L along with V1 and V2L in the mouse model (Charbonneau, Laramee, Boucher, Bronchti, & Boire, 2012; Laramee, Kurotani, Rockland, Bronchti, & Boire, 2011). Therefore, V2L may be a multisensory structure that is conserved across rodent species, however further studies need to be pursued in order to evaluate this hypothesis in the mouse model. As mentioned previously, neural and behavioral studies have evaluated the SC under multisensory conditions in rodents (U. Drager & D. H. Hubel, 1975; Drager & Hubel, 1976; U. C. Drager & D. H. Hubel, 1975; Hirokawa et al., 2011) and in addition there is evidence suggesting that V2L projects to the SC in rodents (May, 2006; Sparks & Hartwich-Young, 1989). This is intriguing since studies have shown that a region of multisensory cortex (i.e. AES) projects to and modulates neural responses in the SC in the cat model system (Jiang et al., 2001; Stein et al., 2002; Wallace & Stein, 1994, 2000). Therefore, these findings offer the possibility that a similar cortical-subcortical circuit may be present in rodents and warrants future investigations.

While prior investigations have focused on the rat, most recently studies have evaluated multisensory processing in the mouse model system. Despite the presence of these exceedingly valuable animal models for clinical and translational research, the mouse has not been a well-studied or well-characterized model species for multisensory research. To this point, while there have been a few investigations evaluating the underlying neural mechanisms of multisensory integration in the mouse (Gogolla, Takesian, Feng, Fagiolini, & Hensch, 2014; Olcese et al.,

2013; Reig & Silberberg, 2014), there currently are no multisensory behavioral paradigms available for this species. Despite this lack of behavioral investigation, there have been intriguing emerging findings into the cellular and molecular mechanisms of multisensory integration in the mouse. For example, Olcese et al recorded from a border region between primary visual and somatosensory cortices (RL) and found a large number of neurons demonstrating multisensory properties (i.e. superadditive responses). Similar to the neural findings in rats, the incidence of these multisensory neurons was greatest in the middle of this multisensory region with unisensory neurons more likely to be found closer to their respective unisensory cortices. In addition, this study demonstrated that the pyramidal cells found in layers II/III were responsible for these multisensory enhancements and by using optogenetics to stimulate the neighboring interneurons it was possible to disrupt these superadditive responses in the pyramidal cells (i.e. resulting in additive responses). Therefore, Olcese et al were the first to identify and characterize cellular and molecular mechanisms of multisensory integration in the mouse model.

Based on these emerging results in wild type mice, Gogolla et al focused on evaluating the neural mechanisms of multisensory processing in mouse models associated with autism spectrum disorder. The rationale being that it has been shown, and will be further discussed in this introduction, that individuals with autism spectrum disorder (ASD) may respond atypically to sensory stimuli with emerging neural and behavioral evidence demonstrating multisensory processing deficits in ASD as well (Baum et al., 2015a). Gogolla et al assessed multisensory function in the insular cortex by recording neural responses to auditory and somatosensory stimuli along with the combined multisensory presentations. It was shown that multisensory responses were atypical and the magnitude or amount of multisensory enhancement was reduced in mouse models associated with ASD compared to wild type animals. In addition, it was shown

that these mouse models had disruptions in the inhibitory circuitry. Based on these findings, the authors then chronically administered diazepam, a benzodiazepine agonist, to mouse models associated with ASD during a critical period of development (i.e. between P15 and P28) for two weeks. Multisensory function was then assessed during adulthood and it was observed that these neural responses under multisensory conditions normalized in mouse models associated with ASD and were now comparable to those found in wild type animals. Intriguingly, this treatment also normalized repetitive behaviors, which were previously observed in these mouse models (Gogolla et al., 2014). Therefore, this was the first study to demonstrate atypical (multi)sensory processing on the neural level in mouse models associated with ASD and provides intriguing evidence suggesting that that this multisensory dysfunction may have cascading effects into core domains such as repetitive or restricted interests that characterize ASD.

Overall, there have been a variety of multisensory studies that have evaluated the neural mechanisms and the resultant behaviors in rodents (Brett-Green et al., 2003; Carandini & Churchland, 2013; Hirokawa et al., 2008; Sakata et al., 2004; M. T. Wallace et al., 2004). However, it has only been until relatively recently that studies have investigated multisensory integration in the mouse model system (Gogolla et al., 2014; Olcese et al., 2013; Reig & Silberberg, 2014). The major advantage of the mouse is the ability to generate genetic models to further study the underlying mechanisms and behaviors associated with disease or disorder (Nestler & Hyman, 2010). While there are emerging neural studies beginning to evaluate multisensory integration in the mouse, there currently are no multisensory behavioral paradigms available for this species. Therefore, the design of novel behavioral paradigms that assess multisensory function in the mouse may be critical for investigators to further understand the

underlying neurobiology and evaluate potential atypical behavioral responses under multisensory conditions in models associated with these clinical disorders.

Introduction to Autism Spectrum Disorder

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder characterized by impairments in social communication as well as the presence of repetitive and restrictive behaviors (APA, 2011). Currently estimates place the prevalence of ASD as 1 in 68 individuals, with the incidence being five-fold higher in males and diagnoses of ASD typically occurring by 3 years of age ((CDC), 2015). Comorbidities with anxiety, ADHD and epilepsy are relatively common in individuals with ASD along with abnormal responses to sensory stimuli, gastrointestinal issues and sleep disturbances (Bauman, 2010; Goldman et al., 2009; Reynolds & Malow, 2011; Simonoff et al., 2008). Genetic studies have found that concordance rates for siblings and fraternal twins may range between 0-10% and for identical twins can range from 70-90% (Bailey et al., 1995). Various genes have been implicated in ASD spanning numerous neurotransmitter systems, yet mainly rare genes compared to common genetic variants have been observed generating relatively small sample sizes (Abrahams & Geschwind, 2008).

In order to investigate the potential causes for the symptoms observed in ASD, there have been a number of investigations evaluating the underlying neurobiology of this disorder (S. E. Levy, Mandell, & Schultz, 2009). As a result, studies have characterized and demonstrated both atypical structural and functional connections in autism spectrum disorder (Courchesne, Redcay, Morgan, & Kennedy, 2005; Rippon, Brock, Brown, & Boucher, 2007). One of these findings in ASD is altered minicolumnar structure (Casanova, Buxhoeveden, Switala, & Roy, 2002). A cortical minicolumn is the fundamental unit in the cortex that is comprised of excitatory

pyramidal cells surrounded by inhibitory interneurons. Postmortem studies revealed that in the frontal and temporal lobes, there were increased numbers of minicolumns, pyramidal neurons in the minicolumnar structure and the overall structure was narrower in individuals with ASD (Casanova, 2007). More excitatory pyramidal neurons along with a greater number of narrower minicolumns could result in over activation; leading to local cortical connections more likely and long range connections less likely to develop (Di Cristo, 2007; Raghanti, Spocter, Butti, Hof, & Sherwood, 2010). To this point, various investigations have studied the role of functional connections between neural structures in ASD (Hughes, 2007; Wass, 2011). Functional connectivity can describe how well brain regions are connected based on the temporal synchronization of the activity between these areas (David, Cosmelli, & Friston, 2004) and it has often been shown that this connectivity is altered in ASD for a variety of tasks (Just, Cherkassky, Keller, & Minshew, 2004; Kana, Libero, & Moore, 2011; Kleinhans et al., 2008; Minshew & Keller, 2010). Based on these structural and functional findings, numerous theories have been proposed to potentially explain the neural, physiological and/or behavioral differences observed in autism spectrum disorder (F. Levy, 2007).

Weak central coherence, the temporal binding deficit hypothesis, the cortical underconnectivity theory and an imbalance of excitation/inhibition signaling are common and related theories that have been proposed in an attempt to explain the neural and behavioral impairments found in ASD. Central coherence is based on the concept that individuals are capable of processing and combining information for a higher level of understanding (Happe, 1999). The theory of weak central coherence (WCC) states that individuals with ASD may have impairments in integrating information from a local or detailed perspective to a more global concept (Happe & Frith, 2006). Thus, if there is a preference for local over global processing,

this could cause the global meaning of social situations and communication to be impaired. Brock's temporal binding deficit hypothesis has attempted to explain weak central coherence by evaluating potential timing deficits in ASD. This theory proposed that long-range connections between brain regions might not be as temporally correlated as brain regions that are in closer proximity, which could result in intact local, but impaired global processing (Brock, Brown, Boucher, & Rippon, 2002). The cortical underconnectivity theory has then explained this hypothesis in more detail; with a number of functional magnetic resonance imaging (fMRI) studies demonstrating that long distance connections between brain regions are less functionally connected in individuals with ASD (Just et al., 2004; Kana et al., 2011). This theory of underconnectivity compliments the previous two theories (i.e. WCC and temporal binding deficit hypothesis) because it provides a potential explanation for deficits in global processing since this requires both the proper timing and integration of information across multiple brain regions. Lastly, a more molecular driven theory of ASD has focused on the potential imbalance in the ratio of excitation/inhibition signaling in the brain (Rubenstein & Merzenich, 2003). This theory has been proposed based on the observations that about 30% of individuals with ASD have a comorbid diagnosis of epilepsy (Maski, Jeste, & Spence, 2011; Sgado, Dunleavy, Genovesi, Provenzano, & Bozzi, 2011) along with the findings of atypical minicolumnar structure in ASD (Casanova et al., 2002), which could result in enhanced local and reduced global cortical connections (Casanova, 2007; LeBlanc & Fagiolini, 2011). Therefore, these theories potentially compliment one another in describing how structural, functional and behavioral impairments may develop and impact individuals with ASD. While no one theory is all encompassing, there are aspects from each of these theories that may lead to greater insights in explaining the underlying neural and behavioral impairments that are observed in ASD.

Autism Spectrum Disorder: Evidence of Atypical Sensory Processing

In addition to the classic domains described in autism spectrum disorder, sensory abnormalities are also highly prevalent with estimates of up to 85% of individuals with ASD being impacted (Iarocci & McDonald, 2006). To this point, the DSM-V now includes sensory disturbances as a criterion for diagnosis (APA, 2011). Kanner first reported sensory disruptions in children with autism spectrum disorder and to date there have been a number of studies demonstrating atypical sensitivity to a variety of sensory stimuli (Kanner, 1943; Klintwall et al., 2011). The nature of these sensory disturbances is highly heterogeneous, with evidence of hyper- or hyposensitivity to numerous different stimuli spanning multiple sensory modalities (Minshew & Hobson, 2008; Rogers & Ozonoff, 2005; Schoen, Miller, Brett-Green, & Nielsen, 2009). Using a visual stimulus as an example, individuals with ASD may immediately cover their eyes or stare at bright lights for long periods of time, depending on the individual's sensitivity to the stimulus. To this point, individuals with ASD can be highly engaged in stimuli that may typically be considered mundane. Therefore, this dysfunction can be classified as sensory aversion or sensory seeking behavior (Ben-Sasson et al., 2009). Based on these observations, studies have also correlated sensory impairments with ASD severity (Hilton et al., 2010; Kern et al., 2007). While numerous studies have demonstrated atypical sensory processing in multiple sensory domains (Baum et al., 2015a; Cascio, 2010; Marco, Hinkley, Hill, & Nagarajan, 2011), the focus of this section will be on dysfunctions in the visual and auditory domains based on being extensively studied and in part because of the central role of these modalities in processes such as communication and social interactions.

Numerous studies have investigated visual processing in individuals with autism spectrum disorder by using simple and complex stimuli (Dakin & Frith, 2005). A consistent

finding is that individuals with ASD tend to perform well on visual tasks that require the processing of individual features (Behrmann, Thomas, & Humphreys, 2006; Y. Chen et al., 2012). For example, individuals with ASD can excel on the Embedded Figures and Block Design tasks, which require the identification of smaller objects within a larger object or the recreation of a detailed pattern (Burnette et al., 2005; Happe, 1999; Happe & Frith, 2006). In addition, individuals with ASD tend to perform more accurately and faster than typically developed (TD) individuals on various visual search tasks (Plaisted, O'Riordan, & Baron-Cohen, 1998). In these tasks, individuals must identify a visual target that is surrounded by numerous distractors that can be either completely different from the target or, to make the task more difficult, can share physical aspects with the target stimulus (i.e. same shape or color). Thus, there is consistent evidence of enhanced behavioral performance for individuals with ASD on tasks requiring the identification of specific visual features. However, there have also been observations of impaired global processing in ASD when these local features are needed to be combined and evaluated in more complex visual tasks (Behrmann et al., 2006). For example, studies have found that individuals with ASD tend to be less accurate for more complex visual tasks such as identifying biological motion (Blake, Turner, Smoski, Pozdol, & Stone, 2003), visual motion coherence (Tsermentseli, O'Brien, & Spencer, 2008) and visual form (Spencer & O'Brien, 2006) compared to typically developed individuals. As a result of these studies, there is evidence of superior performance in ASD when visual tasks require more local or detailed processing, yet impaired performance when these tasks become more complex or require more global processing (Dakin & Frith, 2005).

In addition to the visual domain, a variety of studies have evaluated the auditory system in ASD. Similar to the visual findings, it has been shown that depending on the complexity of the

auditory stimuli and task, individuals with ASD may perform differently compared to TD individuals (O'Connor, 2012). For example, it has been shown that individuals with ASD tend to perform better on tasks that require pitch discrimination specifically for simple pure or musical tones (Kellerman, Fan, & Gorman, 2005; Mottron, Peretz, Belleville, & Rouleau, 1999; O'Riordan & Passetti, 2006) and these findings were replicated with individuals with ASD who had no prior musical training or background (Heaton, Hermelin, & Pring, 1998). Interestingly though, when these auditory stimuli become more complex, deficits in auditory processing tend to be observed in ASD. To this point, studies have shown that children with ASD may be less likely to orient to auditory stimuli, with the greatest deficits being observed for speech stimuli (i.e. calling the child's name) (Dawson, Meltzoff, Osterling, Rinaldi, & Brown, 1998). Also, impairments have been found in ASD when participants were needed to identify speech syllables in varying degrees of background noise (Alcántara, Weisblatt, Moore, & Bolton, 2004). Neural studies have demonstrated that individuals with ASD have similar auditory brainstem responses to simple auditory stimuli such as clicks, however these responses were found to be atypical for more complex auditory stimuli such as speech syllables under both quiet and noisy environment conditions (O'Connor, 2012; Nicole Russo, Zecker, Trommer, Chen, & Kraus, 2009). In addition, atypical neurophysiological findings using EEG and fMRI techniques have been observed in ASD when evaluating more complex auditory conditions such as speech stimuli (Bruneau, Bonnet-Brilhault, Gomot, Adrien, & Barthélémy, 2003; Gervais et al., 2004; Hesling et al., 2010; Lai, Pantazatos, Schneider, & Hirsch, 2012; Lai, Schneider, Schwarzenberger, & Hirsch, 2011; O'Connor, 2012).

Enhanced visual and auditory discrimination to detailed information, yet overall impaired global processing in these individual sensory modalities appear to be consistent findings in ASD

(Dakin & Frith, 2005; O'Connor, 2012). Neural and behavioral based theories such as weak central coherence and the underconnectivity hypothesis have been utilized in an attempt to explain these differences (Happe, 1999; Happe & Frith, 2006; Rippon et al., 2007). Thus, superior performance for specific details may arise from enhanced local connectivity and impaired performance on tasks needed for global processing may then result from decreased connectivity across multiple brain regions (Kana et al., 2011). To further evaluate these differences, investigations in ASD have focused on both behavioral and neural studies that require performance under multiple sensory conditions (Baum et al., 2015a).

Evidence of Atypical Multisensory Processing in Autism Spectrum Disorder

Based on the wealth of evidence highlighting disturbances across a number of sensory systems, there has been increased focus on better characterizing how the integration of information across these different sensory modalities is impacted in autism spectrum disorder. Such multisensory integration plays an integral role in the creation of a unified perceptual gestalt and is fundamental in allowing us to successfully interpret communicative and social signals. Numerous neural and behavioral studies have recently demonstrated atypical multisensory processing in individuals with ASD (Baum et al., 2015a; Foss-Feig et al., 2010; Iarocci & McDonald, 2006; Kwakye et al., 2011; Marco et al., 2011; N. Russo et al., 2010; R. A. Stevenson, Segers, Ferber, Barense, & Wallace, 2014; Wallace & Stevenson, 2014). Evidence of decreased multisensory gain or benefit has been reported utilizing both simple and complex stimuli (Collignon et al., 2013; Mongillo et al., 2008; R. A. Stevenson, Siemann, Schneider, et al., 2014; R. A. Stevenson, Siemann, Woynaroski, et al., 2014), these multisensory deficits have been correlated with symptom severity (Brandwein et al., 2015) and the development of

multisensory processing in ASD has been evaluated as well (Brandwein et al., 2012; Foxe et al., 2013; R. Stevenson et al., 2013; Taylor, Isaac, & Milne, 2010).

One effective way to measure multisensory integration is with the use of cross modal illusions. These illusions can produce interesting behavioral responses based on how one modality affects another. The McGurk effect is a well-known illusion that has been evaluated in children, adults and individuals with neurodevelopmental disorders (Mcgurk & Macdonald, 1976; van Wassenhove, Grant, & Poeppel, 2007; Woynaroski et al., 2013). In this illusion a speaker typically utters the phoneme "ga" while viewers will simultaneously hear an auditory "ba". Interestingly, under these conflicting audiovisual conditions, individuals tend to perceive an entirely new percept and typically report that the speaker uttered the phoneme of either "da" or "tha". Therefore, it is believed that viewers are integrating the visual "ga" with the auditory "ba" and thus perceiving a completely novel percept under these audiovisual conditions (Mcgurk & Macdonald, 1976). Based on these intriguing behavioral findings, investigators were interested in determining the neural structures that are critical for the perception of this illusion. These studies have demonstrated that the superior temporal sulcus (STS) is integral for the perception of the McGurk effect (Audrey R Nath & Beauchamp, 2012; A. R. Nath et al., 2011) and when this brain region is disrupted by transcrancial magnetic stimulation (TMS) the amount of McGurk perception decreases dramatically in typically developed individuals (Beauchamp et al., 2010).

Intriguingly, a variety of studies have also evaluated the McGurk effect in clinical populations such as ASD. Overall, studies have demonstrated that individuals with ASD tend to perceive the McGurk effect less frequently compared to TD individuals (Irwin, Tornatore, Brancazio, & Whalen, 2011; Mongillo et al., 2008; R. Stevenson et al., 2013; R. A. Stevenson,

Siemann, Schneider, et al., 2014; Taylor et al., 2010). However, there have also been observations of no differences in the McGurk perception between these groups (Keane, Rosenthal, Chun, & Shams, 2010; Williams, Massaro, Peel, Bosseler, & Suddendorf, 2004). These discrepancies in the literature may be due to the heterogeneity in ASD, the ages of the participants, differences in the types of stimuli used along with the instructions given during the task, all of which warrant further investigation. However, the majority of the studies that have found a decreased perception of the McGurk effect in ASD have then described this as a decrease in the strength or magnitude of multisensory integration in individuals with ASD. In addition, studies have evaluated the McGurk effect across development (R. Stevenson et al., 2013; Taylor et al., 2010). In childhood, both TD individuals and individuals with ASD perceive this illusion less frequently, which is consistent with prior findings that multisensory function develops and matures throughout life (Hillock et al., 2011; Ross et al., 2011; R. A. Stevenson et al., 2015). However, it was then observed that in adolescence while TD individuals demonstrated an increased perception of the McGurk effect compared to the younger TD participants, individuals with ASD reported a similar (i.e. limited) amount of McGurk perception as found in the younger participants with ASD (R. Stevenson et al., 2013). Therefore, there is evidence suggesting that the development of multisensory integration might be delayed or potentially atypical in individuals with ASD (Brandwein et al., 2012; Foxe et al., 2013; R. Stevenson et al., 2013; Taylor et al., 2010).

Another common multisensory illusion is the simultaneity induced flash illusion (SIFI), which demonstrates that the presence of auditory stimuli can alter an individual's visual perception. In this illusion when two auditory beeps are played in close temporal proximity to a single visual flash, most individuals tend to report seeing multiple visual flashes (L. Shams,

Kamitani, & Shimojo, 2002). In addition, it has been shown that as the amount of time increases before the presentation of the second beep, participants are less likely to perceive the illusion and thus more likely to report a single visual flash (Shimojo & Shams, 2001). The SIFI has been used to evaluate multisensory function in typically developed children, adults and in individuals with ASD as well (Ladan Shams, Kamitani, & Shimojo, 2000; R. A. Stevenson, Siemann, Woynaroski, et al., 2014). It was found that adults with ASD perceived this illusion as frequently as TD controls (van der Smagt, van Engeland, & Kemner, 2007), however when this was evaluated in children it was shown that individuals with ASD perceived the sound induced flash illusion less frequently compared to TD children (R. A. Stevenson, Siemann, Woynaroski, et al., 2014). Therefore, in addition to the McGurk effect findings, this is further evidence that the strength or magnitude of multisensory integration may be decreased or atypical in individuals with ASD and this may be evident specifically earlier in development.

Although the creation of our multisensory worldview involves numerous factors, one of the most important of these is time. As mentioned previously one of the key principles that defines multisensory integration is in the temporal domain (M. A. Meredith et al., 1987). Specifically, there appears to exist a window of time within which events specified in different sensory modalities are perceptually "bound" (Hillock et al., 2011). Such a window makes a great deal of ethological sense, since events happening in close temporal proximity are highly likely to be associated with the same event. This multisensory temporal binding window (TBW) can be described as the time interval in which two cross-modal stimuli are bound together as a single unified perceived event (A. R. Powers, Hillock, & Wallace, 2009). To measure the TBW, participants typically perform psychophysical tasks where auditory and visual stimuli are presented either simultaneously or at delayed time intervals, known as stimulus onset

asynchronies (SOAs). These SOAs can be presented with either auditory or visual stimuli leading and normally range from 10-500ms. In these tasks, participants are asked to respond if they perceived these sensory signals as either occurring synchronously or asynchronously (R. A. Stevenson, Ghose, et al., 2014). Interestingly, individuals are not incredibly accurate at judging temporal synchrony with typically developed participants tending to report that these stimuli occur simultaneously over about a 300ms window or range of time depending on the complexity of stimuli (R. A. Stevenson & Wallace, 2013). To this point, it has been demonstrated that stimulus complexity can greatly affect the size of the TBW, where the most complex stimuli such as speech syllables will result in the widest TBW. In addition, studies have further evaluated multisensory temporal processing by establishing relationships between the width of the TBW and the perception of multisensory illusions, such as the McGurk effect and sound induced flash illusion (R. A. Stevenson, Zemtsov, & Wallace, 2012).

Emerging evidence suggests that this multisensory temporal binding window may be abnormally long in individuals with autism spectrum disorder (Baum et al., 2015a; Baum, Stevenson, & Wallace, 2015b; Foss-Feig et al., 2010; Kwakye et al., 2011; Wallace & Stevenson, 2014), and this widened window could result in degraded perceptual representations. For example, Stevenson et al measured the TBW in both TD children and individuals with ASD across three stimulus complexities: simple visual flashes and auditory beeps, tool stimuli (i.e. a hammer hitting a nail) and a speaker uttering speech syllables. As previously reported (R. A. Stevenson & Wallace, 2013), temporal binding windows increased in width as the stimulus complexity increased for both groups, with the widest windows being observed for speech stimuli. However, significant differences in the TBW widths were observed between the groups under the most complex conditions (i.e. speech), where individuals with ASD demonstrated

wider TBWs compared to TD individuals. In addition, the McGurk effect was also measured and it was found that individuals with ASD perceived the McGurk illusion less frequently compared to TD individuals. Correlations between the TBW width and the perception of the McGurk effect were then determined. Interestingly it was found that for individuals with ASD, a wider TBW, regardless of the stimulus complexity, resulted in less perception of the McGurk effect. Therefore, this study hypothesized that those differences in multisensory temporal processing for even low-level sensory stimuli (i.e. visual flashes and auditory beeps) in ASD could result in potential cascading effects into higher order domains such as speech or communication (R. A. Stevenson, Siemann, Schneider, et al., 2014). In addition to the McGurk effect, the sound induced flash illusion has been utilized in conjunction with the TBW in ASD. Foss-Feig et al showed that individuals with ASD perceived this illusion more frequently when the presentations of the second beep were delayed for longer periods of time. Using the SIFI, it was observed that children with ASD demonstrated an extended temporal binding window that was almost twice as wide compared to TBWs of typically developed controls (Foss-Feig et al., 2010). Therefore this evidence of an extended TBW and overall atypical multisensory temporal processing could result in impacting domains that naturally utilize and integrate multiple sensory stimuli such as communication and social interactions, which are known to be impaired in ASD.

In addition to these studies in clinical disorders such as ASD, various investigations have assessed temporal processing specifically in typical development as well (Hillock et al., 2011; A. R. Powers, 3rd et al., 2012; A. R. Powers et al., 2009; R. A. Stevenson & Wallace, 2013; R. A. Stevenson et al., 2012). For example, it has been shown that multisensory temporal binding windows tend to be wider for children and narrow throughout typical development until adulthood (Hillock et al., 2011). In addition, intriguing evidence has shown that it is possible to

narrow the TBW in adults with the implementation of multisensory perceptual training paradigms (A. R. Powers et al., 2009). Powers et al. demonstrated in TD adults that after training under multisensory conditions it was possible to narrow an individual's temporal binding window and these changes lasted up to 2 weeks, when participants returned for follow up studies. Interestingly, it was shown that the individuals with the widest TBWs demonstrated the greatest narrowing of their TBW after multisensory perceptual training. The neural correlates of the TBW were then investigated and intriguingly the STS was found again to be one of the major brain regions responsible for these changes (A. R. Powers, 3rd et al., 2012). To this point, it was shown that the narrowing of the TBW correlated with the amount of change in activity in the superior temporal sulcus (A. R. Powers, 3rd et al., 2012). In addition, the STS has been shown to be a major cortical hub that is responsible for both multisensory integration and processing speech stimuli (Beauchamp, Argall, et al., 2004; Beauchamp et al., 2010; A. R. Nath et al., 2011; Noesselt et al., 2007; A. R. Powers, 3rd et al., 2012; R. A. Stevenson et al., 2011). Interestingly, the STS has also been implicated in ASD based on structural differences in this region along with studies demonstrating atypical functional responses to both social stimuli and biological motion (Boddaert et al., 2004; Pelphrey, Morris, & Mccarthy, 2004; Redcay, 2008; Zilbovicius et al., 2006). Therefore if it is believed that multisensory function could have cascading effects into higher order domains then the role of the STS, where multisensory information converges and both social and speech stimuli are processed, may be critical for further evaluation specifically in the context of autism spectrum disorder. Lastly, it was mentioned that the temporal binding windows tend to be widest for children (Hillock et al., 2011), may be even wider for children with ASD (R. A. Stevenson, Siemann, Schneider, et al., 2014) and the TD adults who had the widest TBWs demonstrated the greatest narrowing under multisensory

perceptual training paradigms (A. R. Powers et al., 2009). Therefore the plasticity of the temporal binding window along with multisensory perceptual training, specifically in children with ASD, may be a promising avenue in the development of potential remediation tools for individuals with this disorder.

Numerous findings suggest the presence of multisensory and temporal processing deficits in individuals with autism spectrum disorder (Baum et al., 2015b; Brandwein et al., 2015; Collignon et al., 2013; Foss-Feig et al., 2010; Foxe et al., 2013; Irwin et al., 2011; Kwakye et al., 2011; Smith & Bennetto, 2007; R. Stevenson et al., 2013; R. A. Stevenson, Siemann, Schneider, et al., 2014; R. A. Stevenson, Siemann, Woynaroski, et al., 2014; Woynaroski et al., 2013). In addition, there is emerging evidence that (multi)sensory function may be critical as potential building blocks for higher order domains, such as social communication, which is a core deficit of ASD (Baum et al., 2015a; R. A. Stevenson, Siemann, Schneider, et al., 2014). However, further investigation is warranted in order to more fully evaluate this novel hypothesis. Overall, studies have demonstrated deficits in both the amount of behavioral benefit or gain observed under multisensory conditions (Mongillo et al., 2008; N. Russo et al., 2010; R. Stevenson et al., 2013; R. A. Stevenson, Segers, et al., 2014; R. A. Stevenson, Siemann, Woynaroski, et al., 2014; Taylor et al., 2010) as well as impaired temporal processing of multisensory stimuli in individuals with ASD (Bebko, Weiss, Demark, & Gomez, 2006; Foss-Feig et al., 2010; Kwakye et al., 2011; R. A. Stevenson, Siemann, Schneider, et al., 2014; Wallace & Stevenson, 2014). While there is mounting evidence of atypical multisensory integration in ASD on both the behavioral and neural level, one way to further investigate the underlying neural mechanisms and behaviors associated with multisensory processing in the context of autism spectrum disorder is with the use of animal models.

Modeling Autism Spectrum Disorder in the Mouse

First, it is important to clearly state that there is no mouse or animal with a human disease or disorder (i.e. autism, depression, schizophrenia). Instead and importantly we are using animals in order to model and potentially gain a better understanding of the aspects of the disease process along with the possible resultant behaviors associated with disease and/or disorder. Every animal model associated with a disease or disorder is subject to critique and typically animal models are evaluated by three common criteria known as construct, face and predictive validity (Nestler & Hyman, 2010). Construct validity asks the question has this model been designed under genetic, cellular or physiological conditions that are implicated in the human disease of interest? Face validity then asks does this model demonstrate any similar behavioral and/or phenotypic characteristics that are observed in the specific disease or disorder? Lastly, predictive validity questions, does this animal model respond to treatments or therapeutic interventions in a similar way to those being observed in the clinical population? In short, if an animal model passes all three validity tests it may be considered as a good model to study for a specific disease or disorder, however passing even one of the three validity tests is important to note for potential future studies as well (Jacqueline N Crawley, 2007b).

Based on the heterogeneity and numerous genes implicated and associated with autism spectrum disorder along with the known genetic tools available for mice, there have been a variety of mouse models that have been generated with genetic alterations spanning numerous neurotransmitter systems (S. Moy & Nadler, 2008; Provenzano, Zunino, Genovesi, Sgado, & Bozzi, 2012). In order to gain a better understanding of the underlying neural mechanisms, a number of studies have evaluated and demonstrated cellular, physiological and structural abnormalities in these models (J. N. Crawley, 2012; Jill L Silverman & Crawley, 2014). In

addition, atypical behavioral responses have been consistently observed in mouse models associated with ASD (Jacqueline N Crawley, 2007a). Most investigations have evaluated communication, social behavior and repetitive behaviors since these have been the classically defined behaviors associated with autism spectrum disorder (J. L. Silverman, Yang, Lord, & Crawley, 2010). Yet, how is it possible to evaluate these clearly human behaviors in a mouse? One way investigators have studied mouse social behavior is by using a well-established assay known as the three-chambered social task (Nadler et al., 2004). In this task, animals are first placed in a central chamber with two closed dividers on opposite walls that connect to the remaining two chambers. After a few minutes to allow the animals to acclimate to the chamber, these dividers are removed. In one of the remaining two chambers there typically is a novel mouse placed under a pencil wire cup and in the other chamber is either an empty pencil cup or an inanimate object that is placed under a pencil cup. The amount of time spent and number of crossings into each of the three chambers is quantified in normally a 10-15 minute period. Mice tend to be fairly social; therefore a wild type mouse is more likely to spend the most time in the chamber where the novel mouse is held compared to either the central chamber or the chamber containing the inanimate object. However, it has been shown that mouse models associated with ASD tend to spend similar amounts of time in all three chambers or an equivalent amount of time in the chambers containing the novel mouse and inanimate object, which has been interpreted as potential deficits in social behavior (S. Moy et al., 2004; S. S. Moy et al., 2007). In order to measure communication in mice, investigators have evaluated the number of ultrasonic vocalizations (USVs) produced when mice pups are separated from the mother (Jacqueline N Crawley, 2004). When wild type (WT) pups are separated from the dam, these animals tend to produce numerous USVs, which has been interpreted as a form of distress. Interestingly, it has

been shown that mouse models associated with ASD tend to produce less frequent USVs compared to WT mice when separated from the dam, which potentially indicates a communicative deficit in these models (J. N. Crawley, 2012). Finally, in order to measure repetitive behaviors, mice are typically videotaped and behaviors are evaluated within the home cage (Jacqueline N Crawley, 2007a). These assays normally utilize video software to quantify the types and amount of behaviors that both wild type mice and mouse models engage in over normally a 24-hour time period. Repetitive behaviors are compared to those behaviors produced in wild type mice and common repetitive behaviors observed in mouse models associated with ASD include excessive grooming, jumping, flipping/somersaulting or wire hanging from the inside of the home cage (Bechard & Lewis, 2012; J. N. Crawley, 2012; J. Veenstra-VanderWeele et al., 2012).

Communication, social behavior and repetitive behavior are all domains that have been consistently evaluated and characterized in mouse models associated with ASD based on the fact that these features have been classically used to define the disorder (J. L. Silverman et al., 2010). However, with the recent addition of sensory disturbances being included in the diagnostic criteria for ASD, it is critical to utilize these mouse models to study the underlying neural mechanisms and resultant behaviors associated with atypical sensory processing. As mentioned previously, numerous human studies in ASD have demonstrated sensory deficits in individual sensory systems (i.e. vision, audition, touch) (Iarocci & McDonald, 2006; Klintwall et al., 2011; Marco et al., 2011) and now there is growing evidence that when this sensory information is combined across modalities, further deficits are observed under multisensory conditions in individuals with ASD (Baum et al., 2015a). Although emerging psychophysical research suggests the presence of multisensory deficits in ASD, in order to begin to ask mechanistic

questions about the nature of these impairments in multisensory function, it is necessary for these studies to be translated into animal models. While behavioral assays have been used to evaluate basic unisensory processing in mice (Jacqueline N Crawley, 2007b; Pinto & Enroth-Cugell, 2000; Radziwon et al., 2009; J. L. Silverman et al., 2010), there has been no behavioral paradigm to assess performance across sensory modalities in this species. Therefore, it is imperative to develop appropriate behavioral assays to evaluate and assess multisensory processing in the mouse model in order to potentially gain a better understanding into the underlying neural mechanisms and resultant behavioral impairments in multisensory function that have been observed in disorders such as ASD.

Describing the SERT Ala56 Mouse Model: Potential Relationships between Serotonin and (Multi)Sensory Processing in the Context of Autism Spectrum Disorder

The mouse has been a widely used model for autism research, specifically because of the insights that it can provide into genetic, neurobiological, and behavioral factors associated with ASD (Jacqueline N Crawley, 2007a). Most germane to this thesis is a mouse model associated with ASD, which was generated at Vanderbilt University and displays a rare variant in the serotonin transporter (SERT) (Jeremy Veenstra-VanderWeele et al., 2009). SERT and the serotonin (5-HT) system have been consistently studied in the context of autism (Cook & Leventhal, 1996; Hammock et al., 2012; Kepser & Homberg, 2015; C.-J. Yang, Tan, & Du, 2014). For example, it has been demonstrated that about 30% of individuals with ASD display hyperserotonemia (i.e. elevated whole blood serotonin) (Gabriele, Sacco, & Persico, 2014; Mulder et al., 2004). In addition, genetic studies have demonstrated that a group of rare variants of SERT conferred excessive activity (Prasad, Steiner, Sutcliffe, & Blakely, 2009; Prasad et al.,

2005), were associated in families with individuals with ASD and the most common of these variants (SERT Ala56) was found to associate with sensory aversion and rigid-compulsive behavior (Sutcliffe et al., 2005). Based on these intriguing findings in the human clinical population, a mouse model of the most common variant, SERT Ala56, was then generated (Jeremy Veenstra-VanderWeele et al., 2009). Interestingly, this knock-in mouse model recapitulated the biomarker of hyperserotonemia and in addition exhibited similar behaviors to those observed in ASD such as deficits in communication and social behaviors along with the presence of repetitive or restrictive behaviors (J. Veenstra-VanderWeele et al., 2012).

The utility of the SERT Ala56 mouse model is that it allows for a potential bridge to connect altered 5-HT function with potential changes in sensory and/or multisensory processing in autism spectrum disorder. A number of studies have demonstrated the role of serotonin on sensory development (Cases et al., 1996; Gaspar et al., 2003; Lesch & Waider, 2012; van Kleef, Gaspar, & Bonnin, 2012), sensory function (Esaki et al., 2005; L. Hurley, Devilbiss, & Waterhouse, 2004; Jitsuki et al., 2011; Waterhouse, Azizi, Burne, & Woodward, 1990) and have shown that serotonin and SERT are expressed in and project to numerous cortical and subcortical brain regions that are critical for sensory processing (Binns, 1999; Gaspar et al., 2003; Lebrand et al., 1998). In addition, it has been shown that the modulation of serotonin can impact neural responses under unisensory conditions (L. M. Hurley & Pollak, 2001; L. M. Hurley, Thompson, & Pollak, 2002; Waterhouse, Moises, & Woodward, 1986), can result in abnormal connections to individual sensory cortices (X. Chen et al., 2015; Gaspar et al., 2003; Persico et al., 2001; Salichon et al., 2001) and in addition these 5-HT changes can impact neural responses across sensory cortices (Jitsuki et al., 2011). However, most studies to date have evaluated the role of serotonin on unisensory function and the neural structures that are critical for processing

individual sensory stimuli (i.e. visual, auditory, somatosensory) (Esaki et al., 2005; Gaspar et al., 2003; L. Hurley et al., 2004; L. M. Hurley & Pollak, 2005; Murphy, Uzbekov, & Rose, 1980; Waterhouse et al., 1990). Therefore, there is a tremendous opportunity to study the potential impact of serotonin on multisensory processing in the SERT Ala56 model and determine the possible relevance this may have in regards to autism spectrum disorder. Potential results from these studies could then connect abnormalities in the serotonin system to atypical multisensory function in ASD and allow for opportunities for further mechanistic studies in both rodents and human populations.

Introduction to the Current Dissertation Work

A variety of behavioral studies have evaluated (multi)sensory processing in humans and numerous animal models, ranging from monkeys to rats (Murray & Wallace, 2011; R. A. Stevenson, Ghose, et al., 2014). Currently, there are even behavioral assays available to evaluate basic sensory processing in mice with these tests typically focused on determining if there are deficits in one specific sensory system such as vision, audition, or touch (Barneoud, Gyger, Andres, & Van der Loos, 1991; Busse et al., 2011; Horst et al., 2012; Pinto & Enroth-Cugell, 2000; Radziwon et al., 2009). While these tasks are important for assessing sensory function within a specific modality, there were no behavioral paradigms available to evaluate the integration of information across the different sensory systems in the mouse model. This is important since multisensory integration is an essential element in the construction of our perceptual gestalt and can greatly facilitate behavior. Therefore, in Chapter II we demonstrate our findings of the first behavioral study to assess and characterize multisensory processing in the mouse model by using a novel behavioral assay that we developed. We further discuss these

results in Chapter II including the fact that the behavioral benefits observed in wild type mice under multisensory conditions appear to be similar to those findings in humans and larger animals species.

Based on emerging evidence demonstrating specific impairments in multisensory integration in individuals with autism spectrum disorder, it is imperative to study animal models, which can provide important mechanistic insights into the neural bases of these altered multisensory processes. To date most behavioral experiments in mouse models associated with ASD have focused on social, communicative and repetitive or restricted behaviors (J. N. Crawley, 2012). Although, basic unisensory processing differences in these models have been described (J. L. Silverman et al., 2010), no behavioral paradigm to properly assess multisensory processing currently existed. Therefore, in Chapter III, we describe the first behavioral assessments under multisensory conditions comparing wild type mice with a mouse model associated with ASD (i.e. SERT Ala56 animals) (Jeremy Veenstra-VanderWeele et al., 2009). Utilizing the same behavioral paradigm as described previously in Chapter II, we observed that wild type mice exhibit behavioral benefits under multisensory conditions, however SERT Ala56 animals demonstrate striking behaviorally deficits, including impaired behavioral performance and reduced multisensory gain or benefit compared to wild type littermate controls. The results and implications of these findings are further discussed in Chapter III.

Based on the prior behavioral studies discussed in Chapters II and III we observed that mice in general progressed through and completed these operant tasks over a relatively long period of time. Therefore, we were interested in designing a behavioral task variant in Chapter IV that could potentially replicate the behavioral benefits observed in wild type mice and deficits in SERT Ala56 animals under multisensory conditions described in Chapter III in a more timely

manner. Prior studies have shown that an irrelevant sensory stimulus presented simultaneously with a relevant sensory stimulus (i.e. multisensory conditions) can result in behavioral benefits (Lovelace, Stein, & Wallace, 2003; Stein et al., 1989; Van der Burg, Olivers, Bronkhorst, & Theeuwes, 2008). We therefore designed a behavioral paradigm similar to those used in larger animal models. However, in this study with mice, we found no significant differences in behavioral performances when comparing multisensory and visual alone conditions for either wild type or SERT Ala56 animals. Therefore, an irrelevant sensory stimulus (i.e. auditory) did not seem to aid in the detection of a relevant stimulus (i.e. visual) in the mouse model and the results and implications of these findings are further discussed in Chapter IV. Lastly, in Chapter V we will highlight the results, discuss the implications from this work and detail potential studies that may be pursued for future investigations.

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CHAPTER II

A Novel Behavioral Paradigm to Assess Multisensory Processing in Mice[γ]

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Abstract

Human psychophysical and animal behavioral studies have illustrated the benefits that can be conferred from having information available from multiple senses. Given the central role of multisensory integration for perceptual and cognitive function, it is important to design behavioral paradigms for animal models to provide mechanistic insights into the neural bases of these multisensory processes. Prior studies have focused on large mammals, yet the mouse offers a host of advantages, most importantly the wealth of available genetic manipulations relevant to human disease. To begin to employ this model species for multisensory research it is necessary to first establish and validate a robust behavioral assay for the mouse. Two common mouse strains (C57BL/6J and 129S6/SvEv) were first trained to respond to unisensory (visual and auditory) stimuli separately. Once trained, performance with paired audiovisual stimuli was then examined with a focus on response accuracy and behavioral gain. Stimulus durations varied from 50ms to 1s in order to modulate the effectiveness of the stimuli and to determine if the well-established "principle of inverse effectiveness" held in this model. Response accuracy in the multisensory condition was greater than for either unisensory condition for all stimulus durations, with significant gains observed at the 300ms and 100ms durations. Main effects of stimulus duration, stimulus modality and a significant interaction between these factors were observed. The greatest behavioral gain was seen for the 100ms duration condition, with a trend observed that as the stimuli became less effective, larger behavioral gains were observed upon their pairing (i.e. inverse effectiveness). These results are the first to validate the mouse as a species that shows demonstrable behavioral facilitations under multisensory conditions and provides a platform for future mechanistically directed studies to examine the neural bases of multisensory integration.

Introduction

We live in a world comprised of a multitude of competing stimuli delivered through a number of different sensory modalities. The appropriate filtering, segregation and integration of this information is integral to properly navigate through the world and for creating a unified perceptual representation. Having information available from multiple sensory modalities often results in substantial behavioral and perceptual benefits (Murray & Wallace, 2011; Stein, 2012; Stein & Meredith, 1993). For example, it has been shown that in noisy environments, seeing and hearing an individual speak can greatly enhance speech perception and comprehension relative to just the audible signal alone (Sumby & Pollack, 1954). In addition, responses have been shown to be both faster and more accurate under combined modality circumstances (G. Calvert, Spence, & Stein, 2004; R. A. Stevenson, Ghose, et al., 2014). Numerous other examples of such multisensory-mediated benefits have been established (G. A. Calvert & Thesen, 2004; Stein & Stanford, 2008), and serve to reinforce the utility of multisensory processing in facilitating behavioral responses and in constructing our perceptual view of the world. Furthermore, emerging evidence points to altered multisensory processing in a number of human clinical conditions, including autism and schizophrenia, reinforcing the importance of having a better mechanistic understanding of multisensory function (Cascio, Foss-Feig, Burnette, Heacock, & Cosby, 2012; Foss-Feig et al., 2010; Kwakye, Foss-Feig, Cascio, Stone, & Wallace, 2011; Marco, Hinkley, Hill, & Nagarajan, 2011; Martin, Giersch, Huron, & van Wassenhove, 2013; R. A. Stevenson, Siemann, Schneider, et al., 2014).

Numerous animal model and human imaging studies have explored the neural mechanisms that underpin multisensory processing (G. Calvert et al., 2004; G. A. Calvert & Thesen, 2004; Murray & Wallace, 2011; Stein, 2012; Stein & Meredith, 1993; Stein & Stanford,

2008; R. A. Stevenson, Ghose, et al., 2014). These studies have highlighted the neural operations performed by individual multisensory neurons and networks, demonstrating the importance of stimulus properties such as space, time and effectiveness in determining the final product of a multisensory pairing (Ghose & Wallace, 2014; Meredith, Nemitz, & Stein, 1987; Meredith & Stein, 1985, 1986a, 1986b; Royal, Carriere, & Wallace, 2009; Ryan A Stevenson, Fister, Barnett, Nidiffer, & Wallace, 2012; M. T. Wallace & Stein, 2007). In addition, an increasing emphasis is now being placed on detailing how neuronal responses relate to behavioral outcomes under multisensory circumstances (Burnett, Stein, Chaponis, & Wallace, 2004; Hirokawa, Bosch, Sakata, Sakurai, & Yamamori, 2008; Stein, Wallace, Stanford, & Jiang, 2002; Wilkinson, Meredith, & Stein, 1996).

Historically, these multisensory studies have focused on large mammalian models such as the cat and monkey, given the similarities in their sensory systems to humans and the ease with which both neural responses and behavior can be measured. With the advent of molecular genetic manipulations in mouse models and their applicability to human disease, however, there is a growing need to better understand sensory and multisensory function in these lower mammals. As highlighted above, this has become very germane of late as evidence grows for the presence of sensory and multisensory deficits in clinical disorders (Brandwein et al., 2012; Cascio, 2010; Foxe et al., 2013; Iarocci & McDonald, 2006; Keane, Rosenthal, Chun, & Shams, 2010; Kern et al., 2007; Marco et al., 2011; Russo et al., 2010; Smith & Bennetto, 2007; R. Stevenson et al., 2013; R. A. Stevenson, Siemann, Woynaroski, et al., 2014; Mark T Wallace & Stevenson, 2014).

In addition to molecular genetic manipulations such as knock-ins or knock-outs of genes associated with human disease, the rodent offers additional practical advantages spurred by the

development of optogenetic methods to study causal relations in brain circuits (Fenno, Yizhar, & Deisseroth, 2011; McDevitt, Reed, & Britt, 2014). Application of such tools to multisensory questions could be of great utility in developing a better mechanistic understanding of how the integrative features of multisensory neurons and networks arise, and how neuronal and network properties relate to behavior.

For these reasons (and others), recent studies have begun to focus on examining multisensory processes in rodent models (Carandini & Churchland, 2013; Gleiss & Kayser, 2012; Gogolla, Takesian, Feng, Fagiolini, & Hensch, 2014; Hishida, Kudoh, & Shibuki, 2014; Olcese, Iurilli, & Medini, 2013; D. Raposo, Sheppard, Schrater, & Churchland, 2012; Sakata, Yamamori, & Sakurai, 2004; Sheppard, Raposo, & Churchland, 2013; Sieben, Roder, & Hanganu-Opatz, 2013). For practical reasons, this work has initially focused on the rat, and has established strong neural-behavioral links in this species (Hirokawa et al., 2008; Hirokawa et al., 2011; Komura, Tamura, Uwano, Nishijo, & Ono, 2005; Tees, 1999). However, the mouse remains the primary model for molecular genetic and optogenetic manipulations, where limited knowledge concerning multisensory function still exists.

The current study represents the first of its kind to systematically examine unisensory (i.e., auditory alone, visual alone) and multisensory (i.e., paired audiovisual) behavioral function in mice. The core objective of these experiments was to determine if multisensory processing is conserved in the mouse and similar to the features observed in larger animal models. Our ultimate objective is that this behavioral paradigm, in conjunction with neurophysiological methods, could then be used to detail the neural bases of multisensory function. The establishment of such links would then allow for the application of powerful genetic, pharmacologic and optogenetic tools to evaluate questions of mechanistic relevance. Finally,

further studies may assess multisensory processing in mouse models of disease/disorder along with determining the underlying systems that may be atypical under these multisensory conditions.

Materials and Methods

Animals

9 male mice on C57BL/6J and 129S6/SvEv inbred strains were obtained from the Jackson Laboratory (Bar Harbor, ME, USA) and Taconic (Hudson, NY, USA), respectively. All animals were four weeks of age and housed in the Vanderbilt Murine Neurobehavioral Core with one cage mate. Mice were on a 12-hour light/day cycle and given water *ad libitum*. For the first week, at five weeks of age, mice were given food *ad libitum* and handled daily to acclimate to the experimenters and facility. Since the proposed behavioral task requires mice to make a decision in order to obtain a food reward, animals were placed on a food-restricted diet. Mice were only given food *ad libitum* on weekends (non-testing days) and free access to food for 4 hours every weekday, and this food restriction was gradually reached over a 2-week period before behavioral training began. Liquid vanilla Ensure (Abbott Laboratories, Abbott Park, IL, USA) was given in home cages for 60 minutes for 2 days before operant training began to expose animals to the reward. Body weights were recorded weekly and if an animal lost 20% of its initial weight, it would be excluded from the study until it had regained enough weight to participate based on this criterion. All experiments and protocols were approved by the Institutional Animal Care and Use Committee at Vanderbilt University.

Equipment

Mice were placed in adapted mouse operant chambers (Med Associates Inc, St. Albans, VT) that measured 7.0" L x 6.0" W x 7.25" H and were contained in sound attenuating cubicles. The chamber contained three nose poke holes with infrared sensors on the front wall, a house light, fan and clicker positioned on the rear wall and a mounted camera placed on the ceiling of the cubicle above the chamber. A section of metal mesh replaced the standard chamber plate and was placed above the central hole with a 3"x3" horn tweeter (Pyle Pro Audio, Brooklyn, NY) that was located behind the mesh section (**Figure 2-1A.**). LED lights were contained within the left and right nose poke holes and emitted a standard intensity of 1 lux, characteristic of the operant chamber. To minimize the small possibility that outside light may enter into the chambers, training and testing experiments were conducted in dim red light. Auditory stimuli were comprised of either white noise or an 8 kHz tone played at 85db SPL, which were measured and calibrated using a SoundTrack LxT2 sound level meter (Larson Davis, Provo, UT). Non-significant sound level measurements were observed in each chamber as auditory stimuli were played in the remaining chambers to ensure that sounds from one operant chamber could not be heard in another chamber. All rewards were presented in the central nose poke hole and comprised of 0.1cc of liquid vanilla Ensure dispensed by an automated dipper.

Behavioral Task

Initial Training

On the first day of training mice were acclimated to the operant chamber. A reward was given in the central nose poke hole every 60s for 1hr to demonstrate the location where the reward would be dispensed. In the next step of training, only the right nose poke hole was active. Mice were trained to respond in the right nose poke hole, which then resulted in a 500ms visual

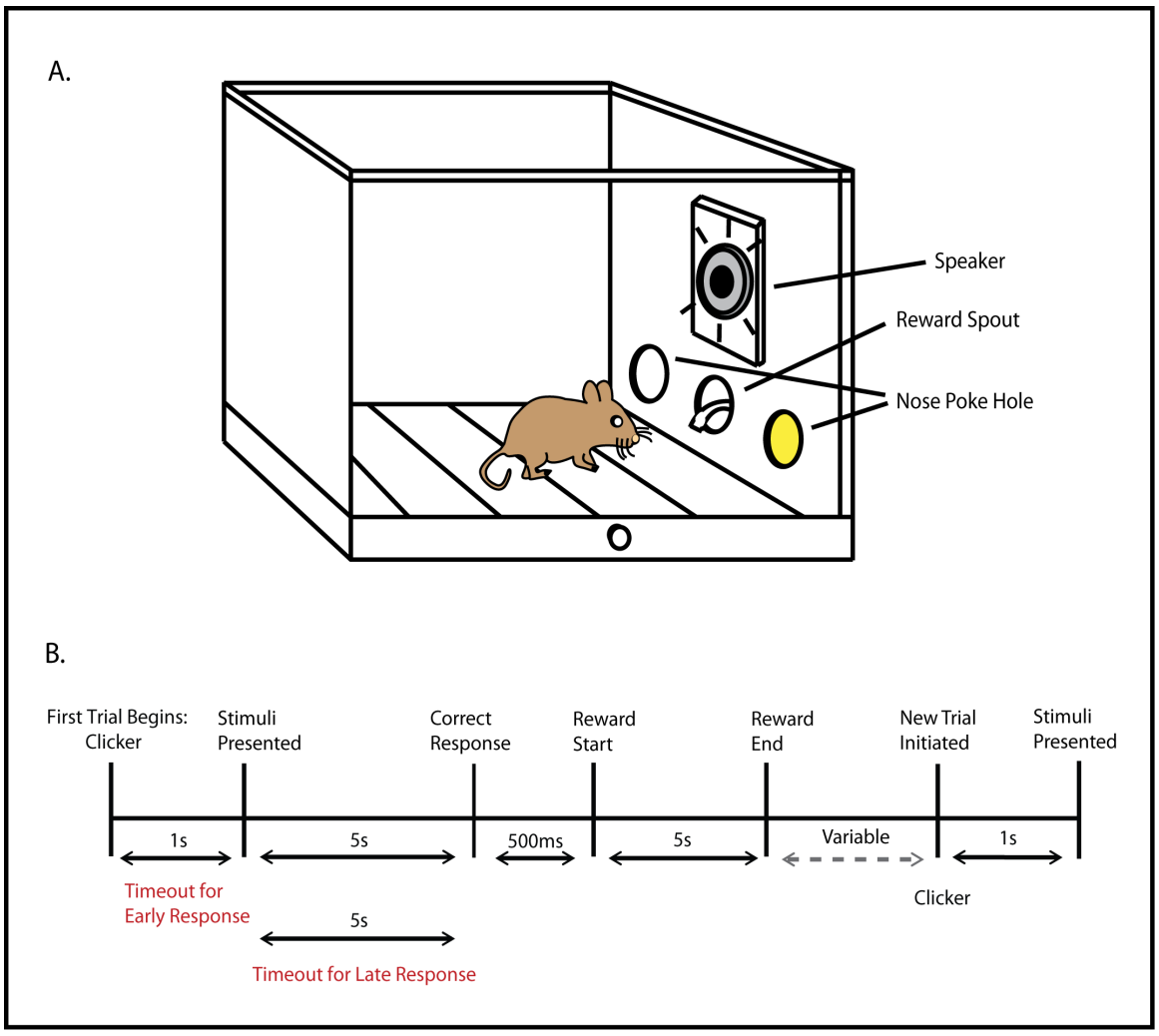

Figure 2-1. Behavioral Task Schematic. (A) Diagram of operant chamber during the presentation of an audiovisual stimulus (represented by the yellow color within the nose poke hole, where the LED was positioned) and by the active speaker. **(B)** Schematic representation of the trial sequence and timing. The phrase variable represents the amount of time that progresses until the animal decides to initiate a new trial by then performing a nose poke in the central hole.

stimulus presentation in the same location immediately followed by an Ensure reward. Once the number of responses was greater for the right vs. left nose poke hole for two consecutive days; the left nose poke hole was then activated and the right was inactivated. The initial training and reversal each lasted 4-5 days to meet the above criterion. This phase demonstrated that responding to either nose poke hole could result in a reward and was an early exposure to a sensory stimulus being paired with a reward.

Unisensory Training

In the next step of the behavioral task, mice were presented with a visual light stimulus in either the left or right nose poke hole. The animal had 5s to make a decision once the stimulus was presented, and a correct trial occurred when the animal responded to the nose poke hole where the visual stimulus was presented. A correct response resulted in an additional 5s time period for the animal to collect the reward. In this training phase, mice also learned to initiate a new trial by nose poking in the central hole. After a correct response and a reward was obtained, there was a 2s "wait" period where any response would not result in the start of a new trial, in order to minimize accidental or impulsive responses. After 2s had passed, mice could then initiate a new trial at any time by nose poking in the central hole. Once a trial was initiated, a clicker (50ms duration) would signal that a stimulus would be presented shortly, and, after a 1s delay, a light stimulus appeared again either in the left or right nose poke hole in a pseudorandom order. Every incorrect trial resulted in a timeout, with the house light illuminating the chamber for 5s, and no further responses could be made during this time period. In addition to timeouts for incorrect responses, timeouts could occur if the animal responded too early, before a stimulus was presented (<1s), and if an animal waited too long (>5s) before making a response (**Figure 2-1B.**). As the mice became more accurate, the duration of the light

stimulus was gradually reduced from 4s to 2s. For each training session mice completed a total of 100 trials (50 per side) for up to 90 minutes. Once the visual task was completed successfully for two consecutive days using a 65% correct response rate criterion, mice progressed to the auditory component of the task. In the auditory task, either white noise or an 8 kHz tone at a constant 85db was played individually. Mice were trained to associate the tone with responding to the left nose poke hole and white noise with responding to the right nose poke hole. The trial description, behavioral sessions and criterion to advance to the next stage of the paradigm were the same as described in the visual component of the task.

Multisensory Testing

After the visual and auditory training components of the task were completed, mice advanced to the behavioral testing phase where multisensory processing was evaluated. For multisensory trials only congruent/paired audiovisual trials were presented. Based on the variability of stimulus duration presentations in the multisensory rodent literature, we pragmatically selected a variety of stimulus durations. Unisensory and multisensory processing was evaluated for 5 days at each of the selected durations and proceeded by gradually shortening the durations. Therefore, mice were initially tested on 1s presentations of visual alone, auditory alone and paired audiovisual stimuli for 5 days, and this was then evaluated at 300ms, 100ms and 50ms stimulus durations. In these behavioral sessions, mice completed 150 trials (50 per condition presented in a pseudorandom order) lasting up to 90 minutes per testing day.

Data Analysis

All behavioral experiments were created utilizing customized Med-PC IV programs (Med Associates Inc). Behavioral accuracies in the initial training phase were calculated by comparing the responses to the left vs. right nose poke hole (and vice versa during reversal learning) using

two-tailed t tests. Accuracies measured for visual and auditory training sessions were calculated as percent correct utilizing a 65% correct response rate for two consecutive days to progress to the multisensory testing phase. Percent gain was calculated as (mean number of correct multisensory trials – mean number of correct best unisensory trials) / (mean number of correct best unisensory trials) x 100. Accuracy data was calculated as correct trials / correct + incorrect trials (misses only). Prism 6 (Graphpad Software Inc, La Jolla, CA) was used to calculate all statistical analyses. Two-way analysis of variances (ANOVA) with repeated measures and Tukey's multiple comparisons tests were utilized for all experiments unless otherwise specified. Mean and standard error of the mean is presented.

Results

Behavioral Performance for Unisensory (Visual alone, Auditory alone) Training

Mice were trained to identify both visual and auditory stimuli on separate and independent tasks. Each training session for these unisensory tasks consisted of 100 trials that occurred once daily. Criterion performance occurred when mice achieved 65% correct performance for two consecutive days. Mice first completed the visual training component of the behavioral task. Once animals reached criterion, they then advanced to the auditory training component. Using this criterion, mice completed the visual task with a final accuracy of 77.3% ± 1.8% and completed the auditory task with a final accuracy of 70.1% ± 1.1%. A paired t test revealed significant differences between visual and auditory unisensory behavioral performance upon achievement of criterion ($p = 0.0002$) **(Figure 2-2.)**. Substantial differences were noted in the time it took the mice to learn the two unisensory tasks. Whereas mice completed the visual task in 12.5 ± 0.95 days, it took 35.1 ± 4.55 days to complete the auditory task. Finally, unpaired t

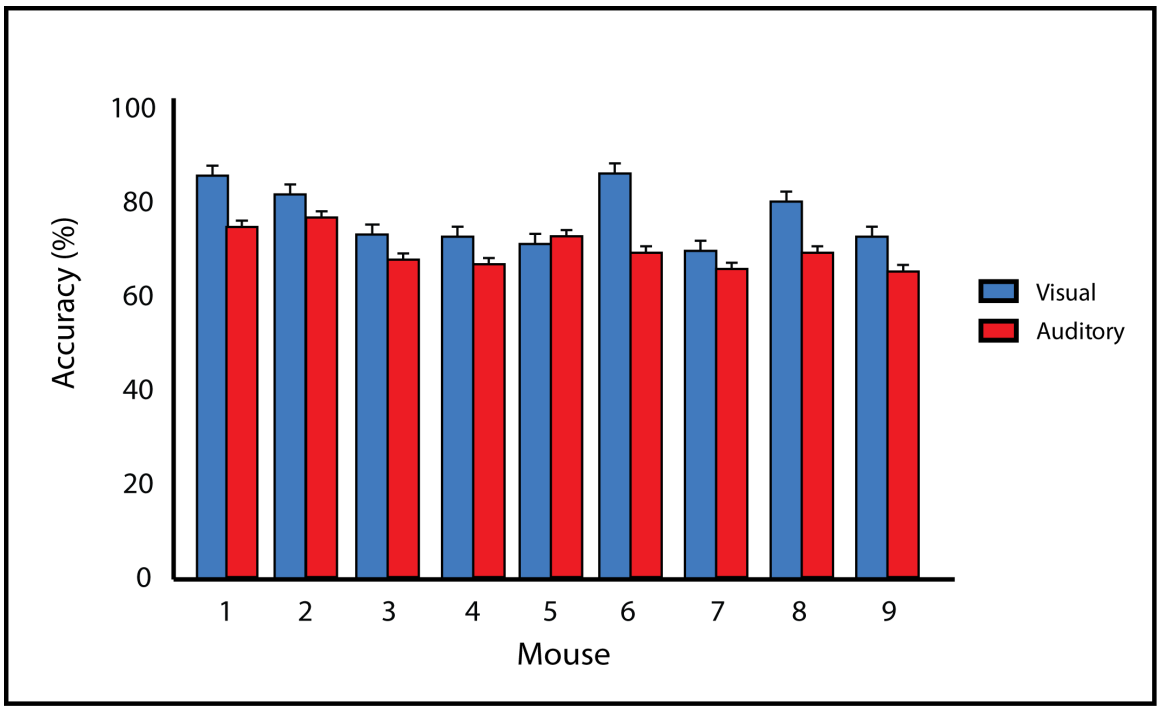

Figure 2-2. Criterion Performance on Unisensory Tasks. Average behavioral accuracies for visual only (blue) and auditory only (red) training conditions for two consecutive days once animals had reached 65% correct criterion performance. A significant difference ($p = 0.0002$) in behavioral performance was found when comparing visual and auditory performance across animals. Error bars represent SEM.

tests revealed no significant differences between mouse strains for behavioral accuracies for either visual training ($p = 0.62$) or for auditory training ($p = 0.29$) **(Figure 2-3.)**.

Unisensory and Multisensory Behavioral Performance as a Function of Stimulus Duration

Once animals had achieved criterion performance for each of the unisensory tasks, they then performed a paired audiovisual version of the task. Furthermore, in order to modulate the effectiveness of the unisensory stimuli, the duration was varied. Mice were initially tested on the longest duration condition (i.e., 1s) in response to visual, auditory and multisensory stimuli for 5 days. Following this, performance was then evaluated at durations of 300ms, 100ms and 50ms. Collapsing across all durations, behavioral accuracies under multisensory conditions were greater than for visual or auditory only conditions (**Figure 2-4A.**). Overall accuracy for these collapsed conditions was as follows: multisensory – 77.8% ± 1.83, visual – 73.7% ± 1.82 and auditory 69.1 ± 1.75. A repeated measures one-way ANOVA was used to evaluate accuracy as a function of sensory modality and revealed a significant difference ($F(1.907, 66.75 = 39.88$; $p < 0.0001$). Using Tukey's multiple comparison post hoc tests, we found significant differences between the multisensory and visual conditions ($p = 0.0007$), multisensory and auditory conditions ($p < 0.0001$) and the visual and auditory conditions ($p = 0.0003$). Next, a repeated measures two-way ANOVA with a Tukey's multiple comparisons post hoc test was used to compare response accuracy for unisensory and multisensory conditions across the different stimulus durations (**Figure 2-4B.**). Main effects of stimulus duration ($F(3, 32) = 31.75$; $p < 0.0001$), sensory modality ($F(2, 64) = 46.65$; $p < 0.0001$) and a significant stimulus duration x sensory modality interaction effect ($F(6, 64) = 2.981$; $p = 0.0125$) were observed. **Table 2-1.** shows response accuracy for each sensory modality and duration. When examined on a duration-by-duration basis, response accuracies under multisensory conditions were consistently greater

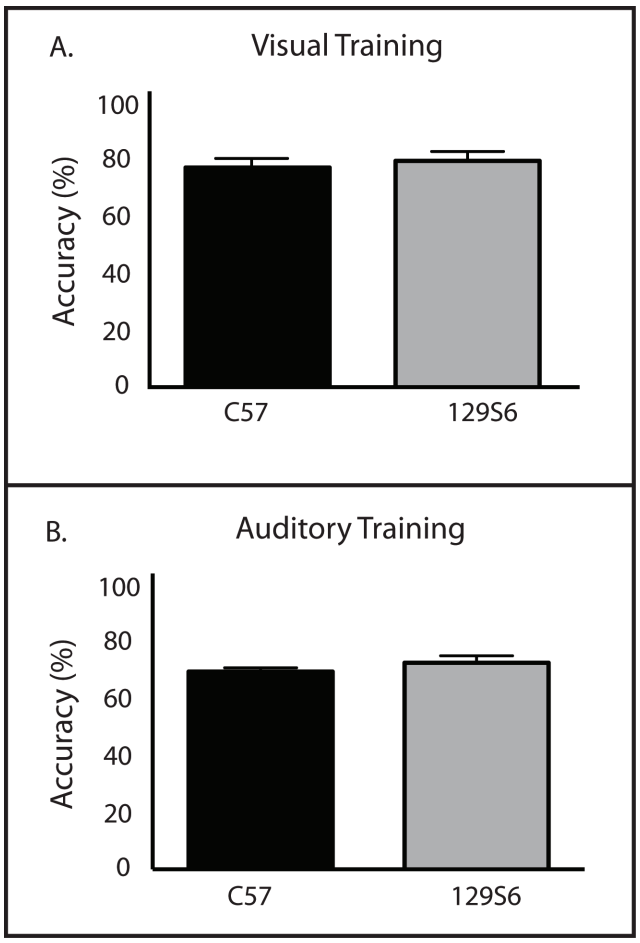

Figure 2-3. Effects of Background Strain on Behavioral Accuracies under Unisensory Training Conditions. No significant differences in accuracies between strains were observed for either visual training ($p = 0.62$) or under auditory training ($p = 0.29$) conditions. Data are presented from five C57BL/6J male mice and four 129S6/SvEv male mice. Error bars represent SEM.

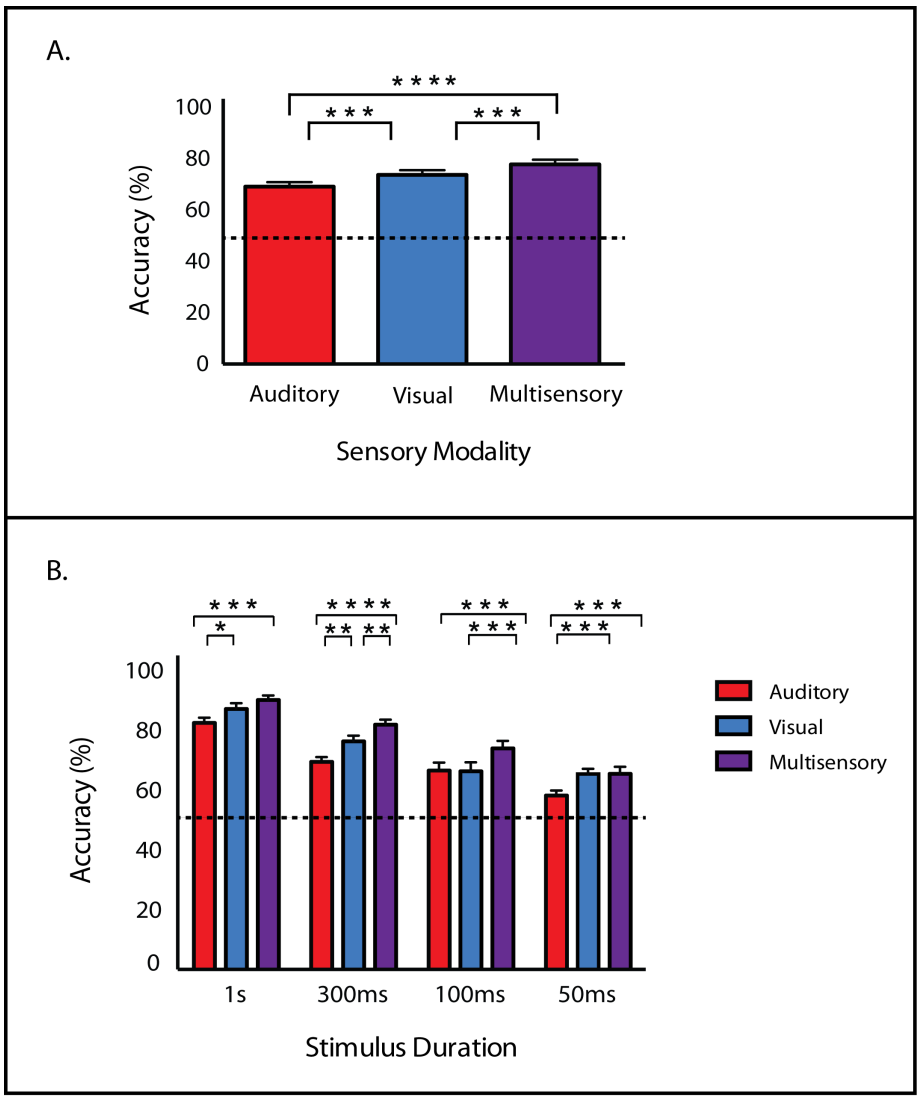

Figure 2-4. Behavioral Accuracy for Auditory, Visual and Audiovisual Conditions Across Stimulus Durations. (A) Accuracy for each of the conditions collapsed across all stimulus durations. **(B)** Accuracies as a function of sensory modality and duration. Note that response accuracy was greatest for multisensory compared to unisensory conditions across all of the tested durations. Data are presented from 9 male mice of both C57BL/6J and 129S6/SvEv strains. Dotted line represents 50% accuracy or chance level. Error bars represent SEM. The significant levels are as follows: (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$).

	Sensory Modality			
Stimulus Duration		Multisensory	Visual	Auditory
	1s	90.0 (1.56)	87.5 (1.94)	82.3 (1.79)
	300ms	81.8 (1.60)	76.2 (1.92)	69.4 (1.58)
	100ms	73.9 (2.52)	66.2 (3.00)	66.5 (2.67)
	50ms	65.4 (2.33)	65.3 (1.70)	58.1 (1.63)

Table 2-1. Behavioral Accuracies for Each Sensory Modality Across Stimulus Durations. Accuracies were greatest under multisensory conditions compared to unisensory conditions for all of the tested stimulus durations. Overall, accuracies decreased as the stimulus duration shortened. Data are presented from 9 male mice of both C57BL/6J and 129S6/SvEv strains. SEM is represented in parentheses.

than under either unisensory condition. We were also interested in assessing any effects of inbred strain. Using a repeated measures two-way ANOVAs main effects of sensory modality were observed at every stimulus duration across both strains (1s; $p = 0.004$, 300ms; $p = 0.001$, 100ms; $p = 0.001$, 50ms; $p = .006$). However, no significant main effects of mouse strain were observed at any stimulus duration (1s; $p = 0.084$, 300ms; $p = 0.29$, 100ms; $p = 0.97$, 50ms; $p = 0.061$). When evaluating multisensory performance across the tested stimulus durations, using a repeated measures two-way ANOVA, a significant main effect of stimulus duration ($F(3,9) = 9.423$; $p = 0.004$) was observed, yet no significant main effect of strain ($F(1,3) = 0.3435$; $p = 0.600$) nor a significant interaction effect ($F(3,9) = 0.5649$; $p = 0.652$) were observed **(Figure 2-5.)**.

Evaluating Multisensory Gain

In order to measure the degree of facilitation attributable to having information available from both senses, we calculated multisensory gain by utilizing the equation (average multisensory correct trials – average best unisensory correct trials) / (average best unisensory correct trials) x 100 (Meredith & Stein, 1983). The greatest multisensory gain was seen for the 100ms duration stimuli, with animals exhibiting a greater than 11% gain in performance. Using this calculation, we found the average multisensory gain at each of the tested stimulus durations was as follows: 1s = 3.40%, 300ms = 7.40%, 100ms = 11.15%, and 50ms = 0.10% **(Figure 2-6.)**. A similar pattern of gain was found for both mouse strains. To further evaluate multisensory gain, we then compared the original behavioral performance data for the multisensory and the best unisensory conditions for each individual mouse across all stimulus durations. A repeated measures two-way ANOVA with factors of stimulus duration and sensory modality with a Sidak's multiple comparisons post hoc test was used. Significant main effects of stimulus duration ($F(3, 24) = 40.1$; $p < 0.0001$) and sensory modality were observed ($F(1, 8) = 12.72$; $p =$

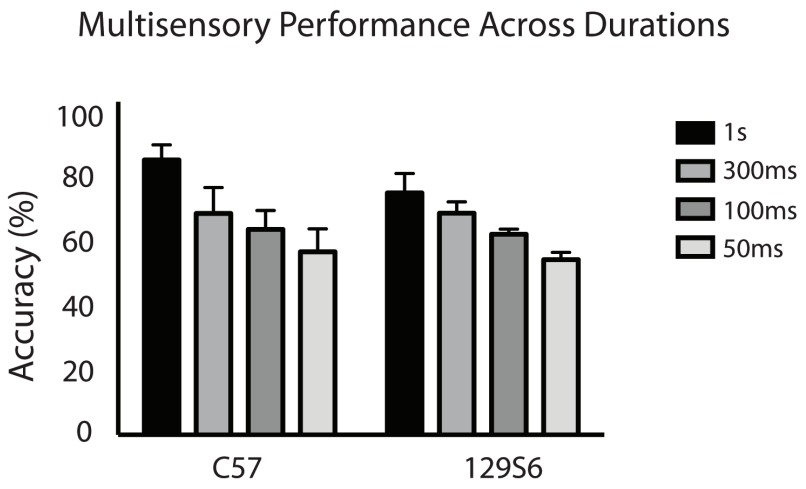

Figure 2-5. Effects of Background Strain on Behavioral Accuracies under Multisensory Conditions. No significant main effect of strain ($p = 0.60$) was observed under multisensory conditions. Data are presented from five C57BL/6J male mice and four 129S6/SvEv male mice. Error bars represent SEM.

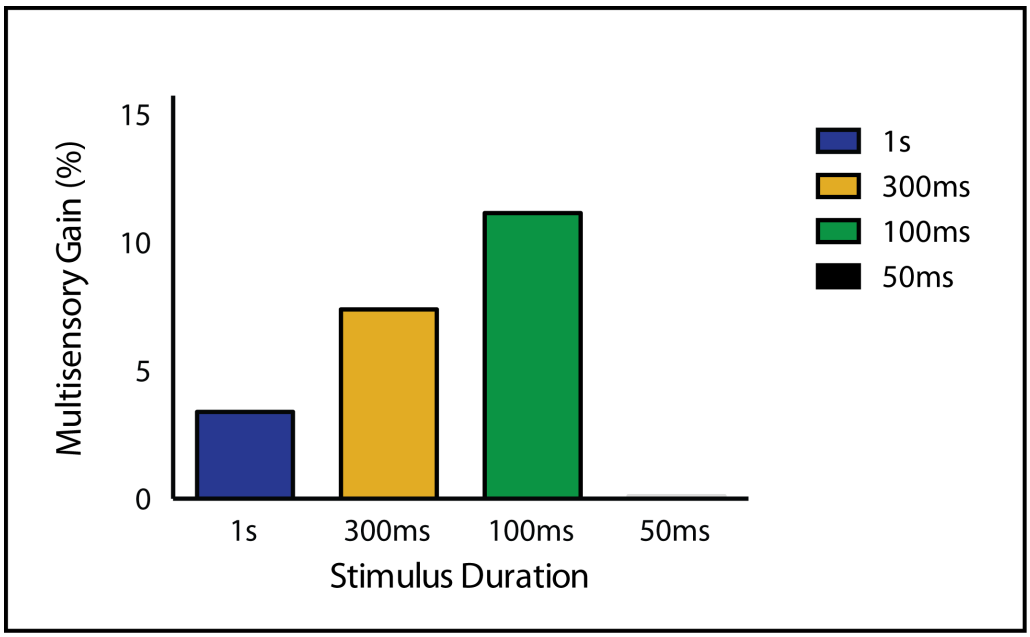

Figure 2-6. Multisensory Gain as a Function of Stimulus Duration. Percent gain was measured for each of the four tested stimulus durations. Gain was calculated as (multisensory correct trials – best unisensory correct trials) / (best unisensory correct trials) x 100. Data are presented from: 9 male mice of both C57BL/6J and 129S6/SvEv strains.

0.0073), but no significant stimulus duration x sensory modality interaction ($F(3, 24) = 2.70$; $p = 0.068$) was observed (**Figure 2-7.**). Utilizing the Sidak's multiple comparison post hoc tests, we found significant differences between the multisensory and best unisensory conditions at the 300ms ($p = 0.011$) and the 100ms stimulus condition ($p = 0.0049$). Overall, multisensory gain was found to gradually increase as stimulus duration was shortened, with gain increasing up to a maximum at stimulus durations of 100ms. Multisensory gain was observed to be significantly different from zero at the 300ms and 100ms conditions. Somewhat surprisingly, however, little gain was seen at the shortest duration (i.e., 50ms), even though animals were performing above chance levels on unisensory trials. One possible explanation for this lack of effect at this shortest duration is the mismatch in performance between the visual and auditory trials. In a Bayesian framework, differences in performance between the two unisensory conditions would be expected to yield little gain because of the differences in reliability of the different sensory channels (here with vision be more reliable) (Beierholm, Shams, Ma, & Koerding, 2007; Deneve & Pouget, 2004; Murray & Wallace, 2011).

Effects of Testing Day

In an attempt to determine if the potential novelty of the multisensory stimuli had any effect on behavioral performance, we evaluated accuracy as a function of testing day. Repeated measures two-way ANOVAs with Tukey's multiple comparisons post hoc tests were used to compare behavioral accuracy for multisensory, visual and auditory conditions across testing days (**Figure 2-8.**). For multisensory conditions, a significant main effect of stimulus duration ($p < 0.0001$; $F(3, 32) = 26.65$) was observed, but neither a significant main effect of testing day ($p = 0.846$; $F(4, 128) = 0.346$) nor a significant interaction effect ($p = 0.465$; $F(12, 128) = 0.987$) was observed. This pattern was also found for both the visual and auditory conditions. For the visual

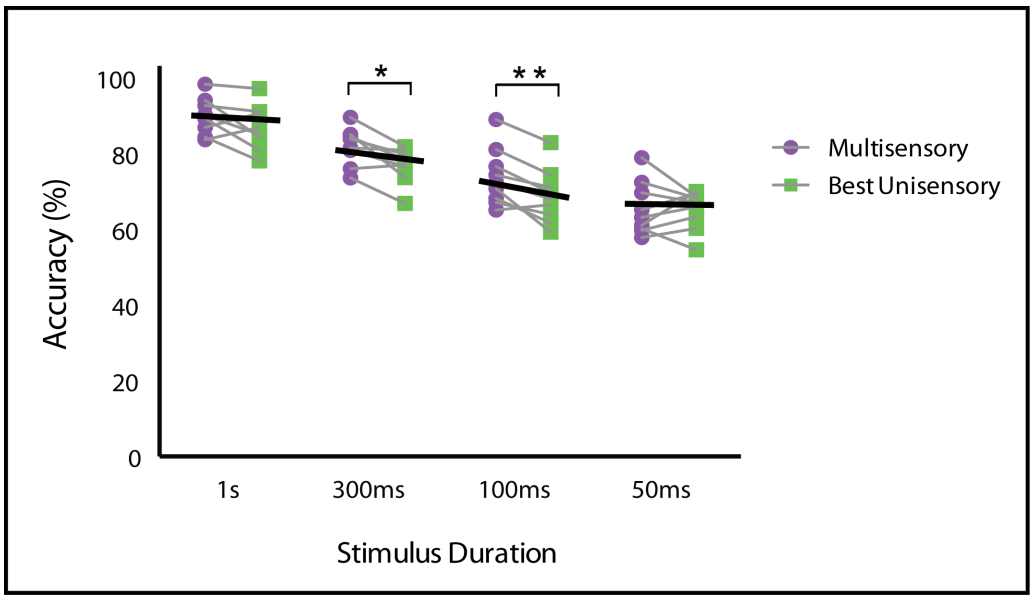

Figure 2-7. Performance Differences Between Multisensory and Best Unisensory Conditions for Individual Animals. Multisensory and the best unisensory performance accuracies were averaged separately for each mouse across the five days of testing for each stimulus duration. Black lines represent the group average performance under multisensory and the best unisensory conditions. Note the descending slope of these lines, which is most apparent for the 300ms and 100ms duration conditions. Data are presented from 9 male mice of both C57BL/6J and 129S6/SvEv strains. The significant levels are as follows: (* = $p < 0.05$, ** = $p < 0.01$).

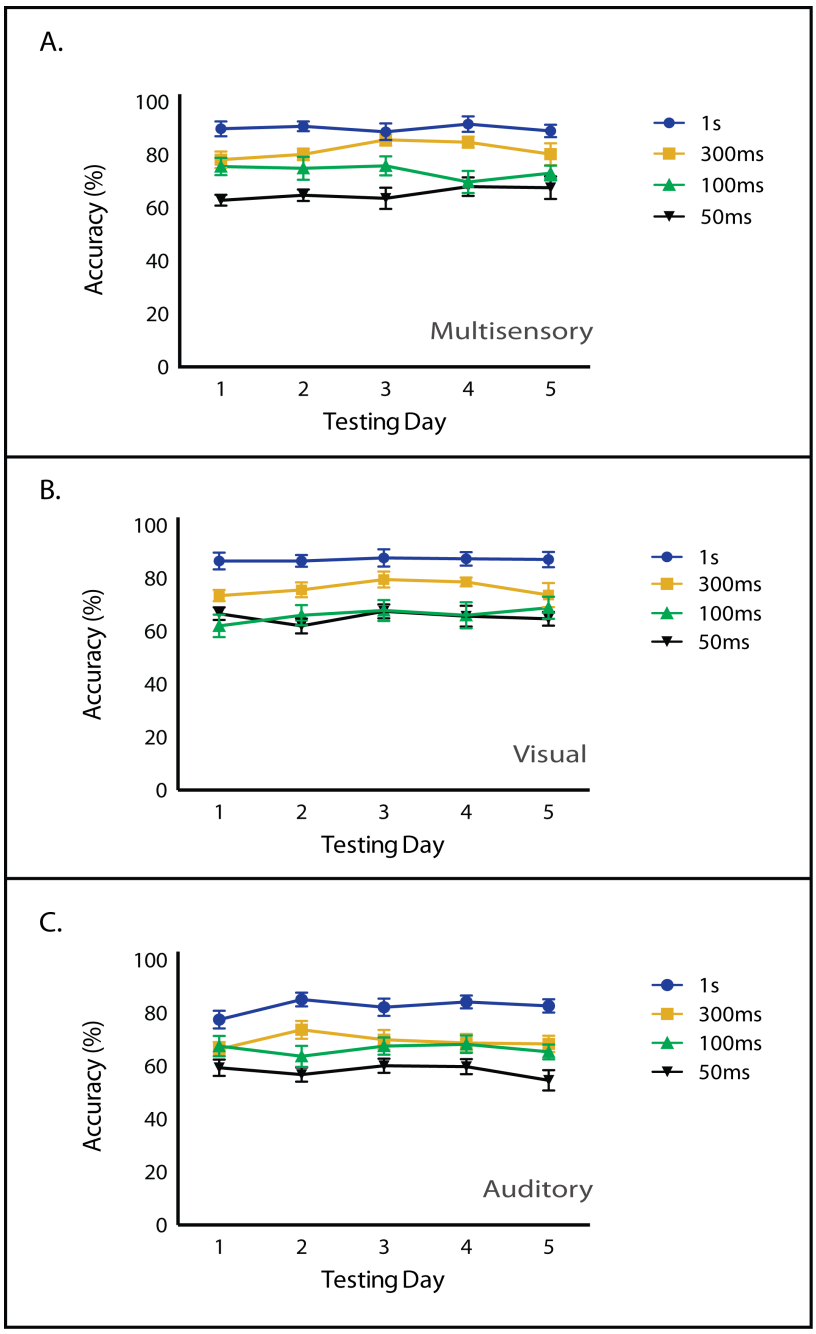

Figure 2-8. Effects of Testing Day on Behavioral Performance. No significant main effects of testing day were observed under **(A)** Multisensory ($p = 0.846$), **(B)** Visual ($p = 0.381$) or **(C)** Auditory conditions ($p = 0.514$) using repeated measures two-way ANOVAs (Tukey's test). Data are presented from 9 male mice of both C57BL/6J and 129S6/SvEv strains. Error bars represent SEM.

condition a significant main effect of stimulus duration ($F(3, 32) = 21.49$; $p < 0.0001$) was observed, yet neither a significant main effect of testing day ($F(4, 128) = 1.056$; $p = 0.381$) nor a significant interaction effect ($F(12, 128) = 0.502$; $p = 0.901$) was observed. Finally, for the auditory condition a significant main effect of stimulus duration ($F(3, 32) = 26.03$; $p < 0.0001$) was found, but neither a significant main effect of testing day ($F(4, 128) = 0.822$; $p = 0.514$) nor a significant interaction effect ($F(12, 128) = 0.831$; $p = 0.619$) was observed. Overall, we found that accuracies were consistent across the testing days, and there were no differences in performance levels for visual, auditory or audiovisual stimuli across the days of testing.

Evaluating Reaction Times Under Auditory, Visual and Multisensory Conditions

Numerous studies in humans and larger animal models have observed behavioral benefits under multisensory compared to unisensory conditions, such as greater accuracies and faster reaction times (Murray & Wallace, 2011; Stein & Stanford, 2008; R. A. Stevenson, Ghose, et al., 2014). In this study we observed that mice were more accurate under multisensory conditions. Therefore, we were also interested in evaluating animals' reaction times under auditory, visual and audiovisual conditions. Using a repeated-measures one-way ANOVA, a main effect of sensory modality was observed ($F(1.541, 53.93) = 19.10$; $p < 0.0001$, **Figure 2-9.**). Tukey's multiple comparison tests then revealed significant differences in reaction times between multisensory and auditory conditions ($p < 0.0001$), visual and auditory conditions ($p = 0.0002$), however no significant differences were observed between multisensory and visual conditions ($p = 0.8443$). Therefore, while reaction times were faster under multisensory conditions compared to auditory alone conditions, no significant differences were observed between multisensory and visual alone conditions. Future studies may need to1) utilize spatially congruent sensory stimuli

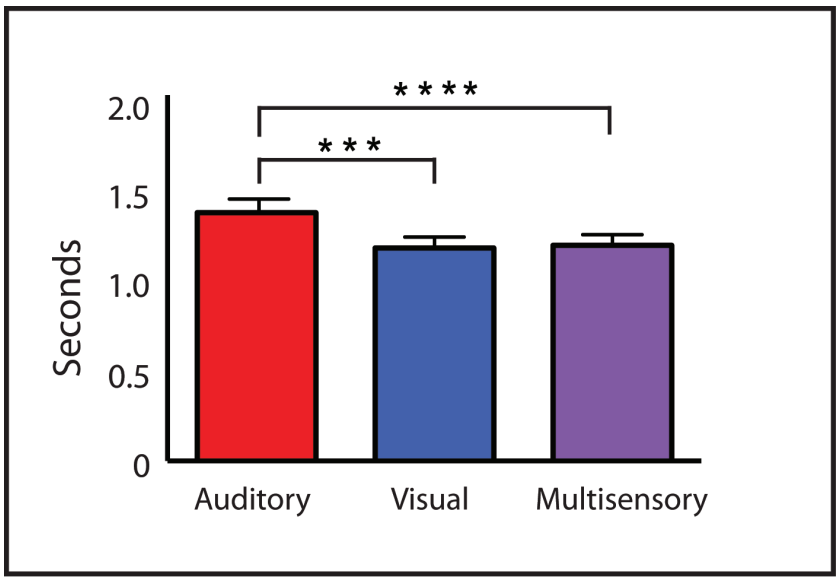

Figure 2-9. Reaction Times Under Auditory Alone, Visual Alone and Multisensory Conditions. Average reaction times were calculated by collapsing across the four tested stimulus durations. Using a repeated-measures one-way ANOVA a main effect of sensory modality was observed ($p < 0.0001$). Data are presented from 9 male mice of both C57BL/6J and 129S6/SvEv strains. Error bars represent SEM.

to potentially observe faster reaction times in mice or 2) design tasks that may be strictly evaluating reaction times (i.e. having an animal hold a position before responding to sensory stimuli) (Burnett et al., 2004; Colonius & Diederich, 2004; Sakata et al., 2004).

Discussion

The current study is the first to evaluate behavioral performance under multisensory conditions in the mouse. A variety of studies have evaluated various facets of either visual or auditory behavioral function in mice (Busse et al., 2011; Jaramillo & Zador, 2014; Klink & Klump, 2004; Pinto & Enroth-Cugell, 2000; Prusky, West, & Douglas, 2000; Radziwon et al., 2009), yet none have focused on determining the behavioral effects when these stimuli are combined. Overall, we found that mice were more accurate at identifying paired audiovisual stimuli compared to either visual or auditory stimuli alone across all of the tested stimulus durations, with significant gains observed at the 300ms and 100ms durations. As a general rule, behavioral accuracy decreased as the stimulus duration was shortened down to the 100ms duration, after which we believe that the stimulus duration was sufficiently short to be close to threshold detection levels. We suggest that this duration effect is in accordance with inverse effectiveness, a key concept in the multisensory literature, and complements a host of similar findings in human, monkey, cat, and rat model systems (Cappe, Murray, Barone, & Rouiller, 2010; Gleiss & Kayser, 2012; Meredith & Stein, 1986b; Murray & Wallace, 2011; Ohshiro, Angelaki, & DeAngelis, 2011). Inverse effectiveness states that as the effectiveness of the unisensory stimuli decreases, greater behavioral benefits can be observed when these stimuli are combined compared to when the individual (visual or auditory) stimulus is presented alone (Meredith & Stein, 1986b). Although effectiveness in the larger animal models has been

typically manipulated via changes in stimulus intensity, in our current study we were able to show corresponding effects in the mouse through manipulations of stimulus duration.

One key limitation from the current study is that we only manipulated one dimension of the sensory stimuli (i.e. duration). In the rat, stimulus intensity has been modulated to examine multisensory function (Gleiss & Kayser, 2012), and we hope to extend our studies into the domain of intensity in the future. Furthermore, due to practical constraints associated with the operant chambers, auditory stimuli were delivered from a single (central) spatial location, thus placing the visual and auditory stimuli somewhat out of spatial correspondence. Future work will add a second spatially congruent speaker to the operant chamber. These optimizations of the stimulus structure will likely reveal even larger multisensory interactions than those revealed in the current study, which we believe to be a conservative estimate of the potential gains in performance. However, we believe that the differences in multisensory gain by using either one or two speakers for this specific task would be minimal. The reasoning is that in this task we used a fairly loud auditory stimulus (85db) from a centrally located speaker in a standard operant chamber that is 7.0" L x 6.0" W x 7.25" H, thus making the stimulus highly effective. Future work will indeed move toward the use of two speakers so that we can begin to manipulate stimulus intensity in a parametric manner, and thus move toward better examining the spatial and temporal aspects of the observed multisensory gain. Another potential caveat to these findings is the role of attention to the unisensory (visual or auditory) stimuli during this task. A variety of studies have demonstrated a relationship between (multi)sensory processing and attention (Spence & Driver, 2004; Stein, Wallace, & Meredith, 1995; Talsma, Senkowski, Soto-Faraco, & Woldorff, 2010). In the current design it is difficult to control for the differential allocation of attention to one modality or the other, yet such attentional biases are likely. Nonetheless, the

pattern of behavioral response argues against a fixed strategy of attending to one of the stimulus modalities, suggesting that there were some attentional resources deployed toward both modalities. Regardless of the distribution of attention, the presence of multisensory gain suggests that even a stimulus in an unattended modality can modulate performance in the attended modality. Future work may also include reversing the training order, where animals would first complete the auditory training followed by visual training before testing under multisensory conditions. In addition, this study focused on the performance under congruent/paired audiovisual trials; however future studies could examine cognitive flexibility or set shifting by utilizing incongruent audiovisual trials. An interesting variant of this task would be to train animals under only one sensory modality condition (ex. auditory) and then pair this with a separate irrelevant sensory stimulus (ex. visual) (Lovelace, Stein, & Wallace, 2003). Finally, future studies may focus on other sensory domains (ex. tactile, olfaction) that may be more relevant or salient to mice.

A number of recent behavioral studies characterizing multisensory processing in the rat are highly relevant to these results (Carandini & Churchland, 2013; David Raposo, Kaufman, & Churchland, 2014; D. Raposo et al., 2012; Sheppard et al., 2013). These studies have demonstrated that behavioral gains can be observed under multisensory conditions similar to those found in larger animal models, and the most recent of these studies have evaluated the underlying circuits that may be crucial for audiovisual integration in the rat (Brett-Green, Fifkova, Larue, Winer, & Barth, 2003; Hirokawa et al., 2008; Hirokawa et al., 2011; Komura et al., 2005). Therefore, with the foundation established for this behavioral paradigm, we believe numerous future studies could be pursued focused on evaluating and linking mechanistic function with the associated behavior under multisensory conditions in the mouse model. More

specifically, we believe that the current work will serve as the springboard for identifying the neurobiological substrates and circuits that support these behavioral effects. Classical studies focused on the deep layers of the superior colliculus (SC) have found this to be a watershed structure for the convergence and integration of information from vision, audition and touch (Meredith et al., 1987; Meredith & Stein, 1983, 1986a, 1986b). Further studies demonstrated that lesions to the deeper (i.e., multisensory) layers of the SC cause not only a diminished neuronal response under multisensory conditions, but also result in a dramatic reduction in the associated behavioral benefits (Burnett, Henkel, Stein, & Wallace, 2002; Burnett et al., 2004; Burnett, Stein, Perrault, & Wallace, 2007). Thus, one likely substrate for the multisensory behavioral effects shown here is the SC, given its central role in audiovisual detection and localization (Hirokawa et al., 2011). In addition, work in the cat model has shown that this SC-mediated integration is heavily dependent upon convergent cortical inputs that appear to gate the integrative features of SC neurons (Jiang, Jiang, & Stein, 2002; M. T. Wallace, Meredith, & Stein, 1992; M. T. Wallace & Stein, 1994; Wilkinson et al., 1996). With the use of neurophysiological and neuroimaging methods, similar corticotectal circuits have been described in the rat, highlighting the conservation of a similar circuit system in smaller animal models (Brett-Green et al., 2003; Menzel & Barth, 2005; Rodgers, Benison, Klein, & Barth, 2008; Sanganahalli, Bailey, Herman, & Hyder, 2009; Sieben et al., 2013; M. T. Wallace, Ramachandran, & Stein, 2004). Specifically the selective deactivation to a higher order cortical region (V2L) resulted in a severe disruption in behavioral performance when responding to audiovisual stimuli (Hirokawa et al., 2008). Of greatest interest to this study, there have been a variety of recent studies focused on determining the underlying brain regions and circuits critical for multisensory processing in the mouse model, although none of these studies have examined

the behavioral response to multisensory stimuli (Charbonneau, Laramee, Boucher, Bronchti, & Boire, 2012; Cohen, Rothschild, & Mizrahi, 2011; Gogolla et al., 2014; Hunt, Yamoah, & Krubitzer, 2006; Laramee, Kurotani, Rockland, Bronchti, & Boire, 2011; Olcese et al., 2013; Reig & Silberberg, 2014). Understanding whether such a cortical dependency exists in the mouse model is a focus of future inquiry and therefore possible targets of multisensory input include the SC and the cortical region V2L. These mechanistically driven studies would then take advantage of the utility of the mouse model by using both genetic and optogenetic techniques to evaluate the underlying neural mechanisms of multisensory processing.

Another avenue of future research is to evaluate and further characterize mouse models of disease/disorder with known (multi)sensory processing deficits in the human population. Most importantly, the use of mouse models allows for the application of powerful genetic, pharmacologic and optogenetic tools to questions of mechanistic relevance that are not readily available for larger animal models. Two clinical populations with known (multi)sensory dysfunction are schizophrenia and autism (Behrendt & Young, 2004; Cascio et al., 2012; Dakin & Frith, 2005; de Gelder, Vroomen, Annen, Masthof, & Hodiamont, 2003; de Gelder et al., 2005; De Jong, Hodiamont, Van den Stock, & De Gelder, 2009; Grossman, Schneps, & Tager-Flusberg, 2009; Iarocci & McDonald, 2006; Javitt, 2009; Marco et al., 2011; Martin et al., 2013; Minshew & Hobson, 2008; O'Connor, 2012; Mark T Wallace & Stevenson, 2014). Recently, there has been an increased focus on linking these behavioral findings with possible neural correlates to gain a better understanding of the atypical (multi)sensory processing observed in these clinical populations (Brandwein et al., 2012; Russo et al., 2010; Stekelenburg, Maes, Van Gool, Sitskoorn, & Vroomen, 2013). Numerous genetic mouse models of clinical disorders such as autism and schizophrenia have shown behavioral deficits and altered neural connectivity

(Hida, Mouri, & Noda, 2013; Karl, 2013; Lipina & Roder, 2014; Provenzano, Zunino, Genovesi, Sgado, & Bozzi, 2012; Silverman, Yang, Lord, & Crawley, 2010); however, behavioral studies of multisensory function have not yet been reported in these animals. In fact, a recent study demonstrated multisensory processing differences between wild type mice and mouse models of autism at the neuronal level and showed the potential to ameliorate these effects under pharmacologic manipulations (Gogolla et al., 2014). This approach has enormous potential to reveal mechanistic contributions of altered multisensory function to these disease states. The use of our behavioral paradigm, along with pharmacologic or optogenetic techniques, could then allow for the assessment of novel therapeutic approaches that may link altered neural mechanisms to the resultant atypical behavior. Finally, these types of studies would offer great promise as a translational bridge that seeks to better link genetic, phenotypic and neural factors in an effort to better elucidate the contributing role of alterations in sensory function in developmental disorders such as autism or schizophrenia.

Overall, this study has shown that multisensory processing is conserved in the mouse model by demonstrating similar behavioral benefits to those observed throughout numerous larger animal models. With the design of the first behavioral paradigm to assess multisensory function in the mouse, we believe this allows for a whole host of future research opportunities. This type of behavioral task will allow for a variety of mechanistically driven studies focused on the neural underpinnings of multisensory processing, in addition to studies dedicated to evaluating these circuits in models of clinical disorders with known (multi)sensory impairments.

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CHAPTER III

An Autism-associated Serotonin Transporter Variant Disrupts Multisensory Processing

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Abstract

Altered sensory processing is observed in many children with autism spectrum disorder (ASD), with growing evidence that these impairments extend to the integration of information across the different senses (i.e., multisensory function). The serotonin system plays an important role in sensory development and function, and alterations of serotonergic signaling have been suggested to play a role in ASD. A gain-of-function coding variant in the serotonin transporter (SERT) Ala56 associates with sensory aversion in humans carrying the variant, and when expressed in mice produces traits associated with ASD, including disruptions in social, communication, and repetitive behaviors. The current study set out to test whether these mice also exhibit changes in multisensory function. Mice were trained to respond to auditory and visual stimuli independently before being tested under visual, auditory and paired audiovisual conditions. Wild type mice exhibited significant gains in response accuracy under audiovisual conditions. In contrast, although the SERT Ala56 animals learned the auditory and visual tasks comparably to wild type littermates, they failed to show behavioral gains under multisensory conditions. These results provide the first behavioral evidence of multisensory deficits in a genetic mouse model related to ASD and implicate the serotonin system in multisensory processing.

Significance Statement

In addition to deficits in social communication function and the presence of restricted interests and repetitive behaviors, sensory and multisensory abnormalities are frequently observed in autism spectrum disorder (ASD). The serotonin system is important in sensory development and function and has been implicated in ASD. This study demonstrates a loss of

multisensory-mediated behavioral facilitation in a genetic mouse model of ASD that expresses a hyperfunctional serotonin transporter. These findings connect long-standing biomarker data in ASD with a basic deficit in multisensory processing; a deficit that may play an important and underecognized role in the core deficits seen in ASD.

Introduction

Autism spectrum disorder (ASD) is characterized by impairments in social communication as well as the presence of restricted interests and repetitive behaviors (APA, 2011; Kanner, 1943). In addition, sensory abnormalities are highly prevalent in ASD and are now part of the diagnostic criteria (Iarocci & McDonald, 2006; Marco, Hinkley, Hill, & Nagarajan, 2011; Rogers & Ozonoff, 2005). These changes in response to sensory stimuli have been described in a number of individual sensory systems (e.g., vision, touch, hearing), with ongoing research continuing to detail both the specific alterations and their mechanistic bases (Cascio, 2010; Kern et al., 2007; O'Riordan & Passetti, 2006; Spencer & O'Brien, 2006). Based on the growing evidence for disturbances across multiple sensory systems, there has been increased focus on examining the integration of information across the different sensory modalities, with several studies detailing impaired multisensory processing in ASD (Brandwein et al., 2012; Cascio, Foss-Feig, Burnette, Heacock, & Cosby, 2012; Foss-Feig et al., 2010; Foxe et al., 2013; Kwakye, Foss-Feig, Cascio, Stone, & Wallace, 2011; Smith & Bennetto, 2007; R. Stevenson et al., 2013; R. A. Stevenson, Siemann, Schneider, et al., 2014; Wallace & Stevenson, 2014; Zaidel, Goin-Kochel, & Angelaki, 2015). The relevance of these multisensory deficits not only extends our understanding of the numerous sensory changes in ASD, but is critical since multisensory integration plays a central role in the construction of social communicative

representations, which can result in profound behavioral and perceptual gains under multisensory circumstances (Murray & Wallace, 2011; B. E. Stein & Stanford, 2008; R. A. Stevenson, Ghose, et al., 2014; Sumby & Pollack, 1954).

The serotonin system has long been implicated in ASD (Cook & Leventhal, 1996; Gaspar, Cases, & Maroteaux, 2003). Elevated whole blood serotonin (5-HT), termed hyperserotonemia, is a well-replicated, heritable biomarker present in more than 25% of children with ASD (Gabriele, Sacco, & Persico, 2014; Mulder et al., 2004; Schain & Freedman, 1961). Genetic studies in autism also point to the 5-HT system, including the identification of a group of rare amino acid variants in the serotonin transporter (SERT) in families with evidence of linkage to the chromosomal region containing SERT (Sutcliffe et al., 2005) and each of these variants confer excess SERT activity (Prasad, Steiner, Sutcliffe, & Blakely, 2009; Prasad et al., 2005). The most common of these SERT variants, Ala56, is carried by about 3 million Americans, and in a study of multiplex ASD families, was associated with both sensory alterations and rigid-compulsive behaviors (Sutcliffe et al., 2005). The SERT Ala56 knock-in mouse model recapitulates the hyperserotonemia biomarker and demonstrates abnormalities in social and communicative behaviors as well as repetitive behaviors (Jeremy Veenstra-VanderWeele et al., 2009; J. Veenstra-VanderWeele et al., 2012).

The SERT Ala56 model is of particular interest because it may represent a bridge connecting altered 5-HT function with changes in sensory and multisensory function in ASD. Numerous animal studies have examined the impact of 5-HT on sensory development (Cases et al., 1996; Gaspar et al., 2003; Lesch & Waider, 2012; van Kleef, Gaspar, & Bonnin, 2012) and processing (Esaki et al., 2005; L. M. Hurley, Thompson, & Pollak, 2002; Jitsuki et al., 2011; Waterhouse, Azizi, Burne, & Woodward, 1990), and have demonstrated that 5-HT and SERT are

found in a number of sensory brain regions (Binns, 1999; Gaspar et al., 2003; Cécile Lebrand et al., 1996; Cecile Lebrand et al., 1998; Lesch & Waider, 2012). Illustrating the importance of 5-HT signaling in these processes, genetic elimination of SERT or the 5-HT metabolizing enzyme MAOA in the mouse disrupts the development and function of somatosensory cortex (Cases et al., 1996; X. Chen et al., 2015; Esaki et al., 2005; Persico et al., 2001; Salichon et al., 2001). Similarly, alterations in serotonin function result in abnormal patterns of sensory connectivity (Esaki et al., 2005; Gaspar et al., 2003; Murphy, Uzbekov, & Rose, 1980; Salichon et al., 2001). Furthermore, 5-HT has been shown to modulate neural responses to a variety of sensory stimuli (L. M. Hurley & Pollak, 2001, 2005; Waterhouse et al., 1990; Waterhouse, Moises, & Woodward, 1986). Whereas almost all of this work has examined the relationship between 5-HT and unisensory (i.e., visual alone, auditory alone) function (L. M. Hurley & Pollak, 2001, 2005; L. M. Hurley et al., 2002; Murphy et al., 1980; Waterhouse et al., 1990), very little is currently known about the role of 5-HT for multisensory function (Jitsuki et al., 2011). As multisensory function necessitates the integration of information across the different sensory channels, and is thus dependent upon connectivity across cortical domains, it may serve as a powerful proxy to probe changes in neural connectivity – changes known to accompany ASD (Just, Cherkassky, Keller, & Minshew, 2004; Kana, Libero, & Moore, 2011; Rippon, Brock, Brown, & Boucher, 2007). Furthermore, sensory and multisensory networks form the foundation for the creation of healthy perceptual and cognitive representations, and thus may represent an important window into deficits in higher-order function.

In an effort to better understand the contributions of the serotonin system to sensory and multisensory function and its potential relevance for autism, we examined aspects of sensory and multisensory function in SERT Ala56 mice. Recent work has begun to detail neurophysiological

changes in multisensory processing in the mouse, and we recently developed a new paradigm to assess behavioral gains under paired audiovisual conditions for the first time in the mouse (Siemann et al., 2015). Here, we show that SERT Ala56 mice have behavioral deficits in multisensory function that extend beyond changes in unisensory (i.e., vision alone, audition alone) performance. These results connect abnormalities in the serotonin system to altered multisensory processing in ASD and provide opportunities for further mechanistic studies in rodents and human populations.

Materials and Methods

All animal procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Vanderbilt University. SERTAla56 knock-in mice were constructed as previously described (Jeremy Veenstra-VanderWeele et al., 2009). Animals were housed and kept under a food-restricted diet previously outlined (Siemann et al., 2015). 8 SERT Ala56 (4 male and 4 female) and 8 wild type littermate control (4 male and 4 female) mice were used and experimenters were blinded to the genotypes throughout the behavioral paradigm. SERTAla56 and wild type animals were the offspring of heterozygous SERT Ala56 parents and began training at 14 weeks of age.

Behavioral Paradigm

Animals proceeded through behavioral training and testing procedures previously described (**Figure 3-1.**) (Siemann et al., 2015). In this behavioral paradigm, mice were initially trained to respond to visual and auditory stimuli separately for a liquid reward. As previously described (Siemann et al., 2015), daily training sessions for these unisensory tasks consisted of 100 trials, with criterion reached when mice achieved 65% correct performance for

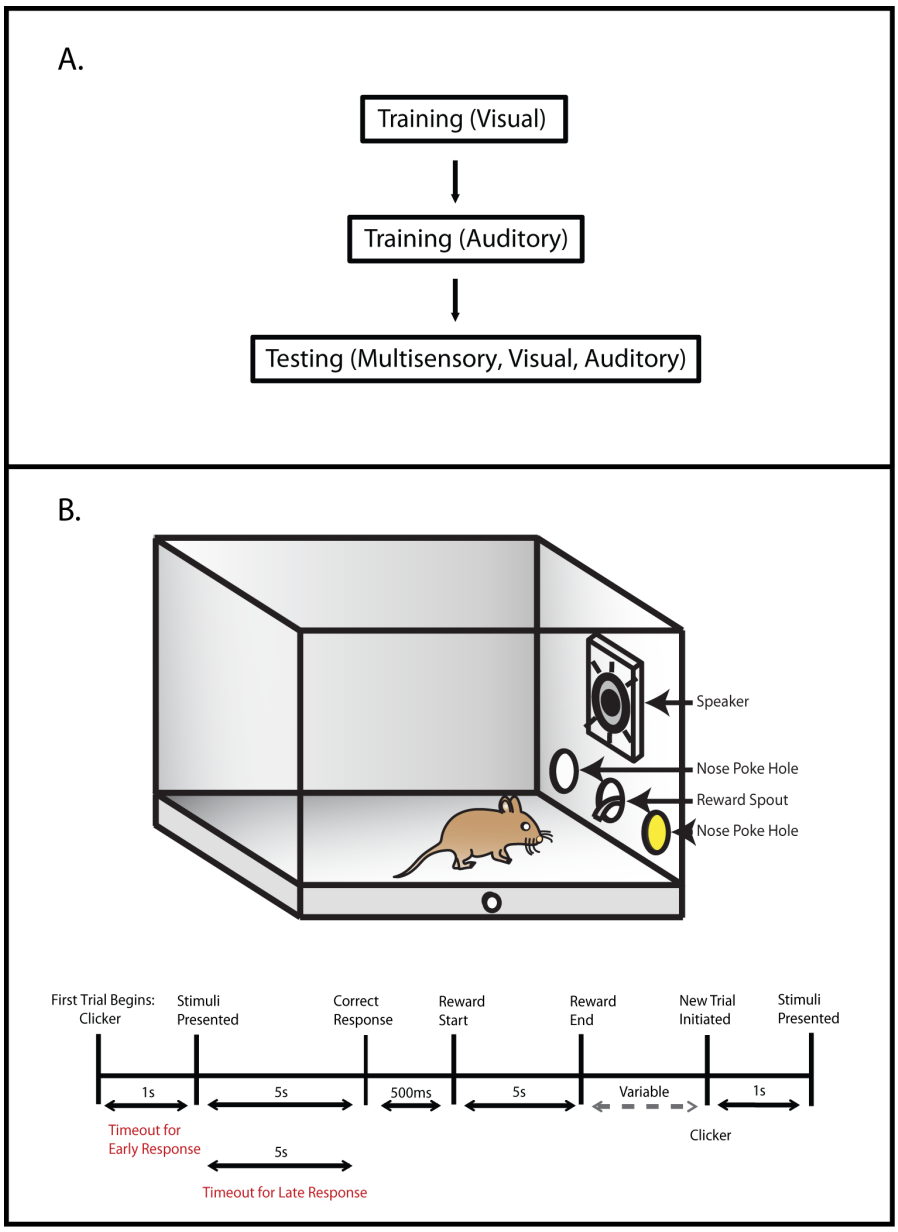

Figure 3-1. Behavioral Paradigm. (A) An outlined progression of the behavioral paradigm. **(B)** Above: A diagram of the operant chamber during the presentation of a congruent audiovisual stimulus (represented by the yellow color within the nose poke hole, where the LED was positioned) and by the active speaker. **(B)** Below: A schematic representation of the trial sequence and timing.

two consecutive days. Once the visual training task was completed successfully, mice progressed to and completed a similar auditory training task. After the unisensory training, animals completed testing sessions where visual, auditory and congruent audiovisual (multisensory) pairings were presented. Animals completed 150 trials (50 per sensory modality) lasting up to 120 minutes per testing session. Animals were tested for 5 days at each of five stimulus durations.

Data Analysis

All behavioral experiments were designed with customized Med-PC IV programs (Med Associates Inc.). As previously described (Siemann et al., 2015), accuracies measured for visual and auditory training sessions were calculated as percent correct utilizing a 65% correct response rate for 2 consecutive days. Multisensory gain was calculated as (mean number of correct multisensory trials – mean number of correct best unisensory trials) / (mean number of correct best unisensory trials) x 100. Accuracies were calculated as correct trials / correct + incorrect trials. Prism 6 (Graphpad Software Inc., La Jolla, CA) was used for all statistical analyses. Repeated measures two-way analysis of variances (ANOVAs), Sidak's multiple comparisons tests and standard error of the mean were used for all experiments unless otherwise specified.

Results

SERT Ala56 Mice Exhibit Comparable Behavioral Performance to Wild Type Mice When Trained on Visual and Auditory Stimuli

SERT Ala56 and wildtype littermate control mice learned to respond to one side of the operant chamber to receive a reward (acquisition), once acquired animals then learned to respond

to the opposite side for a reward (reversal). No significant differences were observed for either acquisition or reversal learning ($p = 0.17$, $p = 0.42$, **Figures 3-2A., 3-2B.**). For unisensory training, mice were first trained to detect and respond to visual stimuli that were presented on either side of an operant chamber in order to receive a liquid reward. Once mice completed the visual component of the behavioral task for two consecutive days using a 65% correct criterion, animals then progressed to the auditory alone component of the task. Under auditory alone conditions, mice were presented with either white noise or and 8 kHz tone at 85dB from a centrally located speaker. When white noise was played animals needed to respond to the right side of the operant chamber, whereas when a tone was played animals needed to respond on the left side in order to receive a liquid reward **(Figure 3-1.)**. For the visual task, no significant differences in accuracy (**Figure 3-3A.**, $p = 0.90$) or days to learn (**Figure 3-3B.**, $p = 0.66$) were observed between SERT Ala56 mice and WT littermate controls. Following the completion of visual training, animals advanced to the auditory training component. Both genotypes took significantly longer to complete auditory training than visual training, but again showed no significant differences between genotypes for accuracies (**Figure 3-3C**, $p = 0.21$) or days to learn (**Figure 3-3D.**, $p = 0.52$). In summary, no significant differences in behavioral performance were observed between genotypes for the visual and auditory tasks.

SERT Ala56 Mice are Less Accurate than Wild Type Mice under Multisensory Conditions

After animals completed the auditory training component of the task for two consecutive days using a 65% correct criterion, mice were tested under visual alone, auditory alone and

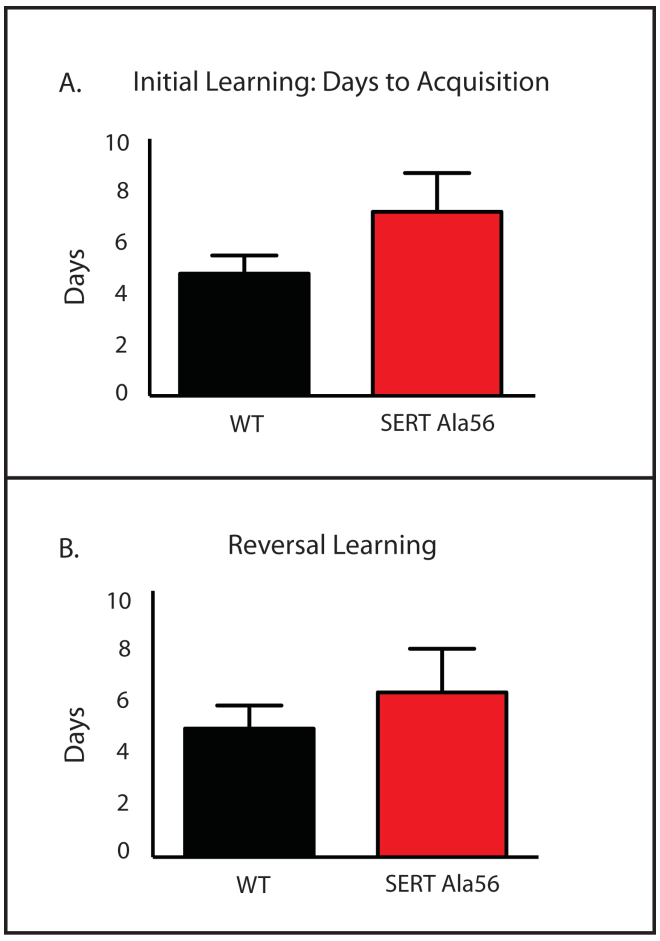

Figure 3-2. Measuring Initial Days to Learn and Reversal Learning Between Genotypes. Unpaired t tests demonstrated no significant differences between wild type and SERT Ala56 mice in either **(A)** initial acquisition ($p = 0.17$) or **(B)** reversal learning ($p = 0.42$).

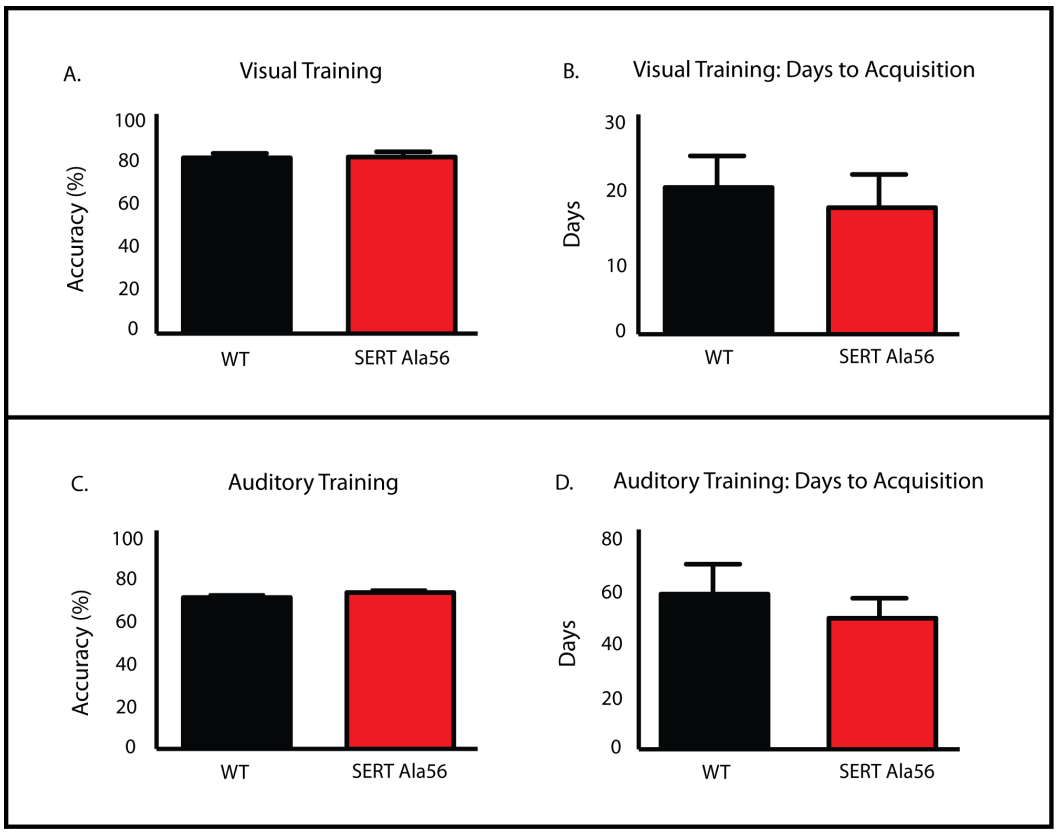

Figure 3-3. Evaluating Behavioral Performance for Wild Type and SERT Ala56 Mice Under Unisensory Training Conditions. Unpaired t tests demonstrated no significant differences between genotypes for either **(A)** accuracies ($p = 0.90$) or **(B)** days to acquisition ($p = 0.66$) under visual training conditions. Wild type mice completed the visual task after 20.6 ± 4.4 days with a final accuracy of 82.2% ± 2.1% while SERT Ala56 mice completed the visual training in 17.8 ± 4.7 days with a final accuracy of 82.6% ± 2.5%. Unpaired t tests demonstrated no significant differences between wild type and SERT animals in **(C)** accuracies ($p = 0.21$) or **(D)** days to acquisition ($p = 0.52$) under auditory training conditions. Wild type animals completed the auditory training in 58.0 ± 11.1 days and with a final accuracy of 70.8% ± 1.4% and SERT Ala56 mice finished this task after 49.0 ± 7.5 days with a final accuracy of 73.2% ± 1.2%.

paired/congruent audiovisual conditions. In order to modulate the effectiveness of the visual and auditory stimuli in an effort to best assess multisensory gain, stimulus duration was varied (Gleiss & Kayser, 2012; Hirokawa, Bosch, Sakata, Sakurai, & Yamamori, 2008; Sakata, Yamamori, & Sakurai, 2004; Sheppard, Raposo, & Churchland, 2013). Mice were initially tested on the longest duration condition (1s) in response to visual, auditory and multisensory stimuli for 5 days. Following this, performance was then evaluated at durations of 500ms, 300ms, 100ms and 50ms in a blocked design. Under multisensory conditions, a repeated measures two-way ANOVA demonstrated a significant main effect of stimulus duration ($F(4, 28) = 32.06$; $p < 0.0001$) and a significant main effect of genotype ($F(1, 7) = 6.645$; $p = 0.0366$) (**Figure 3-4A.**). No significant main effects of genotype were observed under either visual-only conditions ($F(1, 7) = 1.819$; $p = 0.2194$) (**Figure 3-4B.**) or auditory-only conditions ($F(1, 7) = 2.442$; $p = 0.1621$) (**Figure 3-4C.**). Next, we evaluated global behavioral performance by collapsing across stimulus durations. When collapsing across all durations, behavioral accuracies under multisensory conditions were significantly greater than for visual or auditory only conditions for both groups (**Figure 3-5.**). Repeated measures two-way ANOVA revealed significant main effects of genotype ($F(1, 39) = 11.99$; $p = 0.0013$) and sensory modality ($F(2, 78) = 51.12$; $p < 0.0001$). When evaluating within genotype, both SERT Ala56 mice and wild type littermate controls showed significantly improved accuracies under multisensory conditions when compared with both visual and auditory conditions, with no significant differences between the unisensory conditions (**Figure 3-5**). When utilizing Sidak's multiple comparison test and evaluating across genotypes, impaired performance was observed in SERT Ala56 animals in comparison to wild type littermate controls under visual ($p = 0.0215$) and auditory conditions ($p = 0.0014$). Most interestingly, the most substantial impairment between the genotypes was found under

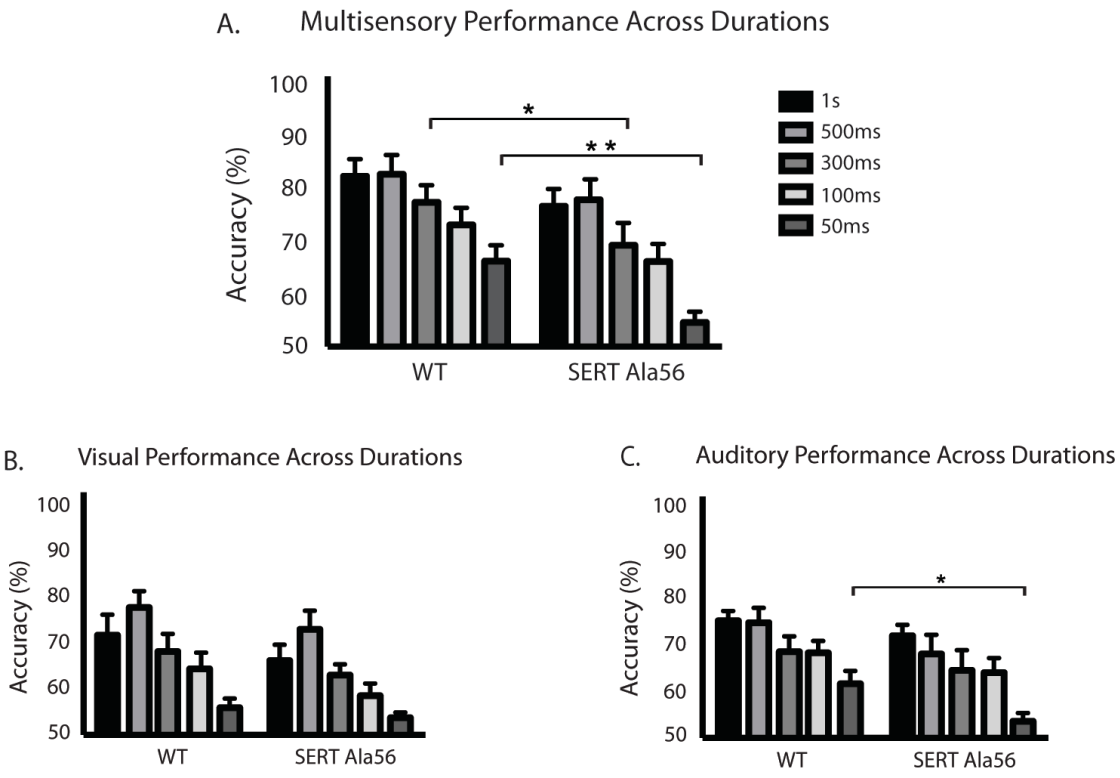

Figure 3-4. Evaluating Behavioral Accuracies Under Multisensory, Visual and Auditory Conditons Across Stimulus Durations. Under multisensory conditions, a repeated measures two-way ANOVA demonstrated a significant main effect of stimulus duration ($F(4, 28) = 32.06$; $p < 0.0001$) and a significant main effect of genotype ($F(1, 7) = 6.645$; $p = 0.0366$) but no significant interaction effect ($F(4, 28) = 0.6097$; $p = 0.6590$) was observed **(A)**. Under visual conditions a main effect of stimulus duration was observed ($F(4, 28) = 31.92$; $p < 0.0001$), yet no significant main effect of genotype ($F(1, 7) = 1.819$; $p = 0.2194$) nor a significant interaction effect ($F(4, 28) = 0.1503$; $p = 0.9613$) were found **(B)**. These findings were similar under auditory conditions, with a main effect of stimulus duration ($F(4, 28) = 24.84$; $p < 0.0001$) being observed, but no significant main effect of genotype ($F(1, 7) = 2.442$; $p = 0.1621$) nor a significant interaction effect ($F(4, 28) = 0.5491$; $p = 0.7012$) were found **(C)**.

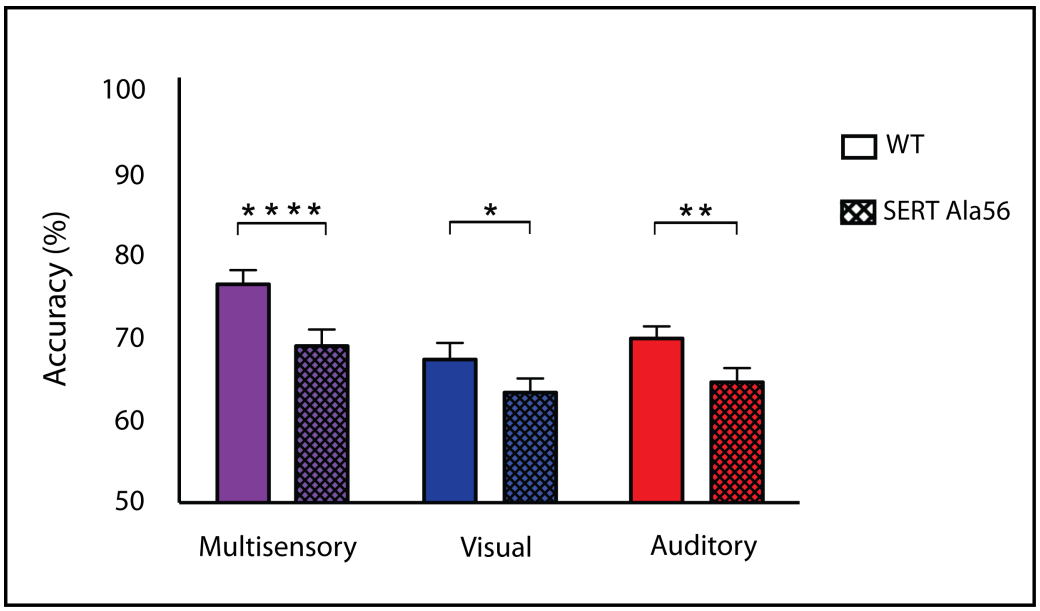

Figure 3-5. Behavioral Accuracies for Multisensory, Visual and Auditory Conditions Collapsed Across Stimulus Durations. Overall accuracies for these collapsed conditions for wild type animals were as follows: multisensory – 76.4% ± 1.71, visual – 67.3% ± 1.98 and auditory 69.8 ± 1.45. Accuracies for SERT Ala56 animals were as follows: multisensory – 68.9% ± 1.99, visual – 63.3% ± 1.68 and auditory 64.5 ± 1.72. Significant main effects of genotype ($F(1, 39 = 11.99$; $p = 0.0013$), and sensory modality ($F(2, 78 = 51.12$; $p < 0.0001$) were observed. Also, significant differences between wild type and SERT Ala56 animals under multisensory ($p < 0.0001$), visual ($p = 0.0215$) and auditory conditions ($p = 0.0014$) were observed. Behavioral performance was then evaluated within each genotype. For wild type animals, significant differences between multisensory and visual conditions ($p < 0.0001$), multisensory and auditory conditions ($p < 0.0001$) and no significant differences between visual and auditory conditions ($p = 0.2000$) were found. Similarly for SERT Ala56 mice, significant differences between the multisensory and visual conditions ($p = 0.0007$), multisensory and auditory conditions ($p = 0.0093$) and no significant differences between visual and auditory

conditions ($p = 0.6816$) were observed. The significant levels are as follows: (* = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$, **** = $p < 0.0001$).

multisensory conditions ($p < 0.0001$). Overall, wild type animals were significantly more accurate than SERT Ala56 animals under multisensory conditions and the greatest differences in behavioral performance between genotypes were observed under these audiovisual conditions.

When comparing performance based on the sex of the animals irrespective of genotype across stimulus durations, a repeated measures two-way ANOVA demonstrated no significant main effect of sex ($F(1, 7) = 3.152$; $p = 0.1191$) (**Figure 3-6.**). Finally, the types of errors were measured for each genotype under multisensory conditions. Impaired performance in SERT Ala56 animals was explained by significant differences in the number of incorrect responses ($p = 0.0070$), with no significant differences in the number of early ($p = 0.9331$) or late responses (0.5016) when compared to wild type animals (**Figure 3-7.**). Overall, these findings demonstrate that wild type mice were significantly more accurate under multisensory conditions compared to SERT Ala56 mice and this was not due to abnormal levels of impulsivity (early errors) or motivation (late errors). In addition, these findings could not be explained by differences in sex or in visual or auditory performance. Lastly, to further evaluate potential behavioral benefits under multisensory compared to unisensory conditions we examined the reaction times for wild type and SERT Ala56 animals. When collapsing across stimulus durations, a main effect of sensory modality ($F(2,78) = 9.337$; $p = 0.0002$) was observed, yet no main effect of genotype ($F(1,39) = 0.6561$; $p = 0.4229$) nor a significant interaction effect ($F(2,78) = 0.0331$; $p = 0.9675$) were found (**Figure 3-8.)**. Interestingly, wild type and SERT Ala56 animals did not differ in reaction times under auditory ($p = 0.0643$), visual ($p = 0.1405$) or multisensory conditions ($p = 0.0745$). Therefore, the behavioral benefits observed under multisensory conditions for wild type animals utilizing this operant task may be limited to behavioral accuracy.

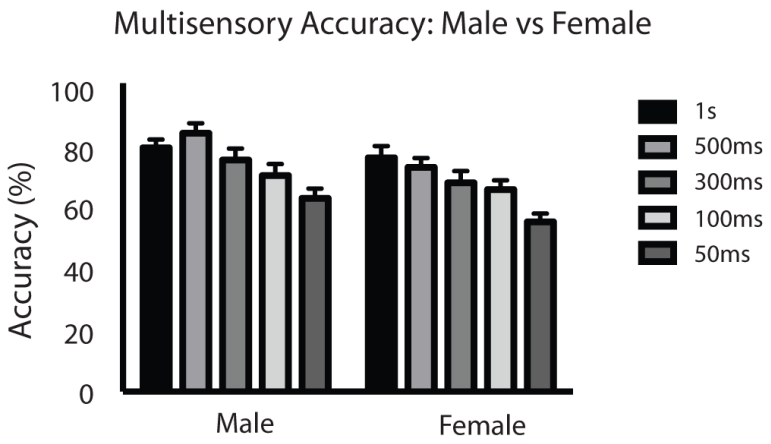

Figure 3-6. Measuring Effects of Sex on Behavioral Accuracies Under Multisensory Conditions. When comparing across sex, a repeated measures two-way ANOVA demonstrated a significant main effect of stimulus duration ($F(4, 28) = 26.30$; $p < 0.0001$) yet no significant main effect of sex ($F(1, 7) = 3.152$; $p = 0.1191$) nor a significant interaction effect ($F(4, 28) = 1.028$, $p = 0.4103$) were observed.

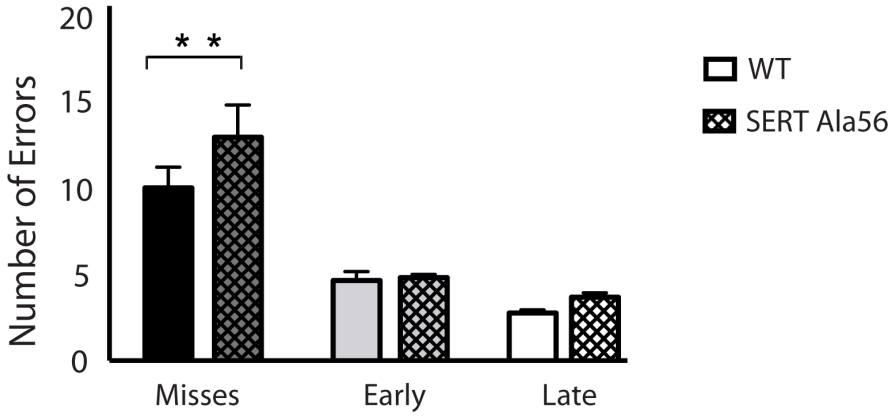

Figure 3-7. Evaluating the Types of Errors Made Under Multisensory Conditions Between Wild Type and SERT Ala56 Mice. Significant main effects of error type ($p = 0.0003$), genotype ($p = 0.0003$) and a significant error type x genotype interaction effect ($p = 0.0475$) were observed.

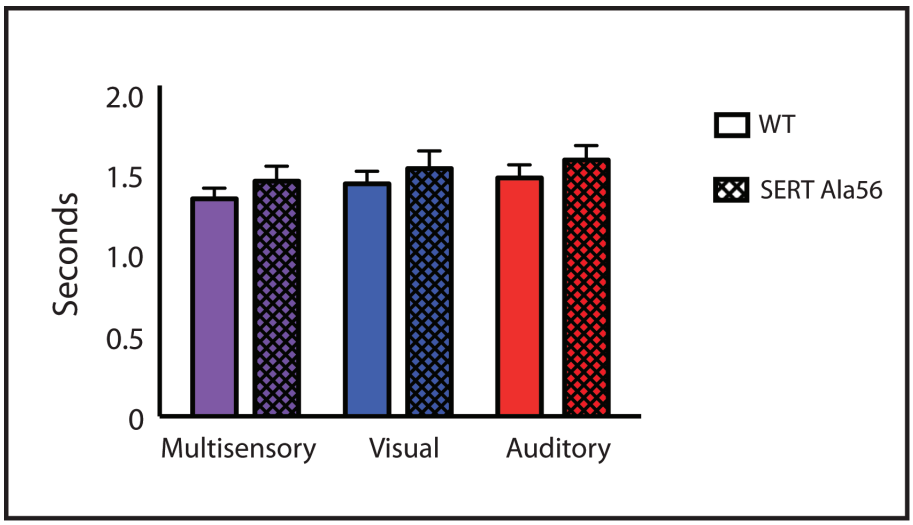

Figure 3-8. Comparing Reaction Times Across Stimulus Durations Under Auditory, Visual and Multisensory Conditions. Utilizing a repeated measures two-way ANOVA, a main effect of sensory modality ($F(2,78) = 9.337$; $p = 0.0002$) was observed, however no main effect of genotype ($F(1,39) = 0.6561$; $p = 0.4229$) nor a significant interaction effect ($F(2,78) = 0.0331$; $p = 0.9675$) was found. Wild type and SERT Ala56 animals did not differ in reaction times under auditory, visual or audiovisual conditions.

Multisensory Gain is Blunted Across All Stimulus Durations in SERT Ala56 Mice

To evaluate the amount of behavioral facilitation resulting from having information available from multiple senses, multisensory gain was calculated using the equation (average multisensory correct trials – average best unisensory correct trials) / (average best unisensory correct trials) x 100 (Meredith & Stein, 1983). The greatest multisensory gain was seen for both wild type and SERT Ala56 mice at the 300ms duration stimuli, with wild type animals exhibiting a greater than 12% gain in behavioral performance. In contrast, SERT Ala56 animals demonstrated a significantly smaller gain in performance (**Figure 3-9A.)**. This pattern of greater multisensory gain for wild type compared to SERT Ala56 animals held for each of the tested stimulus durations. For wild type animals, multisensory gain was found to be significantly different from zero at the 1s ($p = 0.030$), 500ms ($p = 0.027$) and 300ms ($p = 0.005$) conditions. For SERT Ala56 mice, however, there was no statistical evidence of multisensory gain on the group level at any of the stimulus durations. Since this group data could have been due to the performance of a few animals, multisensory gain was further evaluated using individual performance analyses.

We compared the behavioral performance data under multisensory and the best unisensory conditions for each individual mouse at each stimulus duration. When comparing behavioral performance in this manner, significant Pearson correlations were found collapsing across both genotypes (**Figure 3-10A.)**, as well as for wild type mice **(Figure 3-10B.)** and for SERT Ala56 mice alone **(Figure 3-10C.)**. Therefore, if a mouse was accurate under multisensory conditions it was also more likely to be accurate under multisensory conditions, regardless of genotype. At the 300ms duration, a repeated measures two-way ANOVA demonstrated a

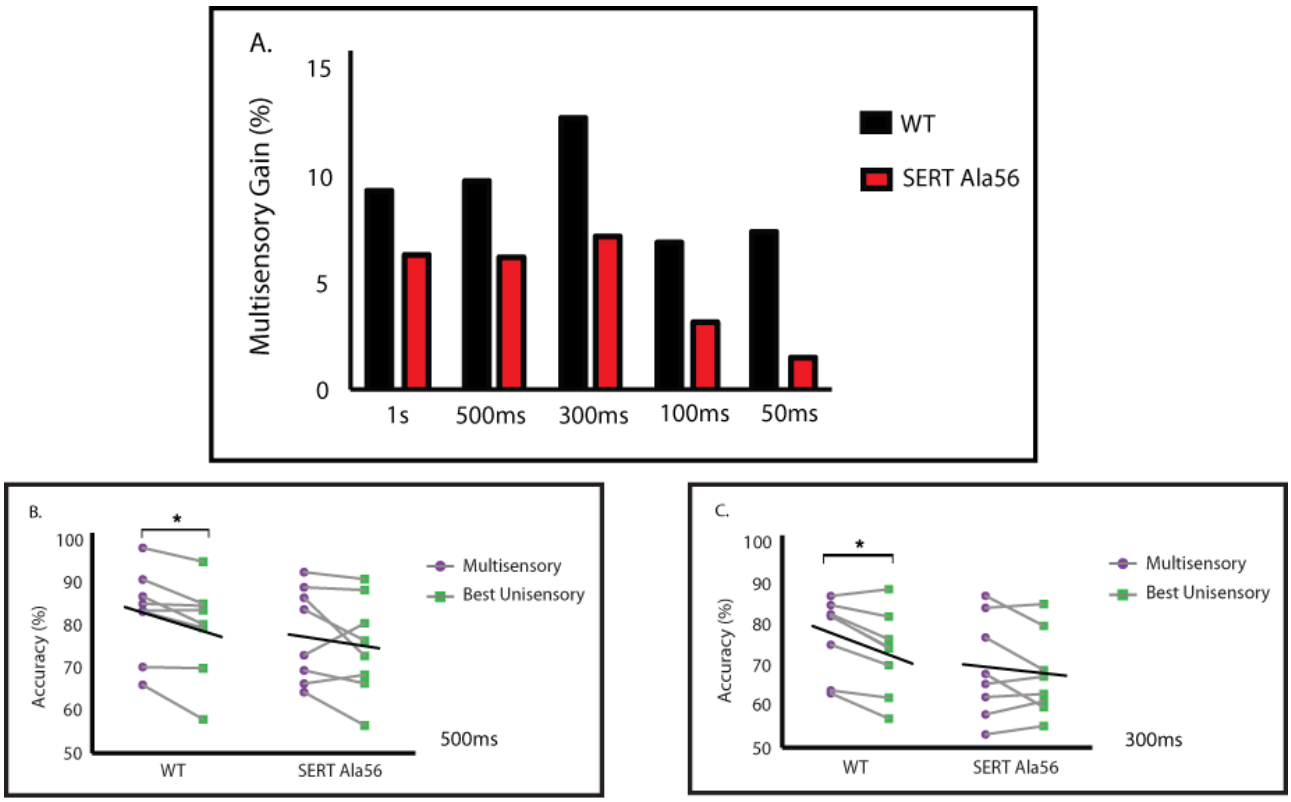

Figure 3-9. Evaluating Multisensory Gain Across Stimulus Durations at Both the Group and Individual Performance Levels. Wild type animals demonstrated greater multisensory gain than SERT Ala56 animals at the group level at all stimulus durations **(A)**. The values for multisensory gain for wild type mice were as follows: 1s – 9.30%, 500ms – 9.74%, 300ms – 12.70%, 100ms – 6.90% and 50ms – 7.40%. Multisensory gain values for SERT Ala56 mice were as follows: 1s – 6.30%, 500ms – 6.20%, 300ms – 7.20%, 100ms – 3.14% and 50ms – 1.50%. Significant differences in accuracies under multisensory and the best unisensory conditions were observed at both the 500ms **(B)** and 300ms **(C)** stimulus durations for wild type animals. At the 300ms duration, a repeated measures two-way ANOVA demonstrated a significant main effect of sensory modality ($F(1, 7) = 6.969$; $p = 0.0334$) and a significant main effect of genotype ($F(1, 7) = 6.159$; $p = 0.0421$) (**C**). Significant differences between

multisensory and the best unisensory conditions were observed for wild type mice ($p = 0.02$) but not SERT Ala56 mice ($p = 0.36$). No significant differences in behavioral accuracies were observed for SERT Ala56 mice for either the 500ms **(B)** or 300ms **(C)** stimulus duration. Black lines represent the group average performance under multisensory and the best unisensory conditions. Note the descending slope of these lines, which is apparent for wild type animals at the 500ms and 300ms durations and is not observed for SERT Ala56 mice. The significant level is: (* = $p < 0.05$)

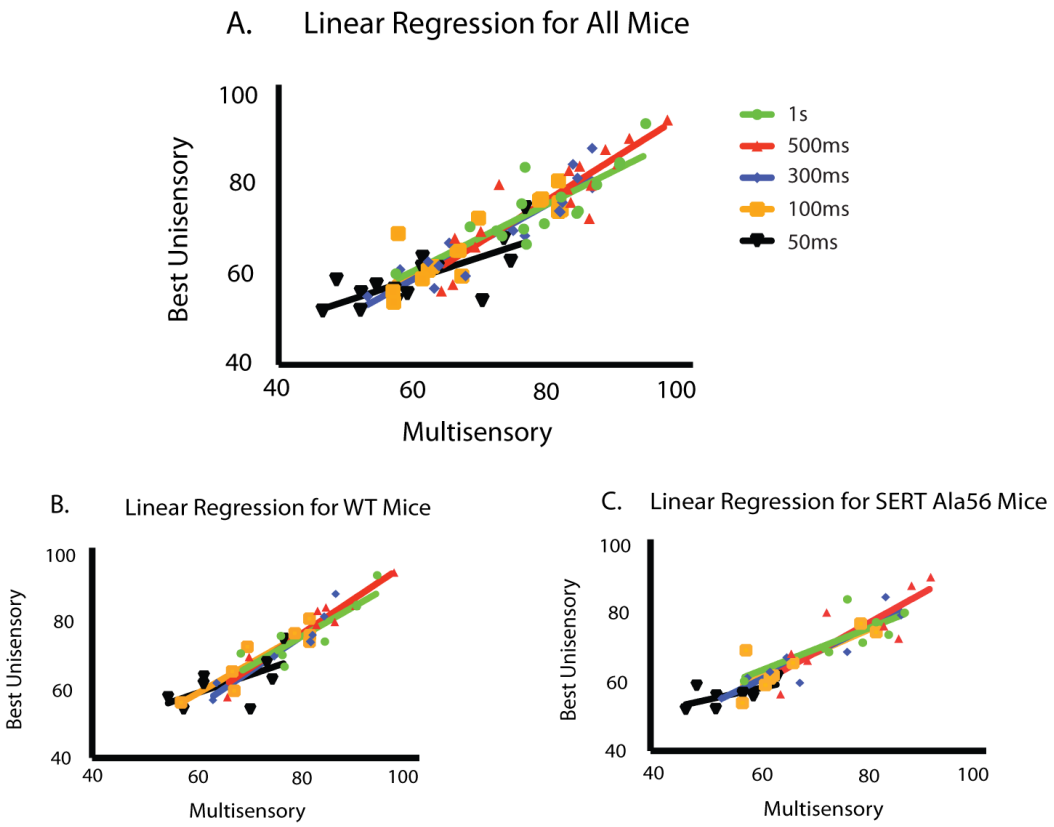

Figure 3-10. Determing the Relationship Between Accuracies Under Multisensory and the Best Unisensory Conditions. Signficant Pearson correlations between accuracies under multisensory and the best unisensory conditions were found at every stimulus duration - 1s: ($r = 0.7017$, $p < 0.0001$), 500ms: ($r = 0.8073$, $p < 0.0001$), 300ms: ($r = 0.8554$, $p < 0.0001$), 100ms: ($r = 0.7759$, $p < 0.0001$) and 50ms: ($r = 0.5414$, $p = 0.0012$) when performance was collapsed for all mice **(A)**. For wild type mice the values were observed as: 1s: ($r = 0.7660$, $p = 0.0044$), 500ms: ($r = 0.9228$, $p = 0.0001$), 300ms: ($r = 0.8980$, $p = 0.0003$), 100ms: ($r = 0.8401$, $p = 0.0014$) and 50ms: ($r = 0.4013$, $p = 0.0917$) **(B)**. For SERT Ala56 mice the values were: 1s: ($r = 0.7714$, $p = 0.0250$), 500ms: ($r = 0.6937$, $p = 0.0103$), 300ms: ($r = 0.8382$, $p = 0.0014$), 100ms: ($r = 0.6712$, $p = 0.0128$) and 50ms: ($r = 0.3733$, $p = 0.1076$) **(C)**.

significant main effect of sensory modality ($F(1, 7) = 6.969$; $p = 0.0334$) and a significant main effect of genotype ($F(1, 7) = 6.159$; $p = 0.0421$) (**Figure 3-9C.**). Significant differences between multisensory and the best unisensory conditions were observed for wild type mice ($p = 0.02$), yet no significant differences were observed for SERT Ala56 mice ($p = 0.36$). Significant differences between multisensory and the best unisensory conditions were found again for wild type mice at the 500ms stimulus duration ($p = 0.04$, **Figure 3-9B.**) but not the 1s, 100ms, or 50 ms durations. No significant differences between multisensory and the best unisensory conditions were observed for SERT Ala56 mice at any of the stimulus durations. Overall, multisensory gain was observed again for wild type animals, yet there was no statistical evidence of multisensory gain on the individual level for SERT Ala56 mice at any of the tested durations.

Discussion

This is the first study to demonstrate changes in multisensory function in a genetic mouse model associated with ASD. SERT Ala56 gain-of-function mutant animals showed no difficulties in learning the visual or auditory components of the task, but showed significantly diminished multisensory performance across multiple stimulus durations in comparison with wild type littermate controls. Behavioral gain in response to paired audiovisual stimulation was found at both the group and individual level for wild type mice. In contrast, multisensory gain was substantially impacted (and often eliminated) for the SERT Ala56 animals. This impaired behavioral performance manifested as more errors under multisensory conditions for SERT Ala56 animals, suggesting that the finding was not a result of changes in impulsivity (which would manifest as differences in early errors) or motivation (which would manifest as differences in late errors).

While overall it was demonstrated that multisensory function is atypical in SERT Ala56 animals and that unisensory (i.e., auditory alone, visual alone) processing appears to be comparable between the genotypes, it is important to provide a few potential alternate explanations/interpretations and caveats associated with these results. First, the current study does not directly measure visual and auditory acuity, but rather evaluates the ability to utilize these individual sensory modalities. Nonetheless, because the animals learned the unisensory components of these tasks in a comparable manner to wild type animals, there appear to be no gross differences in unisensory function. However, it was found that when collapsing across stimulus durations that significant differences were observed between genotypes for each of the three sensory conditions (auditory, visual, audiovisual). Therefore, although the multisensory effects were always greater than those seen within the individual modalities (and were the only significant changes when assessed for a given duration), the audiovisual deficits may reflect an additive or compounding effect of poorer performance under unisensory conditions. However, even if the effects are a result of cumulative effects on auditory and visual function, they do not weaken the importance of the observed multisensory effects, as these effects represent the highly adaptive integration of information across these senses.

Recently, there has been an increased interest in evaluating multisensory processing in individuals with ASD based on the importance of multisensory function for core symptoms such as communication and social interactions (Brandwein et al., 2015; Woynaroski et al., 2013). These human studies have demonstrated atypical multisensory processing in individuals with ASD on both the behavioral and neural levels (Brandwein et al., 2012; Foss-Feig et al., 2010; Keane, Rosenthal, Chun, & Shams, 2010; Kwakye et al., 2011; Marco et al., 2011; Smith & Bennetto, 2007; R. Stevenson et al., 2013; Williams, Massaro, Peel, Bosseler, & Suddendorf,

2004). Most germane in the current context, a number of these human studies have detailed weaker multisensory integrative function (Cascio et al., 2012; Foxe et al., 2013; Russo et al., 2010; R. A. Stevenson, Siemann, Schneider, et al., 2014; R. A. Stevenson, Siemann, Woynaroski, et al., 2014). In addition, a recent study found diminished integration of auditory and somatosensory stimuli at the cellular level in the insular cortex in three mouse lines relevant to autism (Gogolla, Takesian, Feng, Fagiolini, & Hensch, 2014). This study represents the first neural evidence of atypical multisensory responses in mouse models associated with ASD and demonstrated that a pharmacologic intervention early in development could result in normalizing these atypical multisensory responses (Gogolla et al., 2014). Given the absence of any testing for behavioral phenotype in these animals, the current study could be a powerful complement to this work and may represent a useful preclinical tool to test therapeutic strategies.

The current findings can be fit within several of the prevailing neurobiologically motivated theories of autism. For example, central coherence is based on the concept that the construction of coherent perceptual representations entails communication across widely distributed brain regions (Happe, 1999). In the theory of weak central coherence, individuals with autism are suggested to have impairments in integrating information into more global concepts (Y. Chen et al., 2012; Happe, 1999; Happe & Frith, 2006; Mongillo et al., 2008). Therefore, our current findings in the SERT Ala56 mouse model may be reflective of deficits in more complex or global processing, since multisensory function must entail communication across sensory cortical domains. Similarly, evidence suggests autism to be a "functional disconnection" syndrome, in which cortical connectivity is weaker (Belmonte et al., 2004; Courchesne, Redcay, Morgan, & Kennedy, 2005; Just et al., 2004; Rippon et al., 2007; Wass, 2011).

Studies have shown serotonergic projections to cortical and subcortical structures critical for sensory processing (Gaspar et al., 2003; Cecile Lebrand et al., 1998; van Kleef et al., 2012). In addition, 5-HT can modulate signal-to-noise ratio, receptive field size and structure, and the temporal dynamics of neuronal responses to unisensory stimuli (L. Hurley, Devilbiss, & Waterhouse, 2004; L. M. Hurley et al., 2002; Waterhouse et al., 1990; Waterhouse et al., 1986). Thus, one study has demonstrated that serotonin can play an important role in sharpening neural responses to somatosensory stimuli after prolonged visual deprivation in mice (Jitsuki et al., 2011) - the first evidence illustrating the effects of serotonin on cross modal plasticity (Jitsuki et al., 2011). While these studies have focused on the effect of 5-HT on unisensory stimuli (L. Hurley et al., 2004; L. M. Hurley et al., 2002; Waterhouse et al., 1990; Waterhouse et al., 1986), little is known about the role of 5-HT in multisensory function (Jitsuki et al., 2011). The major source of brain 5-HT, the dorsal raphe nucleus, projects to the superior colliculus (Andén, Dahlström, Fuxe, & Larsson, 1965; Binns, 1999), a major hub for multisensory processing (Burnett, Stein, Chaponis, & Wallace, 2004; Hirokawa et al., 2011; Jiang, Jiang, & Stein, 2006; Meredith, Nemitz, & Stein, 1987; Meredith & Stein, 1986a, 1986b; B. E. Stein & Stanford, 2008; Wallace, Meredith, & Stein, 1998; Wallace & Stein, 1996) and which expresses multiple 5-HT receptor subtypes (Binns, 1999; Huang, Mooney, & Rhoades, 1993; R. Mooney, Huang, Shi, Bennett-Clarke, & Rhoades, 1996; R. D. Mooney, Shi, & Rhoades, 1994; Segu, Abdelkefi, Dusticier, & Lanoir, 1986). Furthermore, the superior colliculus is likely to play an important role in the behaviors examined in the current study, given its central role in stimulus detection and orientation (Burnett, Henkel, Stein, & Wallace, 2002; Burnett et al., 2004; Hirokawa et al., 2011; Meredith et al., 1987; Meredith & Stein, 1986a, 1986b). In addition, studies have identified a variety of cortical brain regions important for the processing of multisensory

information (Driver & Noesselt, 2008; Jiang, Jiang, & Stein, 2002; Barry E Stein, Stanford, & Rowland, 2014; B. E. Stein, Wallace, Stanford, & Jiang, 2002; Wallace & Stein, 2000; Wilkinson, Meredith, & Stein, 1996), including areas in the rodent model (Barth, Goldberg, Brett, & Di, 1995; Brett-Green, Fifkova, Larue, Winer, & Barth, 2003; Gogolla et al., 2014; Komura, Tamura, Uwano, Nishijo, & Ono, 2005; Menzel & Barth, 2005; Olcese, Iurilli, & Medini, 2013; Raposo, Kaufman, & Churchland, 2014; Reig & Silberberg, 2014; Sieben, Roder, & Hanganu-Opatz, 2013; Wallace, Ramachandran, & Stein, 2004). One of these regions, area V2L, is of strong interest for the current study given that it has been shown to play an important role in multisensory behavior (Hirokawa et al., 2008) and receives direct projections from primary visual and auditory areas (Charbonneau, Laramee, Boucher, Bronchti, & Boire, 2012; Laramée, Rockland, Prince, Bronchti, & Boire, 2013; Laramee, Kurotani, Rockland, Bronchti, & Boire, 2011). Both the superior colliculus and V2L represent two important brain regions for future investigations into sensory and multisensory processing in rodent models of neurodevelopmental disorders.

Overall, this is the first study to evaluate and characterize multisensory processing behaviorally in a genetic mouse model relevant to autism. Here, we demonstrate a striking deficit in the ability of mice expressing a hyperfunctional SERT to derive behavioral benefits from paired audiovisual stimulation, a result that provides important insights into links between serotonergic signaling, multisensory function and autism. We believe that these findings offer great promise as a translational bridge seeking to link genetic, behavioral and neurodevelopmental findings in an effort to better elucidate the contributing role of alterations in sensory function in autism.

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CHAPTER IV

Task Dependencies Relevant for Behavioral Performance Under Multisensory Conditions in Mouse Models

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Abstract

In addition to social communication deficits and the presence of repetitive behaviors, altered sensory processing has been consistently described in autism spectrum disorder (ASD). A rare variant in the serotonin transporter (SERT) Ala56 is associated with sensory disturbances in individuals with autism and when this variant is expressed in mice produces behaviors similar to those found in ASD. Previously, utilizing a behavioral task that assessed accuracies under visual, auditory and audiovisual conditions, we found altered behavioral performance under multisensory conditions and limited multisensory gain in SERT Ala56 mice compared to wild type animals. In the current study, we were interested in evaluating (multi)sensory behavioral performance in a shorter variant of the original behavioral task. Here we used an irrelevant yet congruent sensory stimulus (i.e. auditory) paired with a relevant sensory stimulus (i.e. visual) to assess multisensory behavioral performance, since it has been shown in larger animal models that under these conditions behavioral benefits can be conferred. SERT Ala56 animals were more accurate under multisensory and visual alone conditions, however there were no differences in performance between the modalities for either genotype. Wild type animals were found to respond faster under multisensory conditions, yet there were no differences in reaction times under multisensory or visual conditions within either genotype. In addition, multisensory gain was found to be negative for both genotypes at multiple tested stimulus durations. Therefore, it appears that an irrelevant sensory stimulus does not result in behavioral benefits under multisensory conditions in the mouse model.

Introduction

Autism spectrum disorder (ASD) is characterized by deficits in social communication, the presence of restricted and repetitive behaviors (APA, 2011) and because of the high prevalence of sensory abnormalities found in this clinical population, are now part of the diagnostic criteria (Iarocci & McDonald, 2006; Marco, Hinkley, Hill, & Nagarajan, 2011; Rogers & Ozonoff, 2005). Atypical responses and processing has been observed for individual sensory systems in ASD and more recently studies have begun to demonstrate multisensory processing deficits when this sensory information is combined across modalities (Brandwein et al., 2012; Cascio, Foss-Feig, Burnette, Heacock, & Cosby, 2012; Foss-Feig et al., 2010; Foxe et al., 2013; Kwakye, Foss-Feig, Cascio, Stone, & Wallace, 2011; Smith & Bennetto, 2007; Mark T Wallace & Stevenson, 2014; Zaidel, Goin-Kochel, & Angelaki, 2015). Multisensory integration plays an integral role in the creation of a unified perceptual gestalt (Murray & Wallace, 2011), is fundamental in allowing for the successful interpretation of communicative and social signals (Stein, 2012; R. A. Stevenson, Ghose, et al., 2014) and may therefore be highly beneficial to study in the context of autism spectrum disorder, where known deficits in social communication exist (Brandwein et al., 2015; Ross et al., 2011; R. Stevenson et al., 2013; R. A. Stevenson, Ghose, et al., 2014; R. A. Stevenson, Segers, Ferber, Barense, & Wallace, 2014; R. A. Stevenson, Siemann, et al., 2014).

The serotonin system has been consistently implicated in autism spectrum disorder, with about 30% of individuals with ASD displaying hyperserotonemia or increased whole blood serotonin levels. To this point, genetic studies identified a group of rare amino acid variants in the serotonin transporter (SERT) in families of individuals with ASD (Prasad, Steiner, Sutcliffe, & Blakely, 2009; Prasad et al., 2005; Sutcliffe et al., 2005). The most common variant, SERT

Ala56, was associated with sensory aversion and rigid-compulsive behaviors in individuals with ASD (Sutcliffe et al., 2005). When SERT Ala56 was later expressed in mice, these animals were found to display the biomarker of hyperserotonemia and demonstrated abnormalities in social and communicative behaviors along with exhibiting repetitive behaviors (Jeremy Veenstra-VanderWeele et al., 2009; J. Veenstra-VanderWeele et al., 2012). The utility of studying this knock-in mouse model is that it allows for the examination of altered serotonin (5-HT) function with changes in sensory and multisensory function, which may be valuable specifically in the context of ASD. Various investigations have studied the role of 5-HT on sensory development (Cases et al., 1996; Gaspar, Cases, & Maroteaux, 2003; Lesch & Waider, 2012; van Kleef, Gaspar, & Bonnin, 2012) sensory processing (Esaki et al., 2005; Hurley, Thompson, & Pollak, 2002; Jitsuki et al., 2011; Waterhouse, Azizi, Burne, & Woodward, 1990), and have demonstrated that 5-HT and SERT are expressed in numerous cortical and subcortical brain regions critical for unisensory processing (Binns, 1999; Gaspar et al., 2003; Cécile Lebrand et al., 1996; Cecile Lebrand et al., 1998; Lesch & Waider, 2012). While the majority of these investigations have focused on individual sensory systems (Hurley & Pollak, 2001, 2005; Hurley et al., 2002; Murphy, Uzbekov, & Rose, 1980; Waterhouse et al., 1990), little is known in terms of the relationship between serotonin and multisensory processing, which should warrant further investigations.

Previously, using a behavioral task that assessed accuracies under visual alone, auditory alone and congruent audiovisual conditions, we observed behavioral deficits under multisensory conditions for these SERT Ala56 animals. In this original paradigm, both wild type and SERT Ala56 mice were trained under visual alone and auditory alone conditions on separate independent tasks before being tested under visual, auditory and congruent multisensory

conditions. The current study aimed to design a shorter variant of the original behavioral paradigm. The rationale for attempting this was that it was previously found that wild type mice learned the visual component of the task after 20.6 ± 4.4 days and SERT Ala56 mice completed the task after 17.8 ± 4.7 days. However, wild type mice completed the auditory component of the task after 58.0 ± 11.1 days and SERT Ala56 animals finished the auditory training after 49.0 ± 7.5 days (Siemann et al, in submission). Therefore, one goal was to make this behavioral paradigm shorter to complete by eliminating the auditory training component, which may then allow for more researchers to potentially assess (multi)sensory processing behaviorally in mice in a more timely manner. In the current task, mice were first trained under visual alone conditions. Once this was completed mice were then tested under visual only conditions and visual conditions that were presented with an irrelevant yet congruent auditory noise burst. It has been shown previously in larger animal models that presenting an irrelevant yet congruent sensory stimulus (i.e. auditory) with a relevant sensory stimulus (i.e. visual) can result in behavioral enhancements under these multisensory conditions (Lovelace, Stein, & Wallace, 2003; Stein, Meredith, Huneycutt, & McDade, 1989; Thorne & Debener, 2008; Van der Burg, Olivers, Bronkhorst, & Theeuwes, 2008). In addition, under these conditions it has been shown that these behavioral benefits under multisensory conditions may be limited or decreased in individuals with ASD (Collignon et al., 2013). Therefore, we were interested in determining if 1) wild type mice would demonstrate behavioral benefits under multisensory conditions in a task similar to those used in larger animal models, 2) if wild type mice would demonstrate similar behavioral benefits as observed in the original behavioral task and 3) if SERT Ala56 mice would demonstrate similar behavioral deficits as found in the original behavioral paradigm.

Materials and Methods

All animal procedures were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by Vanderbilt University. SERT Ala56 knock-in mice were constructed as previously described (Jeremy Veenstra-VanderWeele et al., 2009). Animals were housed and kept under a food-restricted diet previously outlined (Siemann et al., 2015). 8 male SERT Ala56 and 10 male wild type littermate control mice were used and experimenters were blinded to the genotypes throughout the behavioral paradigm. SERT Ala56 and wild type animals were the offspring of heterozygous SERT Ala56 parents and began training between 14 – 17 weeks of age.

Behavioral Paradigm

Animals proceeded through a similar behavioral training and testing paradigm that has been previously described (**Figure 4-1.**) (Siemann et al., 2015). In this variant of the original behavioral task, mice were initially trained to respond to visual stimuli for a liquid Ensure reward. As previously described (Siemann et al., 2015), daily training sessions for visual training consisted of 100 trials, with criterion reached when mice achieved 65% correct performance for two consecutive days. However, once the visual training task was completed successfully, mice then progressed to behavioral testing sessions where either visual or congruent audiovisual (multisensory) pairings were presented. For multisensory stimulus presentations, an auditory white noise burst was played at 85db from a centrally located speaker simultaneously and congruently with the visual stimulus. Mice needed to respond to where the visual stimulus was presented in order to receive a liquid Ensure reward. Animals completed 100 trials (50 per sensory modality) lasting up to 120 minutes per testing session with behavioral sessions

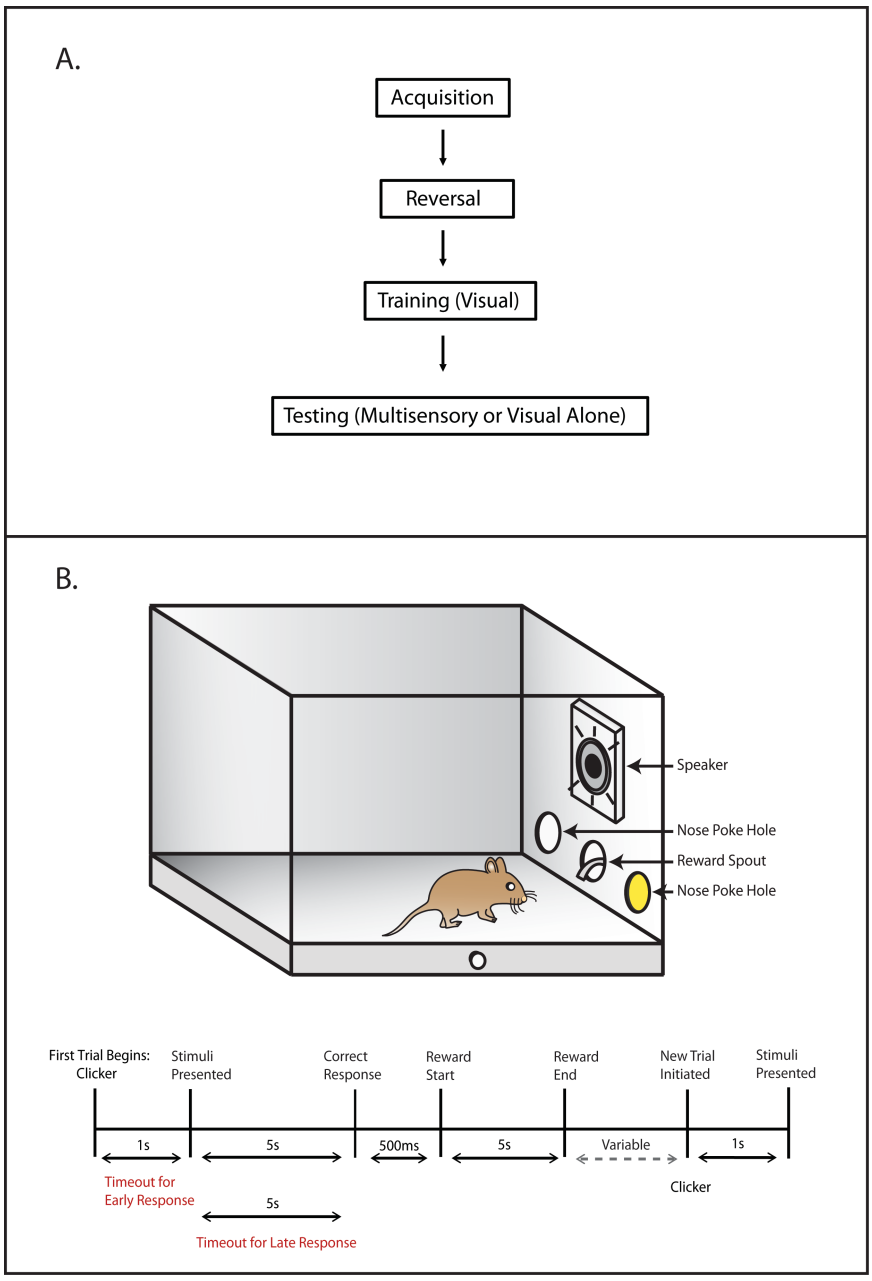

Figure 4-1. Behavioral Paradigm and Trial Schematic. (A) An outline for the progression of training and testing in this behavioral task. **(B)** Above: A diagram of the operant chamber during the presentation of a congruent audiovisual stimulus (represented by the yellow color within the nose poke hole, where the LED was positioned) and by the active speaker playing a white noise burst. **(B)** Below: A schematic of the trial sequence.

occurring once per day. Mice were tested for 5 days at each of four stimulus durations in a block design.

Data Analysis

All behavioral experiments were designed with customized Med-PC IV programs (Med Associates Inc.). As previously described (Siemann et al., 2015), accuracies measured for visual training sessions were calculated as percent correct utilizing a 65% correct response rate for two consecutive days. Multisensory gain was calculated as (mean number of correct multisensory trials – mean number of correct visual alone trials) / (mean number of correct visual alone trials) x 100. Accuracies were calculated as correct trials / correct + incorrect trials. Prism 6 (Graphpad Software Inc., La Jolla, CA) was used for all statistical analyses. Two-way analysis of variances (ANOVAs), Sidak's multiple comparisons tests and standard error of the mean were used for all experiments unless otherwise specified.

Results

SERT Ala56 and Wild Type Mice Exhibit Comparable Behavioral Performance Under Visual Training Conditions

SERT Ala56 and wild type (WT) littermate control mice first learned to respond to one side of an operant chamber to receive a liquid Ensure reward (acquisition) and once acquired animals then needed to respond to the opposite side to obtain a reward (reversal). No significant differences between genotypes were observed for either acquisition or reversal learning ($p = 0.26$, $p = 0.45$, **Figures 4-2A., 4-2B.**). Next animals progressed to the visual training component of the behavioral task. Mice detected and responded to visual stimuli that were presented on

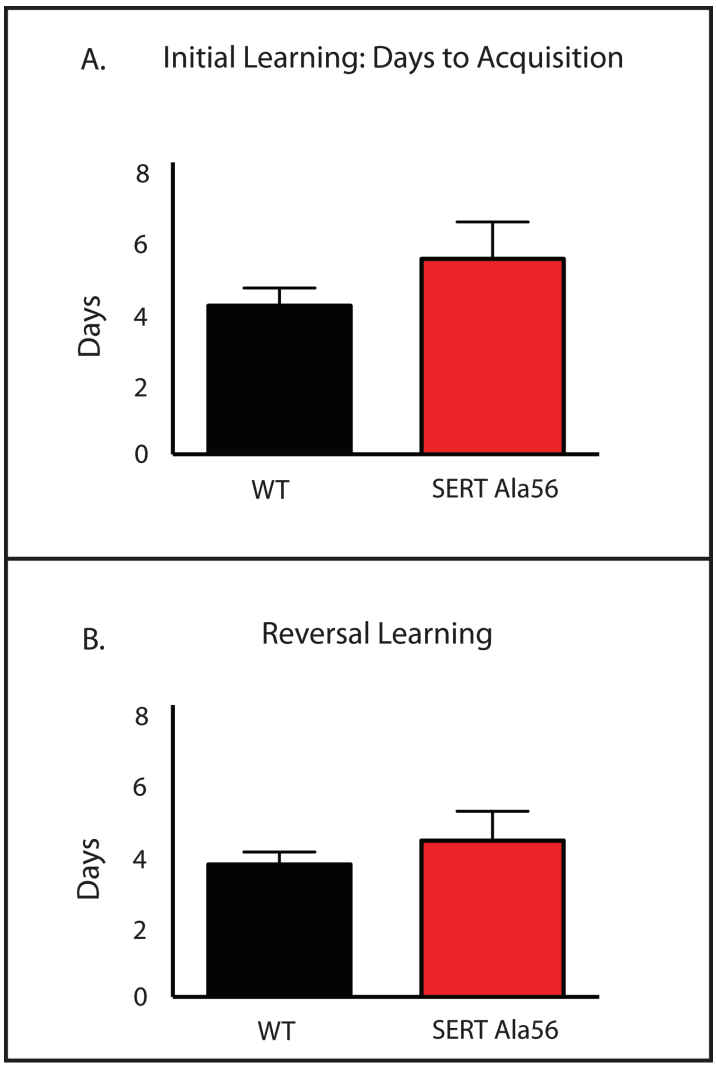

Figure 4-2. Evaluating Initial Acquisition and Reversal Learning in Wild Type and SERT Ala56 mice. Unpaired t tests showed no significant differences between the genotypes for either **(A)** initial acquisition ($p = 0.26$) or for **(B)** reversal learning ($p = 0.45$).

either side of an operant chamber to receive a liquid Ensure reward. Under these training conditions no significant differences in accuracy (**Figure 4-3A.**, $p = 0.32$) or days to learn (**Figure 4-3B.**, $p = 0.73$) were observed between SERT Ala56 mice and WT littermate controls. In summary, wild type and SERT Ala56 mice demonstrated comparable behavioral performance in initial acquisition, reversal learning and under visual training conditions.

SERT Ala56 Mice Are More Accurate Than Wild Type Mice Under Visual Only and Multisensory Conditions

After animals completed the visual training component of the task for two consecutive days using a 65% correct criterion, mice were then tested under either visual alone or congruent audiovisual conditions. Under these multisensory conditions, an auditory white noise burst was presented from a centrally located speaker at the same time and for the same duration as the visual stimulus. In this study mice were never previously exposed to the auditory stimulus. In order to receive a liquid Ensure reward, animals needed to respond to the side of the operant chamber where the visual stimulus was presented. The stimulus durations were varied in order to modulate the effectiveness of the visual and auditory stimuli and to best assess the behavioral benefit under these multisensory conditions (Gleiss & Kayser, 2012; Hirokawa, Bosch, Sakata, Sakurai, & Yamamori, 2008; Sakata, Yamamori, & Sakurai, 2004; Sheppard, Raposo, & Churchland, 2013). The hypothesis in this study was that the presentation of a simultaneous and congruent auditory noise burst would aid in the detection of the visual stimulus especially at the shorter stimulus durations. Mice were initially tested at the longest duration condition (1s) under visual and audiovisual conditions for 5 days. Following this, performance was then evaluated for 5 days at each of the following durations: 500ms, 300ms and 50ms in a block design. We first evaluated behavioral performance when collapsing across the tested stimulus durations. Using a

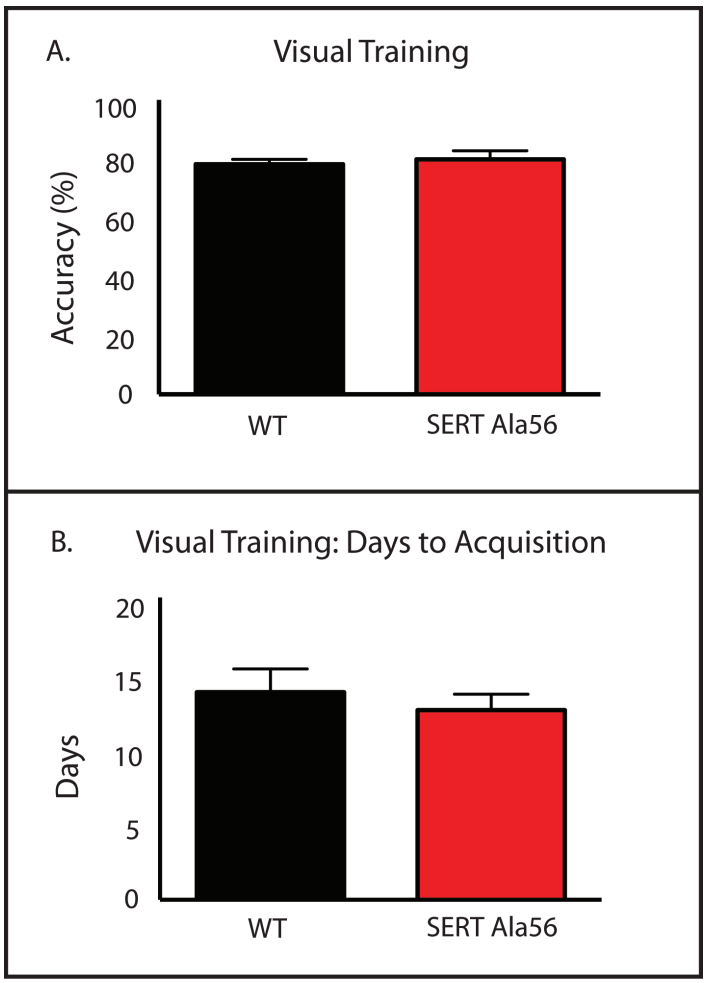

Figure 4-3. Measuring Behavioral Performance Under Visual Training Conditions. No significant differences were observed between wild type and SERT Ala56 mice for either **(A)** accuracy ($p = 0.32$) or **(B)** days to acquisition ($p = 0.73$) under visual training conditions. Wild type mice completed the visual training after 14.1 ± 1.6 days with a final accuracy of 78.23% ± 1.65%. SERT Ala56 animals completed the visual task after 13.4 ± 1.1 days with a final accuracy of 81.43% ± 2.803%.

two-way ANOVA, a significant main effect of genotype ($F(1, 144 = 4.74$; $p = 0.031$) was observed, yet no main effect of sensory modality ($F(1, 144 = 0.5423$; $p = 0.463$) nor a significant interaction effect ($F(1, 144 = 0.0642$; $p = 0.8003$) were observed (**Figure 4-4.**). Post hoc tests revealed no significant differences between the genotypes under either multisensory ($p = 0.321$) or visual alone ($p = 0.168$) conditions. In addition, no significant differences were found when comparing behavioral accuracies under multisensory and visual alone conditions for either wild type ($p = 0.9228$) or SERT Ala56 ($p = 0.7519$) animals. We then determined behavioral accuracies across the stimulus durations. Under multisensory conditions, a two-way ANOVA demonstrated a significant main effect of stimulus duration ($F(3, 66) = 75.71$; $p < 0.0001$) and a significant main effect of genotype ($F(1, 66) = 7.159$; $p = 0.0094$) but no significant interaction effect ($F(3, 66) = 2.604$; $p = 0.0592$) was observed **(Figure 4-5A.)**. Sidak's multiple comparisons test demonstrated a significant difference between the genotypes in multisensory behavioral accuracy at the 500ms stimulus duration ($p = 0.0081$). Under visual alone conditions a significant main effect of stimulus duration ($F(3, 66) = 80.38$; $p < 0.0001$), a significant main effect of genotype ($F(1, 66) = 13.84$; $p = 0.0004$) and a significant interaction effect ($F(3, 66) = 3.081$; $p = 0.0333$) were observed **(Figure 4-5B.)**. Sidak's multiple comparisons test demonstrated significant differences between the genotypes at the 500ms ($p = 0.0076$) and the 300ms ($p = 0.0054$) stimulus durations. Lastly, we compared the behavioral accuracies under multisensory and visual alone conditions for each individual mouse at every tested stimulus duration. Significant Pearson correlations between multisensory and visual accuracies were found for wild type **(Figure 4-6A.)** and SERT Ala56 mice **(Figure 4-6B.)**. Therefore, if a mouse was accurate

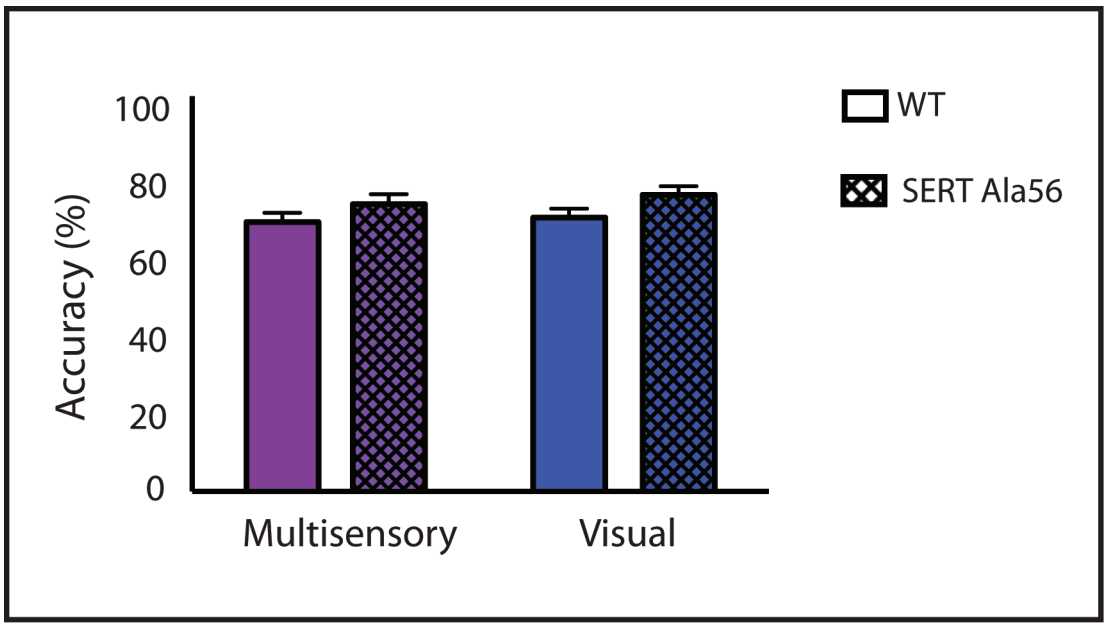

Figure 4-4. Determining Behavioral Accuracies Under Visual Alone and Multisensory Conditions When Collapsing Across Stimulus Durations. Accuracies for these collapsed conditions for wild type animals for multisensory conditions were – 70.02% ± 2.45% and under visual alone conditions – 71.99% ± 2.369%. Accuracies for SERT Ala56 animals were as follows: multisensory – 74.711% ± 2.58% and visual alone – 77.125% ± 2.29%. A significant main effect of genotype ($F(1, 144 = 4.74; p = 0.031)$ was observed, yet no main effect of sensory modality ($F(1, 144 = 0.5423; p = 0.463)$ was found.

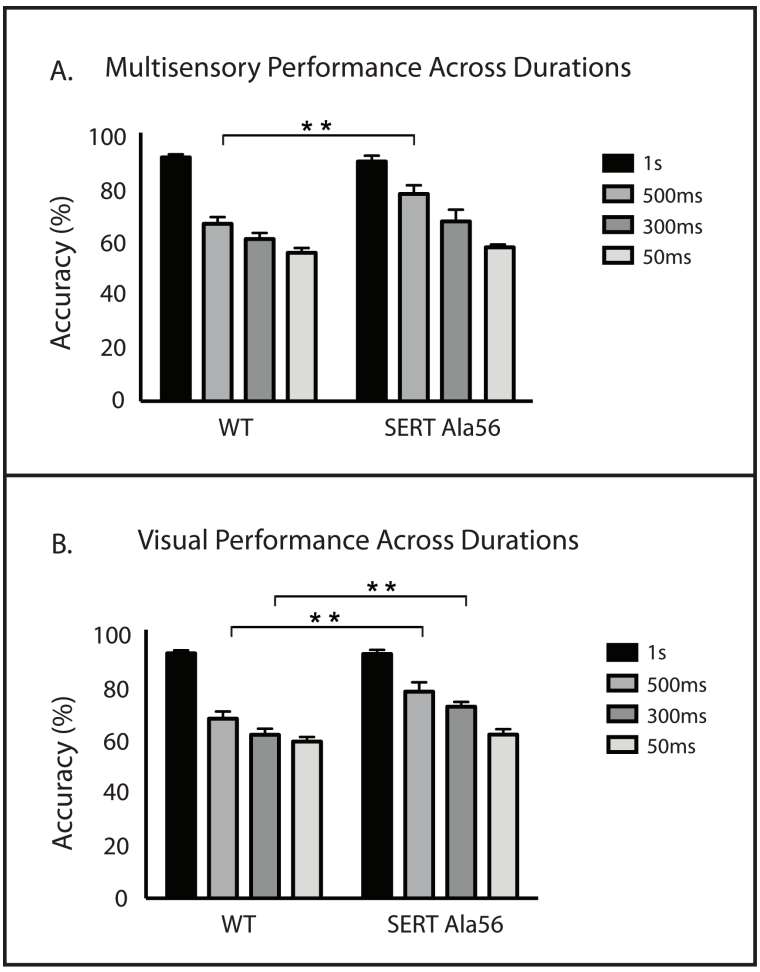

Figure 4-5. Behavioral Accuracies Under Visual Only and Multisensory Conditions Across Stimulus Durations. A significant main effect of stimulus duration ($F(3, 66) = 75.71$; $p < 0.0001$) and genotype ($F(1, 66) = 7.159$; $p = 0.0094$) were observed under multisensory conditions **(A)**. In addition, a significant difference between the genotypes was found at the 500ms stimulus duration ($p = 0.0081$). Under visual only conditions a significant main effect of stimulus duration ($F(3, 66) = 80.38$; $p < 0.0001$) genotype ($F(1, 66) = 13.84$; $p = 0.0004$) and an interaction effect ($F(3, 66) = 3.081$; $p = 0.0333$) were found **(B)**. Significant genotype differences were also observed at the 500ms ($p = 0.0076$) and the 300ms ($p = 0.0054$) stimulus durations.

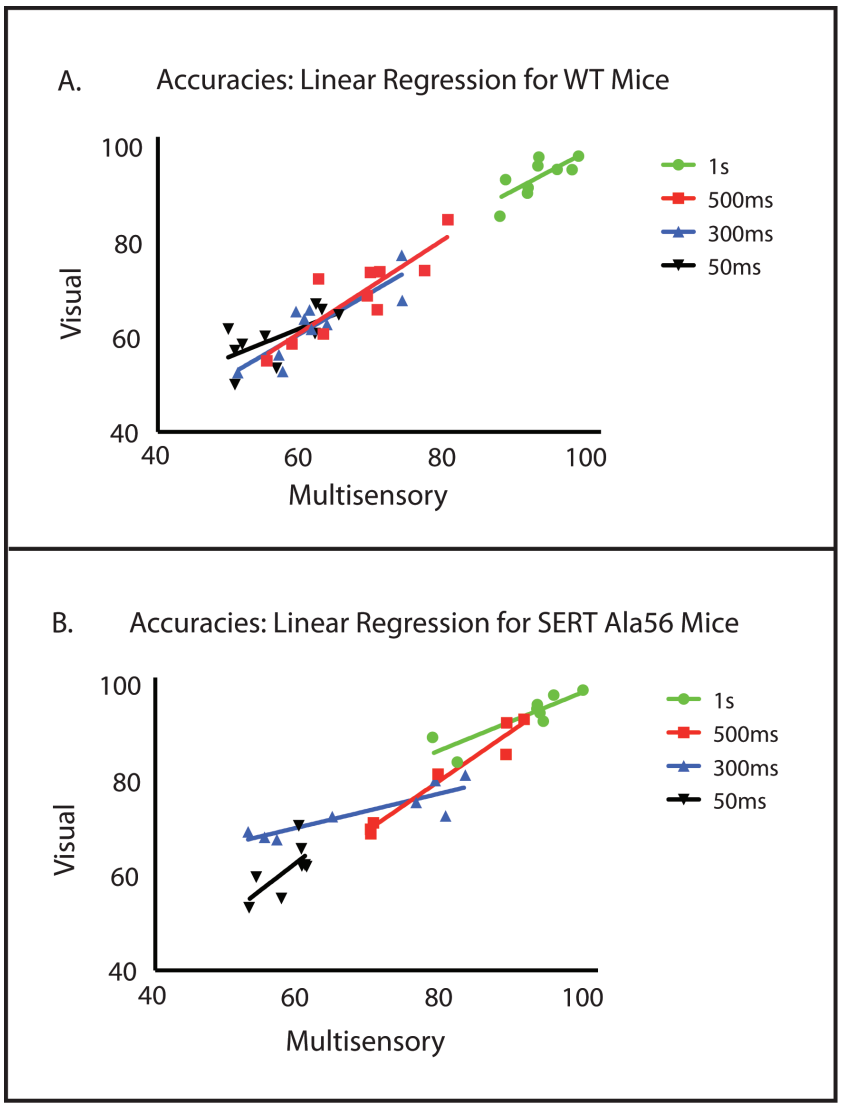

Figure 4-6. Determining the Relationship Between Behavioral Accuracies under Visual Alone and Multisensory Conditions. Significant Pearson correlations between accuracies under multisensory and visual alone conditions were found at every stimulus duration for wild type mice and were significant at most of the stimulus durations for SERT Ala56 mice. For wild type mice the values were as follows: 1s: ($r = 0.7437$, $p = 0.0216$), 500ms: ($r = 0.8727$, $p = 0.0010$), 300ms: ($r = 0.8541$, $p = 0.0017$) and 50ms: ($r = 0.6645$, $p = 0.0361$) **(A)**. For SERT Ala56 mice the values were: 1s: ($r = 0.8759$, $p = 0.0043$), 500ms: ($r = 0.97858$, $p = 0.0001$), 300ms: ($r = 0.8695$, $p = 0.005$) and 50ms: ($r = 0.6807$, $p = 0.0631$) **(B)**.

under visual alone conditions it was also more likely to be accurate under multisensory conditions, regardless of genotype. Overall we observed that, when evaluating behavioral performance across stimulus durations, SERT Ala56 animals were more accurate than wild type mice under both visual and multisensory conditions. However, when collapsing across these stimulus durations, no significant differences in behavioral accuracies were observed between genotypes for either visual alone or audiovisual conditions. Most importantly, when evaluating within either genotype there were no significant differences found in behavioral accuracies when comparing multisensory and visual alone conditions.

Multisensory Gain is Limited Across All Stimulus Durations for Both Wild Type and SERT Ala56 Mice

To evaluate the amount of behavioral facilitation or benefit under multisensory conditions, multisensory gain was calculated using the equation (average multisensory correct trials – average best unisensory correct trials) / (average best unisensory correct trials) x 100 (Meredith & Stein, 1983). Multisensory gain was limited and mostly negative for both wild type and SERT Ala56 animals across all of the tested stimulus durations (**Figure 4-7.)**. The most negative multisensory gain was observed at the 50ms duration for wild type animals (-5.043%) and at the 300ms (-6.59%) and 50ms (-4.03%) stimulus durations for SERT Ala56 mice. Multisensory gain was not found to be significantly different from zero at any of the stimulus durations for either genotype. Since this group data could have been due to the performance of a few animals, behavioral gain was further examined for each individual animal.

We compared the behavioral performance data under multisensory and visual alone conditions for each individual mouse at each tested stimulus duration. Based on the group level data, we

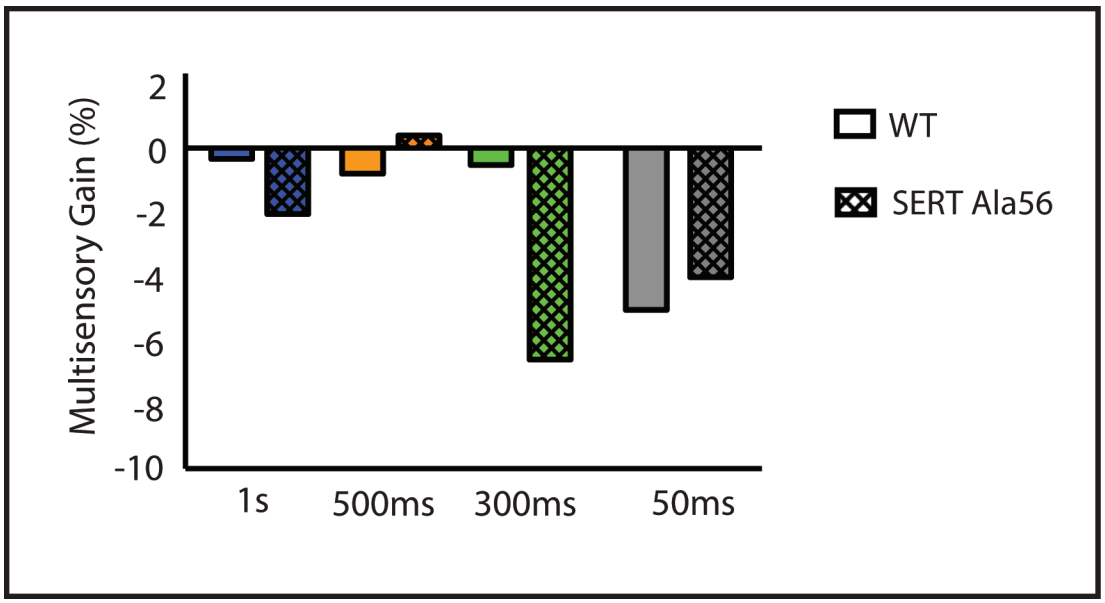

Figure 4-7. Evaluating Multisensory Gain for Wild Type and SERT Ala56 Animals Across Stimulus Durations at the Group Level. Limited multisensory gain was found for wild type and SERT Ala56 animals at the group level at all stimulus durations. The values for multisensory gain for wild type mice were as follows: 1s: -0.337%, 500ms: -0.794%, 300ms: -0.522%, and 50ms: -5.043%. Multisensory gain values for SERT Ala56 mice were: 1s: -2.057%, 500ms: 0.391%, 300ms: -6.59%, and 50ms: -4.03%.

first evaluated potential individual differences specifically at the 300ms and 50ms stimulus durations. At the 300ms duration, utilizing a repeated measures two-way ANOVA, no significant main effect of sensory modality ($F(1, 7 = 2.546$; $p = 0.1546$), genotype ($F(1, 7 = 3.995$; $p = 0.0858$) nor a significant interaction effect ($F(1, 7 = 1.165$; $p = 0.3163$) were observed (**Figure 4-8A.**). At the 50ms stimulus duration, a significant main effect of sensory modality ($F(1, 8 = 12.36$; $p = 0.0079$) was found, yet no significant main effect of genotype ($F(1, 8 = 0.9119$; $p = 0.3676$) nor a significant interaction ($F(1, 8 = 0.1197$; $p = 0.7383$) were observed (**Figure 4-8B.**). Overall, multisensory gain was limited and mostly negative for both wild type and SERT Ala56 animals at the group level. At the individual level, there was no statistical evidence of multisensory gain for either wild type or SERT Ala56 mice at any of the tested durations.

Wild Type Animals Are Faster Than SERT Ala56 Animals Under Multisensory Conditions

To further evaluate potential behavioral benefits under multisensory conditions we examined the reaction times for wild type and SERT Ala56 animals. We first evaluated reaction times across the tested stimulus durations. Under multisensory conditions, no significant main effect of stimulus duration ($F(3, 21) = 2.859$; $p = 0.0614$), genotype ($F(1, 7 = 1.065$; $p = 0.3363$) nor a significant interaction effect ($F(3, 21 = 0.5568$; $p = 0.6494$) were observed (**Figure 4-9A.**). Similarly under visual alone conditions, no significant main effect of stimulus duration ($F(3, 21 = 0.5526$; $p = 0.652$), genotype ($F(1, 7) = 0.5135$; $p = 0.4968$) nor a significant interaction ($F(3, 21 = 0.5184$; $p = 0.6742$) were found (**Figure 4-9B.**). Next we evaluated reaction times by collapsing across the stimulus durations. Using a two-way ANOVA, a main effect of genotype ($F(1,136) = 10.98$; $p = 0.0012$) was observed, yet no main effect of sensory modality ($F(1,136) = 0.3335$; $p = 0.5646$) nor a significant interaction effect ($F(1,136) = 0.1963$; $p = 0.6584$) were found (**Figure 4-10.)**. Sidak's multiple comparisons test revealed significant differences in

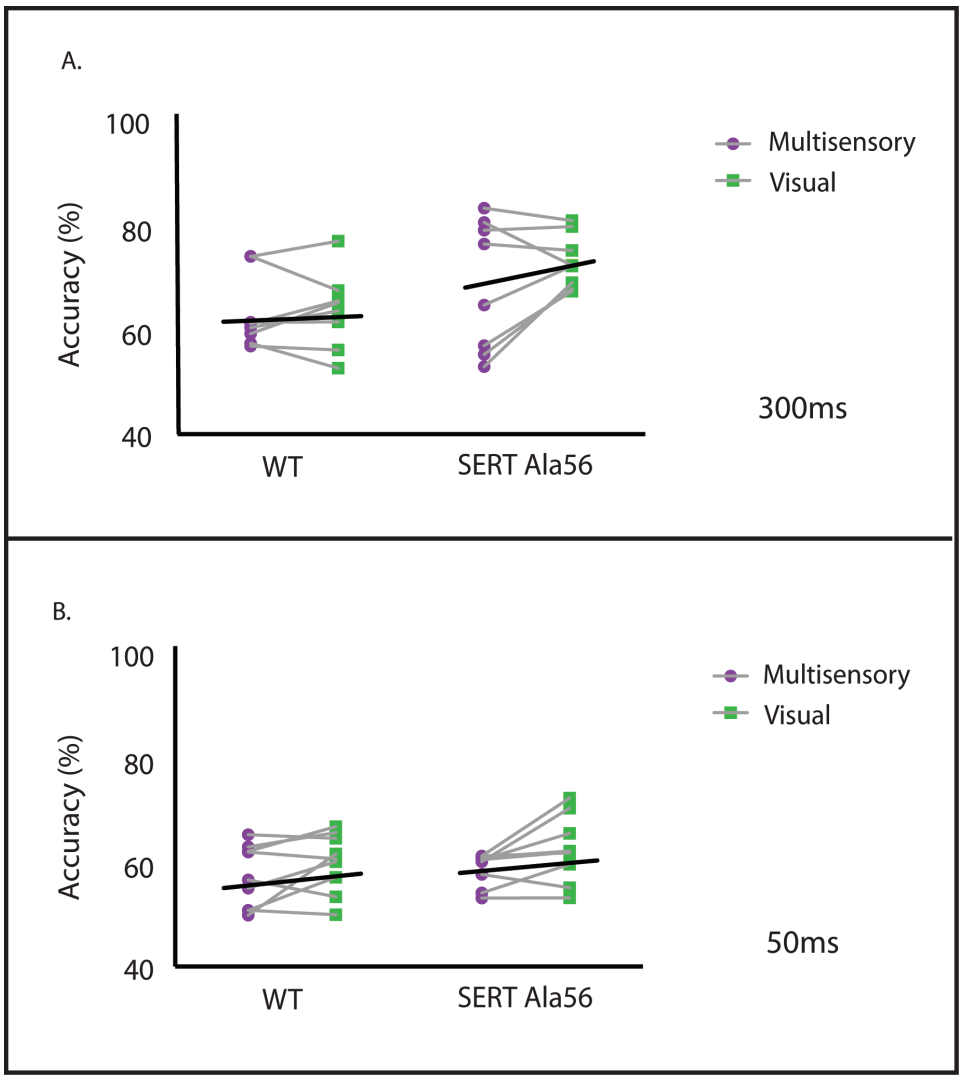

Figure 4-8. Determining Multisensory Gain at the Individual Performance Level for Wild Type and SERT Ala56 Animals. The most negative multisensory gains at the group level were observed at the 300ms and the 50ms stimulus durations. When evaluating performance at the individual level, no significant differences in accuracies between multisensory and visual alone conditions were observed at either the 300ms **(A)** or the 50ms **(B)** stimulus durations for either wild type or SERT Ala56 animals. Black lines represent the group average performance under multisensory and visual alone conditions.

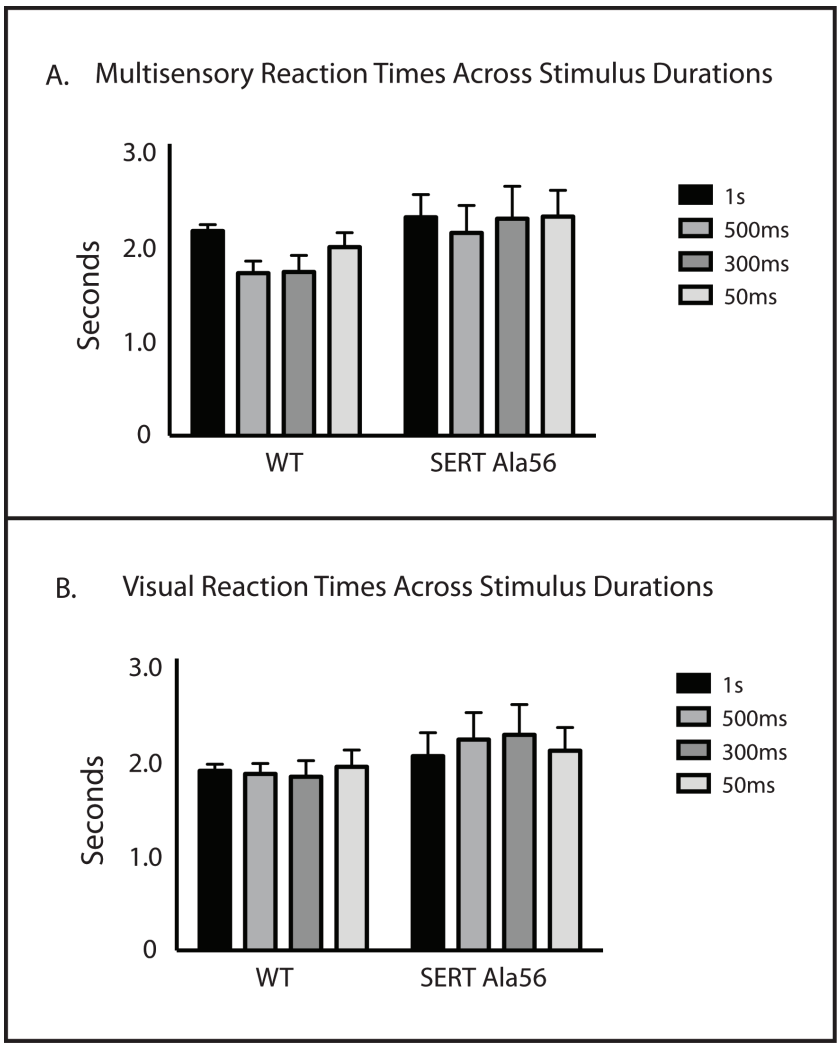

Figure 4-9. Evaluating Reaction Times for Wild Type and SERT Ala56 Animals Across Stimulus Durations. No significant main effect of genotype was observed for either (**A**) multisensory ($F(1, 7 = 1.065$; $p = 0.3363$) or **(B)** visual alone conditions ($F(1, 7) = 0.5135$; $p = 0.4968$) when measuring across stimulus durations.

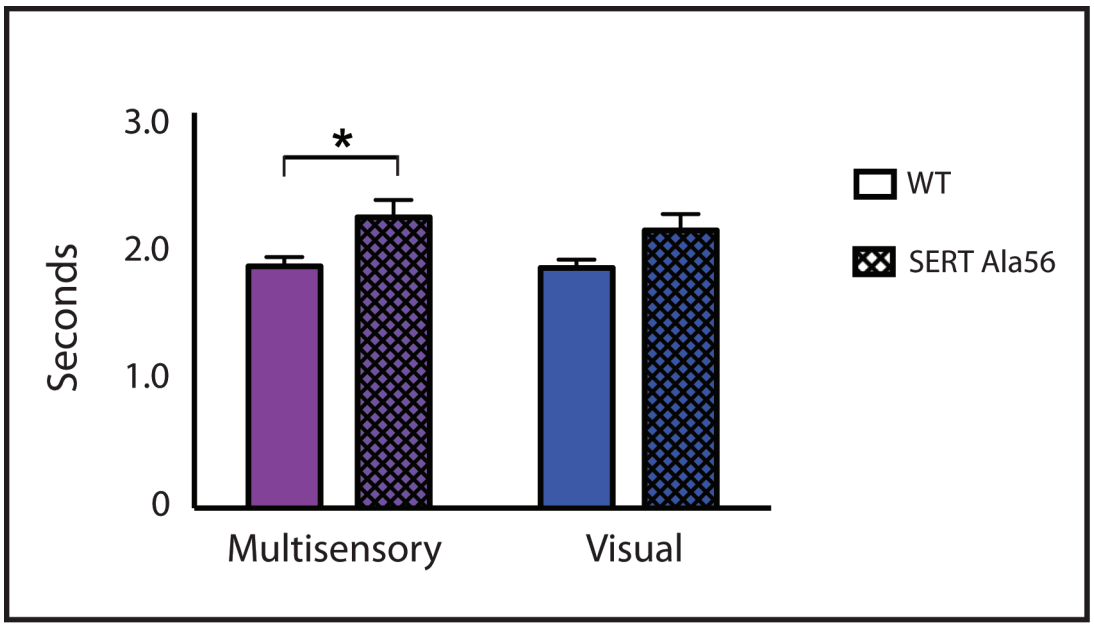

Figure 4-10. Measuring Reaction Times for Visual Alone and Multisensory Conditions When Collapsing Across Stimulus Durations. A main effect of genotype ($F(1,136) = 10.98$; $p = 0.0012$) was observed, yet no main effect of sensory modality ($F(1,136) = 0.3335$; $p = 0.5646$) was observed for reaction times. Post hoc tests revealed that wild type animals were faster under multisensory conditions compared to SERT Ala56 mice.

reaction times between genotypes for multisensory conditions ($p = 0.0176$) but no differences were observed under visual alone conditions ($p = 0.0867$). Interestingly, when evaluating reaction times within each genotype, no significant differences were found when comparing multisensory to visual only conditions for either wild type ($p = 0.9940$) or SERT Ala56 mice ($p = 0.7337$). Lastly, we compared the reaction times under multisensory and visual alone conditions for each individual mouse at each stimulus duration. When comparing behavioral performance in this manner, significant Pearson correlations were found for both wild type **(Figure 4-11A.)** and SERT Ala56 mice **(Figure 4-11B.)**. Therefore, if a mouse was quick to respond under visual alone conditions it was also quicker to respond under multisensory conditions, regardless of genotype. Overall, wild type animals were found to be faster under only audiovisual conditions. However, when evaluating within each genotype, no significant differences in reaction times were observed when comparing multisensory to visual alone conditions.

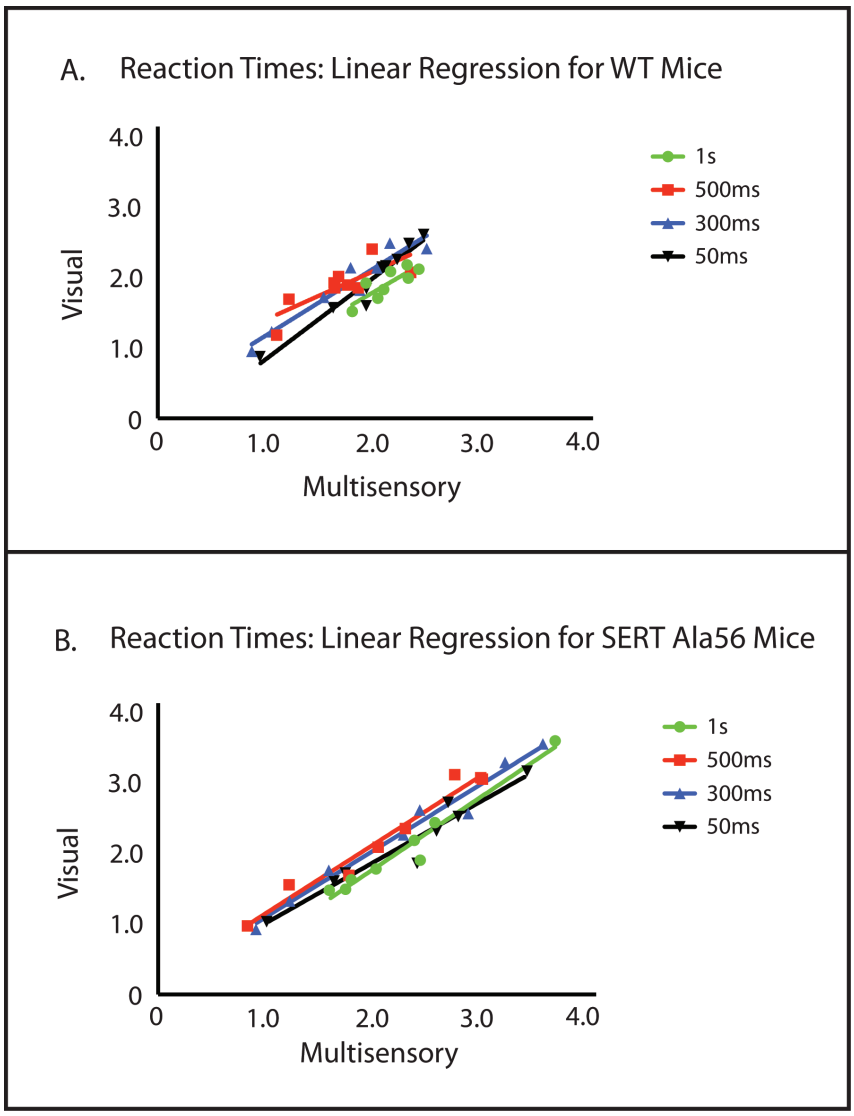

Figure 4-11. Evaluating the Relationship Between Reaction Times Under Visual Alone and Multisensory Conditions. Significant Pearson correlations between reaction times under multisensory and visual only conditions were found at every stimulus duration. For wild type mice the values were observed as: 1s: ($r = 0.8330$, $p = 0.0053$), 500ms: ($r = 0.7887$, $p = 0.0115$), 300ms: ($r = 0.9581$, $p < 0.0001$) and 50ms: ($r = 0.9401$, $p < 0.0001$) **(A)**. For SERT Ala56 mice the values were: 1s: ($r = 0.9802$, $p < 0.0001$), 500ms: ($r = 0.9819$, $p < 0.0001$), 300ms: ($r = 0.9868$, $p < 0.0001$) and 50ms: ($r = 0.9677$, $p < 0.0001$) **(B)**.

Discussion

In this study, we first observed that wild type and SERT Ala56 animals performed comparably under initial acquisition, reversal learning and visual training tasks. These findings are significant because they first indicate no potential differences in learning or unisensory processing between the genotypes and in addition replicate similar results that we found in our previous behavioral study (Siemann et al., in submission). In our current study, it was found that SERT Ala56 mice were more accurate than wild type animals under both audiovisual and visual alone conditions across the tested stimulus durations. However, these genotype differences did not hold for analyses when collapsing across stimulus durations. In addition, when evaluating behavioral performance within each genotype, no significant differences between the visual and audiovisual conditions were observed. This was further evident when multisensory gain was measured at both the group and individual performance levels. At the group performance level, multisensory gain was found to be negative at the majority of stimulus durations for both wild type and SERT Ala56 mice. This demonstrated that at the group level, both genotypes tended to be more accurate under visual alone conditions compared to multisensory conditions. This was further evident at the individual performance level, where no significant differences in behavioral accuracies were observed for either wild type or SERT Ala56 mice when comparing multisensory and visual alone conditions. Lastly, while wild type mice were faster under multisensory conditions compared to SERT Ala56 mice, we observed no significant differences in reaction times between multisensory and visual alone conditions when evaluating within either genotype.

Overall, in the current study, we have shown that an irrelevant sensory stimulus (auditory) paired with a relevant sensory stimulus (visual) does not result in behavioral benefits

for either wild type or SERT Ala56 mice. This study is the first to evaluate the behavioral effects of an irrelevant auditory stimulus on detecting a visual stimulus in the mouse model. Surprisingly, behavioral benefits under these conditions were not conferred in the mouse despite being previously observed in larger animal species (Lovelace et al., 2003; Stein et al., 1989; Thorne & Debener, 2008; Van der Burg et al., 2008). In addition to our findings being different from studies in larger species, we also observed results from the current study that are significantly different from those that we previously observed using a longer behavioral paradigm to assess (multi)sensory function in the mouse (Siemann et al., 2015) (Siemann et al., in submission). In the original behavioral task, both wild type and SERT Ala56 mice were trained under visual alone and auditory alone conditions independently and were then tested under visual, auditory or congruent audiovisual conditions. In this prior study (Siemann et al., in submission), we observed that both wild type and SERT Ala56 animals were more accurate under multisensory conditions compared to either visual or auditory alone conditions. In addition, wild type animals were more accurate under multisensory conditions and displayed significantly more multisensory gain at both the group and individual levels compared to SERT Ala56 animals.

However, in the current study, we believe that this behavioral task variant may not be appropriate to assess behavioral benefits under multisensory conditions in the mouse model. This is evident by the fact that neither wild type nor SERT Ala56 animals performed differently under multisensory compared to visual alone conditions when evaluating either behavioral accuracies or reaction times. Since training under auditory conditions was the main difference between the two behavioral paradigms, we believe that the auditory training component from the original task may be necessary in order to observe behavioral benefits under multisensory conditions in the

mouse model. One hypothesis is that since the behavioral performances were comparable for multisensory and visual alone conditions for both genotypes, mice may have been ignoring the auditory noise burst during audiovisual presentations. This may be plausible since in this behavioral task variant animals were never previously exposed to auditory stimuli. However, in the original paradigm, animals were both trained and rewarded under auditory stimulus conditions (Siemann et al., 2015) (Siemann et al., in submission). As a result, after being trained under auditory conditions, we observed behavioral benefits under multisensory conditions for wild type animals and behavioral deficits for SERT Ala56 mice. Interestingly, it appears that the training (i.e. sensory) environment that mice are exposed to can result in behavioral differences specifically under multisensory conditions. Similar observations of differential responses based on being raised/trained in specific sensory environments have been demonstrated previously in larger animal models (Carriere et al., 2007; Stein, Stanford, & Rowland, 2014; M. T. Wallace & Stein, 2007) and may warrant further examination for future investigations in the mouse model. To more fully address this question, it may be necessary to determine if it is the relevant sensory modality that is important for observing behavioral gains in the mouse. For example, would an irrelevant visual stimulus aid in the detection of a relevant auditory stimulus? Importantly, since this task may not be appropriately evaluating multisensory processing behaviorally in the mouse, the SERT Ala56 mouse model may then still hold promise for further evaluation under both unisensory and multisensory stimulus conditions in the future.

We have now observed under both training and testing conditions in two different behavioral tasks that performance under visual alone conditions are comparable between wild type and SERT Ala56 animals. In addition, we previously demonstrated that wild type and SERT Ala56 mice performed comparably under auditory training and testing conditions. Therefore, this

suggests that the behavioral deficits observed in SERT Ala56 mice may specifically relate to audiovisual processing. Future experiments may first focus on utilizing the original behavioral paradigm to replicate prior findings of enhanced behavioral performance under multisensory conditions for wild type animals and behavioral deficits in SERT Ala56 animals. In addition, studies may then attempt to reverse these potential behavioral deficits observed in SERT Ala56 animals with either pharmacologic, optogenetic or deactivation approaches. Also, recent anatomical and electrophysiological studies in rodents have demonstrated that the border regions of primary sensory cortices contain large numbers of neurons responsive to multisensory stimuli (Hirokawa et al., 2008; Olcese, Iurilli, & Medini, 2013; M. T. Wallace, Ramachandran, & Stein, 2004). Therefore, future studies may focus on these multisensory cortical regions to evaluate the underlying mechanisms critical for multisensory processing and the related behaviors specifically in the context of mouse models associated with disease and/or disorder such as ASD. Finally, prior multisensory studies have demonstrated in numerous animal models that behavioral reaction times can be faster under multisensory compared to unisensory conditions (Calvert, Spence, & Stein, 2004; Murray & Wallace, 2011; R. A. Stevenson, Ghose, et al., 2014). However, reaction times were found to be comparable under multisensory and unisensory conditions for both the original and variant behavioral tasks in the mouse. In order to more appropriately evaluate these reaction time differences, future studies may use spatially congruent sensory stimuli to potentially observe faster reaction times in mice or design a behavioral paradigm which main emphasis is on strictly evaluating reaction times (i.e. having an animal hold a position before responding as quickly as possible to sensory stimuli) (Burnett, Stein, Chaponis, & Wallace, 2004; Colonius & Diederich, 2004; Sakata et al., 2004).

In this study we designed a novel variant of our previous behavioral task and found

evidence of behavioral performance differences between wild type and SERT Ala56 animals. Surprisingly, there were no significant differences in behavioral measures for either genotype when comparing multisensory and visual alone performances. Therefore, this behavioral task variant does not appear to properly evaluate potential behavioral benefits that may result under multisensory conditions for the mouse. Overall, these findings demonstrate that depending on the behavioral task and training procedures differential results can be observed under multisensory conditions, however these results provide future opportunities to design novel paradigms to further assess how these behavioral benefits are conferred under multisensory conditions in the mouse model.

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CHAPTER V

General Discussion

Summary of Results

There are numerous behavioral paradigms available to assess both unisensory and multisensory function in humans and various animal models ranging from monkeys to rats (Calvert, Spence, & Stein, 2004; Murray & Wallace, 2011). However, there previously was no multisensory behavioral paradigm available for the mouse model. The reason why this is specifically important for this thesis is the fact that the mouse allows for a variety of genetic manipulations to then assess and model states of disease and/or disorder (J. N. Crawley, 2012; Nestler & Hyman, 2010). In this thesis we attempted to address the lack of multisensory behavioral paradigms available for mice and specifically to evaluate and characterize multisensory function in a mouse model associated with autism spectrum disorder. First, in Chapter II, we designed and implemented a novel behavioral paradigm to assess multisensory processing in wild type mice. As observed previously in larger animal models (Calvert et al., 2004; Murray & Wallace, 2011; Stein, 2012; Stein & Stanford, 2008; Stein, Stanford, & Rowland, 2014; R. A. Stevenson, Ghose, et al., 2014), we found that mice were more accurate under congruent audiovisual presentations compared to unisensory conditions (i.e. visual or auditory alone) across all tested stimulus durations. In addition, we observed that wild type mice demonstrated multisensory gain at both the group as well as the individual performance level and this steadily increased peaking at the 100ms stimulus duration. Also, using this behavioral task it was found that a key principle in the multisensory literature (i.e. inverse effectiveness) (Meredith & Stein, 1986b) appears to be conserved in the mouse, such that as the stimuli became less

effective (i.e. durations became shorter), a greater gain or benefit was observed under multisensory conditions. Lastly, when evaluating reaction times, no differences in behavioral performance were found when comparing multisensory to unisensory conditions. Overall, in Chapter II we were able to show for the first time that mice exhibit a variety of similar behavioral benefits under multisensory conditions as previously observed in larger animal species (Stein, 2012). Therefore, these results may be of great value to those interested in studying the neural bases of multisensory function, specifically as it relates to critical questions focused on mechanistic relevance.

As mentioned previously, the mouse model allows for investigators to study the underlying mechanisms and resultant behaviors associated with disease and/or disorder (Nestler & Hyman, 2010). Numerous behavioral and neural studies have demonstrated atypical responses to both unisensory (Iarocci & McDonald, 2006; Marco, Hinkley, Hill, & Nagarajan, 2011) and multisensory stimuli in individuals with autism spectrum disorder (Baum, Stevenson, & Wallace, 2015; Brandwein et al., 2015; N. Russo et al., 2010; Wallace & Stevenson, 2014). Therefore, in Chapter III we were interested in evaluating and characterizing sensory and multisensory function behaviorally in a mouse model associated with ASD (i.e. SERT Ala56) (Veenstra-VanderWeele et al., 2012). It was previously observed that rare variants in the serotonin transporter (SERT) were found to associate with ASD with the most common of these being SERT Ala56 (Sutcliffe et al., 2005). Interestingly, individuals expressing this variant demonstrated rigid-compulsive behavior in addition to hypersensitivity to sensory stimuli (Sutcliffe et al., 2005). Once this mouse model was generated, SERT Ala56 animals demonstrated atypical serotonin function (i.e. increased clearance rate, decreased 5-HT receptor sensitivity) and exhibited behaviors associated with ASD such as impairments in social

behaviors, communication and the presence of repetitive behaviors (Veenstra-VanderWeele et al., 2012). Interestingly, studies have evaluated the relationships between serotonin and sensory function (Esaki et al., 2005; Gaspar, Cases, & Maroteaux, 2003; Hurley, Thompson, & Pollak, 2002), however there are relatively few investigations assessing both serotonin and multisensory processing (Jitsuki et al., 2011). Thus, by utilizing the SERT Ala56 mouse model we had a novel opportunity to evaluate multisensory behavioral function in the context of atypical serotonin signaling.

In this chapter we used the same behavioral paradigm as described previously in Chapter II to evaluate (multi)sensory processing in both wild type and SERT Ala56 mice. We first observed no significant differences in initial learning, reversal learning or behavioral performance under either visual or auditory alone conditions. Therefore, these novel findings suggest that SERT Ala56 may not have deficits in reversal learning or unisensory processing, which have been previously observed in mouse models associated with ASD (J. L. Silverman, Yang, Lord, & Crawley, 2010). It was then found that wild type mice were more accurate specifically under multisensory conditions and when compared to wild type littermates, we observed behavioral deficits under multisensory conditions and a complete lack of multisensory gain or behavioral benefit in SERT Ala56 mice. Overall, this was the first study to evaluate and demonstrate multisensory impairments behaviorally in a mouse model associated with ASD. These results are intriguing based on the numerous neural and behavioral studies that have demonstrated atypical unisensory and multisensory processing in individuals with ASD (Dakin & Frith, 2005; Kwakye, Foss-Feig, Cascio, Stone, & Wallace, 2011; Mongillo et al., 2008; O'Riordan & Passetti, 2006). While it is difficult to compare across species, our study is the first to demonstrate behavioral findings of atypical multisensory gain in a mouse model associated

with ASD, which may be similar to some of the deficits found in the human clinical population (Collignon et al., 2013; Mongillo et al., 2008; R. Stevenson et al., 2013). In addition, these findings are the first to describe a potential relationship between multisensory and serotonin function specifically in the context of ASD. However, in order to more fully examine this relationship, future more mechanistically driven studies will need to be investigated. Also, there are a variety of human studies demonstrating atypical multisensory temporal processing in ASD (Bebko, Weiss, Demark, & Gomez, 2006; J. H. Foss-Feig et al., 2010; Kwakye et al., 2011; R. A. Stevenson, Siemann, Schneider, et al., 2014; Woynaroski et al., 2013). Therefore it may be important to design relevant tasks to assess multisensory temporal function in mouse models associated with ASD in the future.

Lastly, based on the length of time it took to complete the original behavioral paradigm utilized in Chapters II and III, we were interested in designing a shorter version of this task. The rationale for attempting this was that it was previously found in Chapter III that wild type mice learned the visual component of the task after 20.6 ± 4.4 days and SERT Ala56 mice completed the task after 17.8 ± 4.7 days. However, wild type mice completed the auditory component of the task after 58.0 ± 11.1 days and SERT Ala56 animals finished the auditory training after 49.0 ± 7.5 days. Therefore, by eliminating the auditory training component we believed that it might have been possible to assess (multi)sensory processing behaviorally in mice in a more timely manner. To address this we designed a similar behavioral assay, in Chapter IV, that has been used in larger animal species to evaluate multisensory function (Lovelace, Stein, & Wallace, 2003; Stein, Meredith, Huneycutt, & McDade, 1989; Thorne & Debener, 2008). Previously in Chapter III, both wild type and SERT Ala56 mice were trained under visual and auditory alone conditions on separate independent tasks before being tested under visual, auditory or congruent

audiovisual conditions. However in Chapter IV, both genotypes were first trained under visual alone conditions and then tested under either visual or congruent audiovisual conditions. The difference in this shorter version was that mice were not previously trained under auditory alone conditions, so the question presented in this study was would an irrelevant auditory stimulus aid in the detection of a relevant visual stimulus?

In Chapter IV, we again observed no significant differences between wild type and SERT Ala56 mice in initial learning, reversal learning or under visual training conditions thus replicating our previous findings in Chapter III. However, when evaluating behavioral performance under testing conditions we found no significant differences between multisensory and visual alone conditions for either behavioral accuracies or reaction times when evaluating within either genotype. In addition, multisensory gain was limited and negative at most of the tested stimulus durations for both wild type and SERT Ala56 mice at the group and individual performance levels. Therefore for mice, it appears that an irrelevant auditory stimulus does not aid in the detection of a relevant visual stimulus. This suggests that at least for audiovisual stimuli, both sensory modalities may need to be relevant in order to confer behavioral gains under multisensory conditions in the mouse model system. Under these conditions in Chapters II and III, mice were not only exposed to the auditory stimuli, but were also rewarded for detecting these stimuli, which speaks to the potential importance of the training procedures. To more fully test this hypothesis, it would be necessary to reverse the relevant sensory stimulus. Would we still observe minimal or a lack of behavioral gain when presenting a relevant auditory stimulus with an irrelevant visual stimulus? These results would help determine if it is the training and relevance of these unisensory stimuli that is critical for observing behavioral benefits under audiovisual conditions in the mouse model. While audiovisual studies are logical based on the

importance and relevance of these stimuli for the human (clinical) population, it may be important for future behavioral paradigms to evaluate sensory domains that are more specialized and typically utilized in the mouse (i.e. somatosensation and olfaction) (Ihara, Yoshikawa, & Touhara, 2013; Petersen, 2014), which may then result in even greater behavioral gains when presented in a multisensory context. Overall, while similar paradigms have demonstrated multisensory benefits in larger animal species (Lovelace et al., 2003; Stein et al., 1989; Van der Burg, Olivers, Bronkhorst, & Theeuwes, 2008), this behavioral task, in Chapter IV, does not appear to confer similar multisensory behavioral gains for the mouse model system. These results may be due to the design and type of behavioral task (i.e. detection vs. discrimination vs. localization), the unisensory training procedures and the spatial location and effectiveness of the stimulus presentations, all of which provide future opportunities to further assess how these behavioral benefits are conferred under multisensory conditions in the mouse model.

Implications of Main Findings

Basic Science Implications

Neurons to Behavior

A variety of studies have evaluated the underlying neural structures and resultant behaviors needed for basic sensory and multisensory function in rodent models (Brett-Green, Fifkova, Larue, Winer, & Barth, 2003; Carandini & Churchland, 2013; Jacqueline N Crawley, 2007; Gleiss & Kayser, 2012; Menzel & Barth, 2005; Sheppard, Raposo, & Churchland, 2013). In the rat, both cortical (Hirokawa, Bosch, Sakata, Sakurai, & Yamamori, 2008; Sieben, Roder, & Hanganu-Opatz, 2013; Tees, 1999; Wallace, Ramachandran, & Stein, 2004) and subcortical (Hirokawa et al., 2011; Komura, Tamura, Uwano, Nishijo, & Ono, 2005) multisensory brain

regions have been identified including the superior colliculus (SC) (Hirokawa et al., 2011; May, 2006; Sparks & Hartwich-Young, 1989), a classical subcortical structure critical for multisensory function in larger species (Stein & Stanford, 2008). In terms of behavioral tasks, investigations have mainly focused on utilizing rats to evaluate multisensory function because of their ability to complete more complex cognitive tasks when compared with mice (Gleiss & Kayser, 2012; Raposo, Sheppard, Schrater, & Churchland, 2012; Sakata, Yamamori, & Sakurai, 2004). The most interesting of these studies, which have been previously accomplished in the cat model, are those that either transiently or permanently disrupt these brain regions and then assess the effects on the resultant multisensory behavior (Burnett, Stein, Chaponis, & Wallace, 2004; Jiang, Jiang, & Stein, 2002; Wilkinson, Meredith, & Stein, 1996). Based on these classical studies, investigators are beginning to design similar studies to determine these brain-behavior relationships in rats (Hirokawa et al., 2008; Tees, 1999).

In the rat, it has been shown that the border regions between primary sensory cortices contain multisensory responsive neurons (Wallace et al., 2004). Hirokawa et al extended these intriguing neural findings by then evaluating multisensory processing in a behavioral context. In this study, when rats performed a multisensory behavioral detection task it was observed that the greatest neural activity, by evaluating c-Fos expression, occurred in a cortical border region between V1 and A1 (i.e. V2L). The authors then injected muscimol, a GABA A agonist, into cannula targeting V2L in a new cohort of animals. It was shown that the selective deactivation of V2L resulted in slower reaction times specifically under multisensory conditions compared to control animals that received saline injections into V2L. Therefore, this was one of the first deactivation studies to identify a neural structure that is critical for multisensory processing in awake behaving rats (Hirokawa et al., 2008). Interestingly, there are emerging studies in the

mouse demonstrating anatomical connections between A1 and V2L as well as V1 and V2L (Charbonneau, Laramee, Boucher, Bronchti, & Boire, 2012; Laramee, Kurotani, Rockland, Bronchti, & Boire, 2011) suggesting that this multisensory structure may be conserved in rodents and a potential target for future studies in the mouse. In addition, emerging evidence in the mouse model has demonstrated the presence of multisensory neurons in border regions between unisensory cortices (Olcese, Iurilli, & Medini, 2013) similar to those findings observed in rats (Hirokawa et al., 2008; Wallace et al., 2004), thus providing further evidence that some of the neural circuits and structures needed for multisensory processing may be conserved in rodents.

In addition to these cortical structures, studies have shown that multisensory neurons are present in the deep layers in the SC in rodents and exhibit similar response properties to those observed in larger animal species (U. Drager & D. H. Hubel, 1975; Drager & Hubel, 1976; U. C. Drager & D. H. Hubel, 1975; Hirokawa et al., 2011; Sparks & Hartwich-Young, 1989). Also, there is evidence that the multisensory cortical region V2L projects to the SC (Harvey & Worthington, 1990; Sparks & Hartwich-Young, 1989). This is of interest because it suggests that there may be a similar cortical-subcortical circuit that has been previously demonstrated in the cat model system (Stein, Wallace, Stanford, & Jiang, 2002). Classic single cell electrophysiology experiments have demonstrated in the cat that neurons in both the deep layers of SC (Meredith, Nemitz, & Stein, 1987; Meredith & Stein, 1986a, 1986b) and in the cortical anterior ectosylvian sulcus (AES) (Wallace, Carriere, Perrault, Vaughan, & Stein, 2006; Wallace, Meredith, & Stein, 1992) display multisensory response properties, intriguingly though it is the projections from the AES that allows the SC neurons to display these neural enhancements under multisensory conditions (Wallace, Meredith, & Stein, 1993; Wallace & Stein, 1994). For example, it has been shown with pharmacology (Wilkinson et al., 1996) and deactivation studies (Jiang et al., 2002;

Jiang, Wallace, Jiang, Vaughan, & Stein, 2001; Wallace & Stein, 1994) that the AES is critical for modulating the neural responses in the SC in addition to being integral for the behavioral enhancements observed under multisensory conditions as well. Therefore, based on these foundational observations in the cat and rat model systems, it would be logical to determine and potentially establish a similar circuit in the mouse model.

By using the behavioral task described in this thesis, it would first be possible to assess and determine the functional roles of specific multisensory brain regions such as V2L in the mouse model. For example, it would be possible to deactivate V2L with either pharmacology or optogenetics during the multisensory behavioral task described in this thesis. If V2L were important in a multisensory behavioral detection task, one would hypothesize that once V2L was deactivated, behavioral accuracies would decrease and/or behavioral reaction times would increase under multisensory conditions, similar to those observations found in rats (Hirokawa et al., 2008). Once V2L was reactivated, these multisensory behavioral enhancements would then return comparable to those observed under control/baseline conditions (i.e. no deactivation). In addition, in order to more fully determine the role of V2L on behavior, future studies may focus on and evaluate the type of behavioral task employed (i.e. detection, discrimination or localization) in conjunction with these deactivation studies in mouse models. If V2L were important in a behavioral context, it would then be critical to evaluate the neural responses to multisensory stimuli in V2L. First, with electrophysiological techniques, it would be possible to determine if there are multisensory responsive neurons in V2L in the mouse and if the distribution of these neurons is similar to those previously observed in rats. For example, in these cortical border regions it has been shown in both rats and mice that the majority of multisensory neurons tend to be located in the middle of these structures whereas the majority of unisensory

responsive neurons are more likely to be distributed closer to their respective unisensory cortices (Olcese et al., 2013; Wallace et al., 2004). In addition to this cortical study, it would be an important proof of concept to record from the deep layers of the SC in order to replicate and further validate prior findings that multisensory responsive neurons are present in this subcortical structure in the mouse (U. Drager & D. H. Hubel, 1975; Sparks & Hartwich-Young, 1989). These studies would provide novel findings in identifying cortical and subcortical structures that contain multisensory responsive neurons and thus may be critical for multisensory behavioral function in the mouse model.

In order to establish a potential circuit in the mouse, it would be necessary to utilize similar techniques previously used in the cat and rat model systems (Hirokawa et al., 2008; Stein et al., 2002). To this point, investigators could perform single cell electrophysiology recordings from multisensory neurons in the SC, while simultaneously deactivating the cortical region V2L. In order to accomplish this, investigators could deactivate V2L with pharmacology or optogenetics, while performing electrophysiological recordings from the SC in the mouse. If V2L projects to and modulates neural responses similarly to the circuit observed in the cat model (i.e. AES modulating neural responses in the SC), it would be hypothesized that once V2L was inactivated, multisensory neural responses would be diminished in the SC resulting in additive or subadditive responses. However, when V2L was reactivated these neural enhancements or superadditive responses under multisensory conditions would then return in the SC. In addition, based on these hypothetical results from deactivation studies assessing behavioral and neural responses individually, it would also be possible to evaluate this circuit in awake behaving animals. Thus, by deactivating V2L, recording in the SC and simultaneously evaluating behavioral performance during our multisensory detection task it would be possible to truly

relate physiology with the resultant behaviors. By utilizing electrophysiology, deactivation/reactivation studies along with the current behavioral paradigm described in this thesis it would be possible to gain novel insights into the underlying mechanisms, neural structures and establish potential relationships between physiology and behavior that would be of critical relevance to multisensory function in the mouse model.

Clinical & Translational Science Implications

The studies outlined above would address mechanistically driven questions focused on the functional role and neural underpinnings of multisensory processing in the mouse. However, in order to begin to address translational applications, it is first important to gain a better understanding of these deficits in the clinical population. Or more simply stated in the context of this thesis: what does the multisensory experience look like in ASD? As mentioned previously, numerous studies have observed atypical sensory and multisensory processing in individuals with ASD (Baum et al., 2015; Iarocci & McDonald, 2006; Marco et al., 2011). In addition, to decreased multisensory behavioral gain (Mongillo et al., 2008; R. Stevenson et al., 2013), multisensory temporal processing appears to be atypical in ASD as well (J. H. Foss-Feig et al., 2010; Kwakye et al., 2011). However, similar to those observations in unisensory processing (Dakin & Frith, 2005; O'Connor, 2012), these multisensory deficits may arise once this information is needed to be combined into more of a global or complex concept (i.e. communication or social behaviors) (Foxe et al., 2013; Nicole Russo, Zecker, Trommer, Chen, & Kraus, 2009; R. A. Stevenson, Siemann, Schneider, et al., 2014). Therefore, in this clinical population the relative timing and integration of more basic or simple stimuli (i.e. visual flashes and auditory beeps) may be in tact. However, once this multisensory information becomes more complex, temporal processing and/or multisensory function appear to be impacted which may

result in more degraded and/or confusing perceptual representations observed in ASD. Gaining a better understanding into these multisensory impairments for this population is critical not only for clinical research, but may be highly relevant for designing behavioral tasks and experiments for animal models associated with this disorder in the future.

Utilizing Psychophysical Behavioral Tasks for Genetic Rodent Models and Clinical Populations

Numerous animal models have been used to gain a better understanding into the neural mechanisms and behaviors associated with disease and disorder. However, the most applicable of these models currently may be rodents (i.e. rats and mice). The advantages that rodents have now over most other animal model species include the fact that rodents are relatively inexpensive, large population studies can be accomplished with genetically identical animals, large pharmacological studies and screens for novel compounds can be evaluated and most importantly it is possible to design and generate novel genetic rodent models associated with disease and/or disorder (Nestler & Hyman, 2010). However, as with any animal model rodents do have some disadvantages. These limitations include: the behavior observed in these models is not as related or comparable to human behavior, the rodent brain and homology is less similar to humans compared to larger animal species and specifically for sensory related questions, rodents are not typically audiovisual animals and rely more heavily on olfactory and tactile domains (Jacqueline N Crawley, 2007). In the multisensory literature, various studies have demonstrated that behavioral benefits such as increases in accuracies and the reduction in reaction times can be conferred under multisensory conditions (Murray & Wallace, 2011; Stein & Stanford, 2008). Classically, humans and animal models such as the monkey and cat have been used in these behavioral studies (Stein, 2012; R. A. Stevenson, Ghose, et al., 2014), however, more recently

rodents are emerging as novel animal models to assess multisensory function (Gleiss & Kayser, 2012; Hirokawa et al., 2008; Hirokawa et al., 2011; Sakata et al., 2004). Recently, the rat has been the model of choice to investigate these questions based on the ability to complete complex cognitive tasks and relatively low hyperactivity compared to other rodents such as mice. Interestingly, psychophysical studies have evaluated multisensory processing in both humans and rats using comparable behavioral tasks and have demonstrated strikingly similar results and benefits under multisensory conditions (Carandini & Churchland, 2013; Raposo et al., 2012; Sheppard et al., 2013). These studies therefore demonstrate the utility of rodent models for studying multisensory function and provide evidence that these behavioral results could be compared across species. Interestingly, despite numerous behavioral investigations, no study had previously evaluated or characterized multisensory behavioral function in the mouse model. Therefore, the behavioral paradigm described in this thesis may then allow for a variety of novel opportunities to study multisensory function in mouse models associated with disease and/or disorder (i.e. ASD). Studies are just beginning to evaluate the neural structures critical for multisensory processing in wild type mice (Olcese et al., 2013; Reig & Silberberg, 2014), along with emerging evidence of smaller multisensory neural responses and limited multisensory neural enhancements in mouse models associated with ASD (Gogolla, Takesian, Feng, Fagiolini, & Hensch, 2014). Therefore, with the field just beginning to pursue the underlying mechanisms of multisensory processing in mouse models, the use of this behavioral paradigm offers the unique opportunity to potentially assess multisensory function in genetic mouse models. Importantly, based on prior behavioral studies (Carandini & Churchland, 2013; Raposo et al., 2012; Sheppard et al., 2013), it may also be possible to compare these behavioral results in rodents to those observed in the human clinical population as well.

By utilizing this behavioral task or similar versions, it may be possible to assess multisensory behavioral function for rodents in order to mirror/relate these findings to those being observed in human psychophysical tasks. If this were possible, a number of important translational and clinically relevant questions could be evaluated and described. For example, would we observe similar multisensory behavioral deficits in rodent models associated with ASD as observed in clinical populations? If rodent models demonstrate atypical multisensory function it may be possible to screen and identify potential subsets of individuals with ASD with similar genetic mutations in order to determine if similar multisensory deficits exist in this clinical subset. In addition, rodent models would allow for screening and testing of specific pharmacological agents, which could reverse/normalize multisensory function. These results could then be translated and evaluated in the clinical population as well. If so, would pharmacologic treatment in this subpopulation with similar genetic alterations and multisensory impairments result in similar improved multisensory behavioral performance? By identifying these subsets of individuals and evaluating sensory/multisensory function as a potential biomarker, it may be possible to determine the efficacy of specific pharmacological compounds. While these studies may be difficult and may not result in these hypothetical results, they do highlight the fact that with genetic rodent models it may be possible to assess behavioral function across species, which may prove to be highly relevant for clinical and/or translational science. In order to investigate more basic science questions a variety of techniques (i.e. pharmacology, deactivation, optogenetics) could be used in conjunction with our behavioral paradigm to gain a better understanding of the underlying circuits and potential atypical neural structures which are driving this aberrant behavior observed in mouse models of ASD.

Evaluating the Potential Underlying Mechanisms of Atypical Multisensory Function in a Mouse Model Associated with ASD

As a continuation to the studies described above in the basic science implications section, it would be possible to assess multisensory function utilizing behavioral deactivation and neural/electrophysiology experiments in a mouse model associated with ASD (SERT Ala56). By performing similar studies to those outlined in wild type animals, it would be possible to determine if atypical multisensory neuronal responses were present in either subcortical (SC) or cortical (V2L) structures in SERT Ala56 mice. For example, with electrophysiological studies one could hypothesize that diminished (i.e. subadditve or additive) neural responses to multisensory stimuli may be observed in SERT Ala56 compared to wild type animals in either or both neural structures. This would demonstrate more of a mechanistic explanation for why there are behavioral deficits observed under multisensory conditions in SERT Ala56 mice. In addition, investigators could determine if this potential circuit from V2L to the SC is comparable between wild type mice and SERT Ala56 animals by utilizing deactivation studies focused on multisensory neural and behavioral function. First, it would be possible to deactivate V2L while recording from the SC. If V2L modulates neural responses in the SC for SERT Ala56 mice, as hypothesized for wild type animals, then these responses under multisensory conditions would be diminished or additive once V2L is silenced. However, if V2L does not modulate these responses in SC for SERT Ala56 animals then the deactivation of this cortical region would result in having limited or no effect on the SC neural responses under multisensory conditions. In addition to these neural studies, it would be possible to evaluate if V2L is critical in a behavioral detection task for SERT Ala56 mice. If similar behavioral deficits to those found in wild type animals are observed by deactivating V2L this would suggest that this cortical brain region is

being utilized in both genotypes for this multisensory detection task, whereas if there is no difference in the behavioral performance for SERT Ala56 mice during deactivation of V2L then this may be further evidence that this specific circuit is not being utilized in SERT Ala56 animals. Finally, as mentioned before, to more fully assess these relationships between physiology and behavior it would be possible to assess multisensory behavioral and neural function in awake behaving mouse models associated with ASD by combining the electrophysiological, deactivation and behavioral studies as outlined above. Overall, these future studies would be critical in understanding the neural structures and circuits needed for multisensory integration in mouse models and may provide novel insights into the potential underlying mechanisms and behaviors of atypical (multi)sensory processing in disorders such as ASD.

Translational Approaches for Autism Spectrum Disorder: Relationships between Multisensory and Higher Order Cognitive Function

As mentioned above, with the use of animal models it is possible to design and implement novel pharmacologic and therapeutic approaches in the study of clinical disorders such as ASD. While most intervention studies in humans have focused on treating the core symptoms of ASD this unfortunately has resulted in relatively limited success (Dove et al., 2012; Warren et al., 2011). To this point there are only two current FDA approved drugs for ASD, yet these focus mainly on treating behavioral issues/irritability such as self-injurious behaviors (Ghanizadeh, Sahraeizadeh, & Berk, 2014). In addition to these human studies, a variety of pharmacologic compounds have been developed and found to be successful in the treatment of these core behaviors observed in animal models (Unpublished work from the Blakely and Veenstra-VanderWeele laboratories) (Gogolla et al., 2014; J. Silverman et al., 2015; Jill L

Silverman et al., 2012). However, most of these compounds have failed to be effective when evaluated in human clinical trials (Jill L Silverman & Crawley, 2014). Therefore, it is evident that novel therapeutics for the treatment of this disorder are still needed. While most investigations have focused on core symptoms, it may be beneficial to design treatments or interventions for more understudied symptoms found in ASD. As mentioned previously, because of the high prevalence, sensory disturbances have just recently been included as a criterion for ASD diagnosis (APA, 2011). In addition, both neural and behavioral studies have demonstrated atypical (multi)sensory processing in individuals with ASD (Baum et al., 2015; Brandwein et al., 2015; Iarocci & McDonald, 2006; Marco et al., 2011; N. Russo et al., 2010; Wallace & Stevenson, 2014). Since, multisensory integration is critical for our perception of the world and is integral for the interpretation and understanding of both communicative and social signals, it may be the treatment of these sensory and/or multisensory disturbances that can have therapeutic effects for these core symptom domains. One hypothesis that has been proposed is that these (multi)sensory deficits may be important building blocks that can then have cascading effects into higher order cognitive processes, which may impact the core symptoms that define ASD (Baum et al., 2015; R. A. Stevenson, Siemann, Schneider, et al., 2014). Before, therapeutic approaches are even considered though, it is first necessary to establish and determine the possible relationships between (multi)sensory processing and higher order domains, such as social communication in ASD.

Currently, there is limited yet emerging evidence to support this hypothesis in both humans and animal models. Stevenson et al demonstrated behaviorally that atypical multisensory temporal processing in individuals with ASD for low-level sensory stimuli resulted in poorer performance in perceiving a multisensory illusion that utilizes speech related stimuli (R. A.

Stevenson, Siemann, Schneider, et al., 2014). In addition, it is known that the brain region (STS) used in the perception of this illusion (McGurk effect) (Beauchamp, Nath, & Pasalar, 2010; Audrey R Nath & Beauchamp, 2012; A. R. Nath, Fava, & Beauchamp, 2011) is also necessary for processing both social and communicative stimuli (Pelphrey, Morris, & Mccarthy, 2004; R. A. Stevenson, VanDerKlok, Pisoni, & James, 2011; Zilbovicius et al., 2006) and has been found to be both structurally and functionally atypical in ASD (Boddaert et al., 2004; Redcay, 2008). In addition, studies have shown that increased sensory disturbances can correlate with greater deficits in social communication along with the presence of more repetitive behaviors in individuals with ASD (Boyd et al., 2010; Boyd, McBee, Holtzclaw, Baranek, & Bodfish, 2009; Jennifer H Foss-Feig, Heacock, & Cascio, 2012; Hilton et al., 2010; Kern et al., 2007; Schauder, Muller, Veenstra-VanderWeele, & Cascio, 2015). Lastly, in addition to these human findings, these relationships have been evaluated in emerging animal model studies. Gogolla et al demonstrated that a pharmacological intervention in mouse models associated with ASD not only normalized atypical neural responses under multisensory conditions, but it also reversed the presence of repetitive behaviors found in these animal models (Gogolla et al., 2014). Therefore, there is intriguing evidence beginning to suggest a relationship between the core domains and multisensory processing deficits in ASD. Thus, if sensory and/or multisensory disturbances do have cascading effects into these higher order or core domains, the treatment of atypical (multi)sensory processing may be a novel therapeutic approach for individuals with ASD. While studies in humans can certainly investigate these relationships, animal models make it possible to study the underlying mechanisms and associated behaviors by utilizing novel techniques and evaluating pharmacological compounds that are not readily available for the human clinical population.

In order to evaluate the relationships between (multi)sensory function and higher order domains in ASD, it may be necessary to use rodent models. To this point, optogenetics, electrophysiology or pharmacology could be used to manipulate the known circuits and neural populations needed for either multisensory processing or higher order domains to determine the underlying relationships between these processes. For example, if relevant brain regions critical for multisensory processing are selectively silenced does this deactivation result in impairments in more cognitive processes or modulate the core behaviors associated with ASD? In terms of a potential animal study, would the disruption to a multisensory brain region result in impairments in social or communicative behaviors in wild type animals and/or would this manipulation in multisensory function specifically worsen these core behavioral deficits observed in mouse models associated with ASD? In addition, since a variety of behavioral assays are currently available and with the recent development of touch screen operant chambers, investigators have utilized and developed more complex and cognitive demanding tasks for rodent models. In an attempt to further assess these potential relationships, investigators could train animals on a multisensory behavioral task to first possibly improve multisensory function and then determine if this training results in improvement in social or communicative domains. Thus, by utilizing rodent models it would be possible to evaluate the relationships between (multi)sensory processing and higher order or core domains associated with ASD by either enhancing and/or impairing these potential interactions.

A variety of pharmacological agents could be utilized to assess these relationships in the future. However, based on the mouse model associated with ASD (i.e. SERT Ala56) that we have described and utilized in this thesis we may have a novel opportunity to evaluate serotonin signaling, multisensory processing and possibly normalizing atypical multisensory behavioral

function with selective serotonin reuptake inhibitors (SSRIs). As discussed previously, serotonin has been implicated in ASD (Cook & Leventhal, 1996; Mulder et al., 2004), has been shown to be important for sensory cortical development (Cases et al., 1996; Gaspar et al., 2003; Salichon et al., 2001), responses to sensory stimuli (Esaki et al., 2005; Hurley et al., 2002; Waterhouse, Azizi, Burne, & Woodward, 1990) and with this model it is possible to assess multisensory function and serotonin signaling, a relationship that has been greatly understudied. SSRIs in general have been used for the treatment of ASD with a variety of mixed results and effectiveness in addition to how it relates to the age of the patients (i.e. children vs. adults) (Kolevzon, Mathewson, & Hollander, 2006; West, Brunssen, & Waldrop, 2009; Williams, Brignell, Randall, Silove, & Hazell, 2013). One major reason why there may be such a varied level of effectiveness of SSRI treatment may be due to the heterogeneity of the ASD population. The SERT Ala56 mouse model would allow for the evaluation and hypothesis that by normalizing serotonin signaling with SSRI treatment this may reverse multisensory behavioral deficits observed in these animals (which will be described in greater detail in the future directions below). In addition, in order to determine potential developmental vs. dynamic changes it would be possible to treat SERT Ala56 animals with SSRIs either early or late in development and assess multisensory function. Interestingly, it has previously been shown that multisensory integration can be normalized with pharmacological treatment of a different neurotransmitter system (GABA) in a mouse model associated with ASD if given early in development as opposed to later in life (Gogolla et al., 2014). These behavioral pharmacology studies in SERT Ala56 animals will be discussed in further experimental detail in the future directions section, but highlight the fact that treatment of mouse models with specific genetic mutations may be important for further understanding the development and underlying processes

for multisensory function in the context of ASD. These studies may be pursued in two different yet applicable directions, which have been discussed above. The first is that with this mouse model associated with ASD it would be possible to evaluate SSRI treatment on potential changes in (multi)sensory processing and possible cascading effects into areas such as communication or social interactions. Two, if these hypothetical pharmacological results are found in this mouse model, it could be beneficial or relevant for the human clinical population because it may be possible to identify individuals with genetic abnormalities in the serotonin system along with (multi)sensory processing deficits, which may have some therapeutic value in the future. To this point, a hypothetical example of this would be that sensory/multisensory processing could be used as a biomarker to predict the efficacy of an SSRI in a subset of individuals with ASD (i.e. individuals with (multi)sensory deficits and genetic alterations in 5-HT signaling). The ultimate goal is to design novel therapeutic interventions for individuals with ASD. There is emerging evidence that sensory and/or multisensory deficits may have cascading effects that could impact the core symptoms that define ASD. Therefore, with the advantages of rodent models it is the ultimate goal to determine if the relationships between multisensory function and higher order domains exist and if the treatment of these multisensory disturbances may be a novel therapeutic approach for the core features found in ASD.

Future Directions

While there are a variety of potential studies that may be pursued from this work, some of which have already been mentioned and discussed in this thesis and general discussion, we will focus on and detail two main future directions below.

Utilizing Behavioral Pharmacology to Potentially Reverse Atypical Multisensory Processing in a Mouse Model Associated with Autism Spectrum Disorder

It has previously been demonstrated that the SERT Ala56 mouse model recapitulates the biomarker of hyperserotonemia (i.e. elevated whole blood serotonin levels) (Veenstra-VanderWeele et al., 2012) found in individuals with ASD (Mulder et al., 2004). In addition, this point mutation causes a gain of function SERT resulting in a higher clearance rate of serotonin (5-HT) in the central nervous system compared to wild type SERT (Veenstra-VanderWeele et al., 2012). Based on these observations, it was hypothesized that a decreased availability of serotonin in the synaptic cleft may result in a compensatory hypersensitivity of 5-HT receptors in SERT Ala56 animals (Veenstra-VanderWeele et al., 2012). In order to assess 5-HT receptor sensitivity a $5\text{-HT}_{2A/2C}$ receptor agonist, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), and a $5\text{-HT}_{1A/7}$ agonist, 8-hydroxy-2-(di-n-propylamino)-tetraline (8-OH-DPAT) were used to test this hypothesis (Veenstra-VanderWeele et al., 2012). Physiological measures were evaluated for DOI and 8-OH-DPAT such as head twitches and hypothermia respectively during these challenge studies. Veenstra-VanderWeele et al observed 5-HT receptor hypersensitivities in SERT Ala56 animals such that SERT Ala56 mice demonstrated a greater number of head twitches and increased hypothermia when compared to wild type animals for these experiments. In addition to these molecular findings, behavioral changes in this mouse model were observed including deficits in the social domain (i.e. tube test) (Veenstra-VanderWeele et al., 2012). In previous unpublished work in the Veenstra-VanderWeele lab, it was shown that chronic injection of a common selective serotonin reuptake inhibitor (SSRI), fluoxetine, resulted in normalizing/reversing this social behavioral deficit in SERT Ala56 animals. It has been shown previously that SSRIs bind in a typical manner to SERT Ala56 (Prasad, Steiner, Sutcliffe, &

Blakely, 2009) suggesting that by slowing the 5-HT clearance rate in SERT Ala56 animals with an SSRI it is possible to reverse a social behavioral phenotype in this mouse model associated with ASD. To this point, prior unpublished work in the Blakely and Veenstra-VanderWeele labs also demonstrated that chronic treatment with an SSRI normalized 5-$HT_{2A/2C}$ and 5-$HT_{1A/7}$ receptor sensitivity in SERT Ala56 animals with DOI and 8-OH-DPAT challenge studies. Therefore, these studies demonstrated that normalizing serotonin signaling could result in behavioral changes relevant to those behaviors associated with ASD (i.e. social deficits). Interestingly there are established relationships between serotonin and unisensory function, in which serotonin has been shown to modulate responses to sensory stimuli (Hurley & Pollak, 2005; Hurley et al., 2002; Waterhouse et al., 1990) along with projecting to brain regions critical for (multi)sensory processing (Lebrand et al., 1996; Mooney, Huang, Shi, Bennett-Clarke, & Rhoades, 1996; Murphy, Uzbekov, & Rose, 1980). To this point, studies have shown aberrant connections to sensory cortices in rodent models with genetic alterations in the serotonin system (Cases et al., 1996; Gaspar et al., 2003; Salichon et al., 2001) and in addition these animal models can demonstrate atypical responses to unisensory stimuli (Esaki et al., 2005). However, there are relatively few investigations that have evaluated the role of serotonin on multisensory function (Jitsuki et al., 2011). Since serotonin is important for sensory cortical development it is possible that SERT Ala56 animals, which have a limited availability of serotonin in the synaptic cleft, may develop atypical connections to brain regions important for (multi)sensory processing. Thus, with SERT Ala56 animals it would be possible to assess potential relationships between multisensory processing and serotonin along with utilizing SSRIs to potentially reverse atypical multisensory function in a behavioral context. Therefore, would the reversal of atypical serotonin signaling result in normalizing multisensory processing in a mouse model associated with ASD?

In these future studies, (multi)sensory processing would be evaluated again in both wild type and SERT Ala56 animals utilizing the original behavioral paradigm as previously described in Chapters II and III. This would be important because it would first potentially replicate our previous findings of behavioral benefits in wild type mice and behavioral impairments in SERT Ala56 animals under multisensory conditions. Next, based on these findings, it would then be necessary to determine at which of the tested stimulus durations were the greatest behavioral differences observed between the genotypes. Previously in Chapter III we found that at the 500ms and 300ms stimulus durations, wild type mice demonstrated significantly greater multisensory gain at both the group and individual levels compared to SERT Ala56 animals. Once investigators determine the stimulus duration in which animals demonstrate the greatest multisensory gain, mice of both genotypes would be chronically injected with either saline or fluoxetine for 21 consecutive days (Naudon, El Yacoubi, Vaugeois, Leroux-Nicollet, & Costentin, 2002; Siesser et al., 2012) (unpublished work from Veenstra-VanderWeele lab). After chronic dosing is completed, both groups would undergo testing under visual alone, auditory alone and multisensory conditions for five consecutive days at the selected stimulus duration in order to determine if multisensory function either completely or partially normalized in SERT Ala56 animals. In addition to this main question, it would be important to determine if either unisensory processing was altered in SERT Ala56 mice or if (multi)sensory function changed in wild type animals with chronic SSRI treatment. If multisensory processing does normalize in SERT Ala56 animals, this would suggest a dynamic change and could possibly hold therapeutic value in the future for individuals with ASD who have both (multi)sensory deficits and 5-HT or SERT genetic mutations. However, if these multisensory deficits are still present in SERT Ala56 animals it may suggest that these changes are more developmentally based and would require

future studies that focus on SSRI treatments earlier in development.

Lastly, it would be important to determine if normalizing multisensory behavioral function also results in molecular changes by evaluating 5-HT receptor sensitivities. After chronic administration of fluoxetine and reevaluation of (multi)sensory behavioral performance, it would be possible to use pharmacologic challenge experiments (i.e. DOI and 8-OH-DPAT) to test this hypothesis. Therefore, if chronic treatment with an SSRI normalizes 5-HT receptor sensitivity in addition to restoring multisensory function behaviorally in SERT Ala56 animals, this would be the first neural and behavioral comparisons linking multisensory and serotonin function specifically in the context of ASD. By utilizing SSRIs it may be possible to reverse atypical serotonin signaling which in turn could normalize multisensory function. However, more basic science driven studies would need to evaluate the underlying mechanisms and anatomy of these changes. For example, would SSRI treatment normalize cortical connections to unisensory and/or multisensory brain regions in this mouse model, which could result in reversing these multisensory behavioral responses? Would these changes be possible during adulthood (i.e. dynamic changes) or would this treatment be most effective during a potential critical period of development (i.e. developmental changes)? By pursuing these questions, these future studies would allow for investigators to gain a better understanding into the potential mechanistic, developmental and behavioral changes that may relate and impact serotonin and multisensory function in the context of autism spectrum disorder.

Designing Behavioral Tasks to Further Assess Multisensory Processing and Novel Paradigms to Evaluate Multisensory Temporal Processing

In this thesis, we have described and demonstrated novel behavioral paradigms that assess (multi)sensory processing in the mouse model. When animals were trained under visual and auditory conditions alone and then tested under visual, auditory and congruent audiovisual presentations, behavioral benefits were observed and these findings were similar to those in humans and larger animal species (Murray & Wallace, 2011; R. A. Stevenson, Ghose, et al., 2014). However, even with these novel results it may be possible to design tasks where larger behavioral gains could be observed in the mouse. One of the key three principles in the multisensory literature is the spatial rule or principle, where greater behavioral benefits can be observed when unisensory stimuli are spatially aligned (Meredith & Stein, 1986a). In addition, the principle of inverse effectiveness importantly states that when unisensory stimuli (i.e. auditory and visual) are presented together near threshold detection levels that larger behavioral benefits can be observed as compared to if the individual sensory stimulus is presented alone (Meredith & Stein, 1986b). In our behavioral task a centrally located speaker was used to present auditory stimuli whereas visual stimuli were presented in nose poke holes to the left and right of the speaker in the operant chamber. Therefore, while these stimuli were relatively close in space, they were not completely spatially congruent. Also, in these behavioral studies only the duration was manipulated in order to evaluate stimulus effectiveness. Thus, if two speakers were used and aligned with the nose poke response holes (i.e. where the visual stimuli were presented) and the effectiveness of the stimuli were modulated by diming the visual stimulus or reducing the amplitude (i.e. loudness) of the auditory signal, even greater multisensory behavioral benefits could potentially be observed in the mouse model. In addition to the spatial and effectiveness

principles, the temporal principle is an area of great interest that will be discussed in detail in upcoming paragraphs.

What is somewhat surprising is that in these behavioral investigations, mice, which typically do not rely as heavily on auditory and visual stimuli compared to larger species, still demonstrated multisensory gains under audiovisual conditions. Therefore, by presenting modalities that tap into sensory structures which are more sensitive and developed in the mouse (i.e. olfaction or somatosensation) (Ihara et al., 2013; Petersen, 2014) even greater multisensory enhancements may be observed in these behavioral paradigms. Lastly, it was observed in all three chapters of this thesis that reaction times did not differ between unisensory and multisensory conditions. In order to potentially observe reaction time differences in the mouse, it may be necessary to change this paradigm drastically to resemble tasks used for humans and larger animal species that have focused on evaluating behavioral reaction times (Bell, Meredith, Van Opstal, & Munoz, 2005; Colonius & Diederich, 2004). For example, in these tasks animals are usually trained to hold a position (Sakata et al., 2004; Wallace, Meredith, & Stein, 1998) and respond to both unisensory and multisensory stimuli as quickly as possible to receive a liquid or food reward and typically these studies find that animals respond faster under multisensory compared to unisensory conditions (Cappe, Murray, Barone, & Rouiller, 2010; Gleiss & Kayser, 2012). While these types of behavioral tasks can and have been implemented in larger species such as monkeys, cats and rats this may prove to be more difficult in the mouse model. Rats in general have been chosen to be the main rodent model over mice for perceptual and more cognitively demanding studies because of their relatively low hyperactivity and ability to learn these tasks faster when compared to mice (Carandini & Churchland, 2013; Jaramillo & Zador, 2014). Thus certain behavioral tasks which require animals to hold a specific position and then

react to (multi)sensory stimuli as quickly as possible could be assessed with mice, but may be more well suited for larger species such as rats. Another point that may impact the field in the future is that in general, the mouse has had a major advantage over most animal models because of the known genetics in this system, however recent molecular genetic approaches are now becoming available in generating rat models associated with disease/disorder as well (Cong et al., 2013). These findings may result in larger animal species being evaluated for these types of perceptual behavioral tasks over mouse models in the future (Carandini & Churchland, 2013). While there are some clear disadvantages with mice and recent intriguing discoveries that may impact larger animal species, the mouse still offers a host of advantages. There are still a variety of well known, characterized and relevant mouse models associated with disease/disorder and in addition the breeding and relative costs allow for these animals to be generated and available for studies in much shorter time periods compared to larger species. Despite some of these difficulties in the mouse, overall in this thesis we were able to evaluate and characterize multisensory processing behaviorally for the first time in both wild type mice and a mouse model associated with ASD. Thus, we believe that the mouse offers a variety of advantages that make it still highly relevant for studying and assessing multisensory function in the future. To note though, our current paradigm may in fact not be fully capturing the behavioral benefits that could be observed in mice, thus this may be accomplished in the future by utilizing more appropriate behavioral tasks as outlined above for the mouse model.

The paragraph above detailed potential ways of altering the current behavioral paradigm in order to better assess multisensory function in the mouse by utilizing the principles of space and effectiveness. However, in this paragraph we will focus on discussing how the third multisensory principle (i.e. temporal) may be very important in designing novel tasks to evaluate

and characterize multisensory temporal function in both wild type mice and mouse models associated with disease/disorder. Recently, studies have designed and utilized psychophysical tasks similar to those used in humans and monkeys to evaluate sensory and multisensory processing in rodent models (Carandini & Churchland, 2013; Gleiss & Kayser, 2012; Hirokawa et al., 2008; Raposo et al., 2012; Sheppard et al., 2013). Interestingly, the results (i.e. psychometric curves) found are strikingly similar for both humans and rodents performing similar behavioral tasks (Raposo et al., 2012; Sheppard et al., 2013). This demonstrates that rodents are not only useful animal models for assessing (multi)sensory function, but because of the capability to design genetic rodent models, more appropriate behavioral tasks may need to be developed to mirror those paradigms used in the human clinical population in order to gain a better understanding into these (multi)sensory processing impairments. As mentioned previously, numerous studies have demonstrated atypical unisensory and multisensory processing in individuals with ASD (Baum et al., 2015; Iarocci & McDonald, 2006; Marco et al., 2011). In these studies, two main observations have been found: 1) the magnitude of behavioral gain under multisensory conditions appears to be decreased in ASD (Collignon et al., 2013; Foxe et al., 2013; Mongillo et al., 2008; N. Russo et al., 2010; R. Stevenson et al., 2013; R. A. Stevenson, Siemann, Woynaroski, et al., 2014; Taylor, Isaac, & Milne, 2010) and 2) multisensory temporal processing seems to be disrupted in ASD (Bebko et al., 2006; J. H. Foss-Feig et al., 2010; Kwakye et al., 2011; R. A. Stevenson, Siemann, Schneider, et al., 2014).

Based on prior studies focused on the temporal domain, there appears to exist a window of time within which events specified in different sensory modalities are perceptually "bound" (Hillock, Powers, & Wallace, 2011). Such a window makes a great deal of ethological sense, since events happening in close temporal proximity are highly likely to be associated with the

same event. This multisensory temporal binding window (TBW) can be described as the time interval in which two cross-modal stimuli are bound together as a single unified perceived event (A. R. Powers, Hillock, & Wallace, 2009). Investigators have used psychophysical tasks to evaluate the temporal binding window and assess multisensory temporal function in both typical development (TD) and ASD (Hillock et al., 2011; A. R. Powers, 3rd, Hevey, & Wallace, 2012; A. R. Powers et al., 2009; R. A. Stevenson, Zemtsov, & Wallace, 2012; Wallace & Stevenson, 2014). It has been shown that individuals with ASD tend to have a wider or extended TBW compared to TD individuals and it is thought that by binding these multisensory stimuli over longer periods of time, this could result in degraded and confusing perceptual representations (Baum et al., 2015; J. H. Foss-Feig et al., 2010; Kwakye et al., 2011; Woynaroski et al., 2013). Therefore, it would be logical to design a behavioral task to assess multisensory temporal function in both wild type mice and mouse models associated with ASD. Our hypothesis being that mouse models associated with ASD would exhibit wider TBWs compared to wild type mice. By designing this type of task it would be possible to first determine if multisensory temporal function can be assessed and evaluated behaviorally in mice and second if deficits are present in mouse models associated with ASD compared to wild type animals, are these impairments similar to those observed in the human clinical population? If similar behavioral deficits do exist in mouse models this would allow for further evaluations into the underlying genetic and neural mechanisms that may be driving these behaviors and would offer the opportunity to utilize pharmacologic manipulations to potentially restore or reverse atypical multisensory temporal function in these mouse models. Therefore, designing a behavioral paradigm that assesses multisensory temporal function in the mouse may be important because it would mirror similar tasks that are currently being used in the human (clinical) population and could potentially

provide novel insights into the underlying mechanisms driving these behaviors in both typical development and ASD.

Conclusions

The studies proposed here sought to develop a series of behavioral tests to assess sensory and multisensory function in the mouse model and to then use these tests to characterize (multi)sensory function in a mouse model associated with autism spectrum disorder. The significance of these studies lay in several domains. This work represents the first of its kind to systematically examine sensory and multisensory function in mice and demonstrates that the mouse is a valid animal model system to study multisensory integration. This is of extraordinary value to those interested in studying the neural bases of multisensory function and allows for the application of powerful genetic, pharmacologic, optogenetic and electrophysiological tools to questions of mechanistic relevance. Second, the work applies this behavioral paradigm to a well-established mouse model associated with ASD. Based on the multisensory deficits observed in this mouse model, these studies have provided an essential foundation for future work aimed at better characterizing the neural underpinnings of these deficits along with the associated behavior. Finally, we believe this work offers great promise as a translational bridge that seeks to better link genetic, phenotypic and neural factors in an effort to better elucidate the contributing role of alterations in sensory and multisensory function in autism spectrum disorder.

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