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ABSTRACT

THE EFFECTS OF FASTIGIAL NUCLEI INACTIVATION ON SOCIAL BEHAVIOR IN THE RAT

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Research has implicated the cerebellar deep nuclei in autism. This study questioned whether fastigial nuclei damage accounts for abnormal social behaviors seen in autism. Bilateral cannulation surgery was performed on 13 rats. An ABABAB reversal design was implemented. All animals received a microinfusion of saline during the A phases (baseline). Social interactions were tested using a social interaction chamber and an open field. Seven animals received microinfusions of bupivacaine in the B phase (treatment), which temporarily inactivated the fastigial nuclei. Six control animals received saline again, and social interaction was retested. This sequence was executed three times over six days to achieve an ABABAB design. Results indicate animals with inactivated fastigial nuclei engage in less intense social interactions and engage in more behaviors to prevent social interaction. Knowledge that the fastigial nuclei mediate social interaction can further the understanding of pathology in the autistic brain and lead to breakthrough treatments.

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Introduction

Research on autism and autism spectrum disorders (in this paper, together referred to as autism) has increased in the past few decades. Since autism spectrum disorders affect more than one percent of children (Minshew & Williams, 2007), this line of research is likely to continue as a prevalent topic in psychology, namely neuroscience and behavior analysis. Autism presents with numerous cognitive and behavioral symptoms including repetitive behaviors and rigid cognitive thinking. One telltale characteristic of individuals with autism is abnormal social behavior (Acosta & Pearl, 2003; Critchley, 2000), but the underlying pathology contributing to this symptom is largely unknown. Examining the brain regions responsible for symptoms of autism is important in understanding the disorder and can contribute to potential treatments and cures.

Autism has been widely attributed to abnormal brain connectivity and an irregularity in the inhibition or excitation of certain brain regions or circuits. While some symptoms of autism relate to functions of the frontal lobe, other brain regions have been implicated as well, especially areas of the forebrain. However, many of the symptoms in autism can be attributed to the cerebellum and its connections with those forebrain regions and the cerebral cortex. Upon post-mortem analysis of autistic brains, 95 percent of patients exhibited cerebellar abnormalities (Allen & Courchesne, 2003). Within the cerebellum, its deep nuclei have been specifically implicated in autistic symptoms. Research on the dentate nuclei suggests malfunctions in its connectivity or structure lead to motivational deficits, which are common in autism. The fastigial nuclei have been shown to contribute to social abnormalities, but results of studies on this topic have been contradictory (Berntson & Schumacher, 1980; Bobèe, Mariette, Tremblay-Leveau, &

Caston, 2000). This paper intends to clarify how the fastigial nuclei contribute to abnormal social interaction seen in individuals with autism.

Overview

The external appearance of the cerebellum was described as early as the 16th century, and the deep cerebellar nuclei were described as early as the 17th century, although these structures were not explicitly named until the mid-19th century (Glickstein, Strata, & Voogd, 2009). These early descriptions and studies involving the cerebellum almost unanimously ascribe a motor function to the structure. It was not until more recently that researchers began to explore its involvement in higher-order functioning, which has now become the primary focus of cerebellar examination.

The cerebellum lies posterior to the fourth ventricle and has its own encompassing cortex distinct from the cerebral cortex. The cerebellum consists of two hemispheres connected by a vermis, which is a core-like structure properly named using the Latin word for "worm," as it describes the approximate shape of the structure. Studies performed on the vermis suggest its role in affect (Riva & Giorgi, 2000) and motor functions of core muscles (Coffman, Dum, & Strick, 2011). Directly lateral to the vermis is the intermediate zone of the cerebellar hemisphere, followed by the bulk of the hemisphere itself. The hemispheres are known to control the motor functions of distal body parts. The areas of the hemispheres closer to the vermis control gross limb movements, and the more lateral areas control fine motor movements (Coffman et al., 2011). The most lateral parts of the cerebellum connect extensively with the frontal cortex (Appollonio, Grafman, Schwartz, Massaquoi, & Hallett, 1993). Research has been inconclusive pertaining to whether the hemispheres of the cerebellum have specialized left-right functions like the cerebral hemispheres. However, the hemispheres have recently been identified as having

microzones (sometimes referred to as microcomplexes), which are small parts of the cerebellum with a common, specific function. Massao Ito (2006) suggests there may be upwards of 5,000 of these microzones in the cerebellum.

The outermost layer of the cerebellum is its cortex, which consists of many sulci and gyri. The middle layer is made up of white matter, and the innermost layer is gray matter. The cerebellar deep nuclei exist as this innermost gray matter. The dentate, or lateral, nuclei, the interpositus nuclei, which consist of the eboliform and globose nuclei, and the fastigial nuclei make up the cerebellum's core. Each of the deep nuclei exist bilaterally on both sides of the cerebellum.

Anatomy

The cerebellum's cortex consists of three distinct layers and several types of neurons unique to the cerebellum. The innermost cortical layer is the granule layer, which is named for its concentration of cerebellar granule cells (cerebellar granule cells are distinct from cerebral granule cells). Numerous granule cells packed together form the interior section of the cerebellar cortex. This layer extends from the white matter of the cerebellum to its neighboring layer, the Purkinje layer. Granule cells are glutamatergic neurons and are the smallest, but most numerous neuron in the brain (Voogd & Glickstein, 1998). Because granule cells are packed tightly together, yet are so small, the thickness of the granule layer varies depending on the number of granule cells at that particular site in the cortex. The thickness of the granule layer has been observed between 30μ and 200μ (Gray, 1961).

The middle layer of the cerebellar cortex is arguably the most unique. This is the Purkinje layer, named for the Purkinje cells it houses. Purkinje cells are named after Jan Evangelista Purkinje, the Czech physiologist who discovered this unique cell type in 1837 (Glickstein et al.,

2009). He noted the cells' specific shape, orientation, and arrangement. Each cell consists of a cell body, an axon, and an extensively branched dendritic tree. Each Purkinje cell is oriented the same way: the axon reaches inward to the granule layer, the cell body lies in the Purkinje layer, and the dendritic tree extends superiorly to the molecular layer. Although technically they are three dimensional, the Purkinje cells may be best understood in two dimensions. The dendritic tree of each Purkinje cell exists perpendicular to the cortical edges. The dendrites are closest to the surface, and the axon is closest to the core of the cerebellum. Their structure allows them to be packed closely together, and each cell extends the entire length of the cortex, so the Purkinje layer itself houses only one row of Purkinje cell bodies. Purkinje cells are not found anywhere else in the brain outside their layer of the cerebellar cortex. They are GABAergic neurons and are inhibitory in action (Voogd & Glickstein, 1998).

The outermost layer of the cortex is the molecular layer. It houses the dendritic trees of the Purkinje cells, and it is made up of basket cells and stellate cells. Basket cells and stellate cells are inhibitory interneurons in the cerebellar cortex (Marr, 1969). Because they perform the same function, albeit in slightly different ways, basket and stellate cells are often jointly referred to as the inhibitory interneurons in the molecular layer.

There are two types of fibers that run through the cerebellar cortex: climbing fibers and mossy fibers. Together, these fibers form the afferent connections of the cerebellar cortex. It is presumed that mossy fibers carry information about upcoming movements, and climbing fibers carry information about how to execute those movements (Glickstein et al., 2009). Climbing fibers are unique to their cerebellar loop, and have no counterpart in the cerebrum (Ito, 2006). They originate from the inferior olive and connect to the cerebellar cortex. Every cell in the inferior olive sends signals to the cerebellum through its own climbing fiber. This single

climbing fiber finds exactly one Purkinje cell to innervate, and it intertwines itself in the dendritic tree of that Purkinje cell. One climbing fiber makes many synapses on its one Purkinje cell's dendritic tree, resulting in a highly excitatory reaction (Marr, 1969). Marr's 1969 theory of cerebellar function holds that the inferior olive has one cell responsible for each possible movement the body can perform, and when that cell sends a climbing fiber signal to the appropriate Purkinje cells, the Purkinje cells are then activated to inhibit the correct muscles to perform that specific motion or action.

In addition to the climbing fibers, mossy fibers also play a crucial role in the cerebellum. Mossy fibers are less specific than climbing fibers in the sense that they originate from many sources including the spinal cord, brain stem, and peripheral nerves (Ito, 2006). Mossy fibers target the granule cells of the cortex and synapse with their dendrites. Unlike the one-to-one connection of a climbing fiber, a mossy fiber can synapse with 400-600 granule cells. However, to aid in minimizing interference, a single granule cell usually receives signals through synapses from only four or five mossy fibers (Ito, 2006). Because of their extensive connections with granule cells, mossy fibers are thought to provide contextual information about the task at hand. In other words, the connection between mossy fibers and granule cells functions as a pattern separator, meaning it distinguishes a branching point between two similar circumstances or settings and identifies one as a new situation (Marr, 1969).

The connection between the granule cells and the Purkinje cells happens indirectly through parallel fibers. Granule cells send their unmyelinated axons through the Purkinje layer to the molecular layer (Voogd & Glickstein, 1998). In the molecular layer, the axons branch out to form two fibers, one in each direction parallel to the cortical boundary. These parallel fibers intersect Purkinje dendritic trees. It has been calculated that parallel fibers synapse with

approximately 54% of the dendritic trees they pass through (Ito, 2006). If the parallel fiber makes contact with the dendritic tree, it will form at most only one or two synapses with that cell, but parallel fibers are long enough to cross many Purkinje dendritic trees. This contrasts with climbing fiber connections, which contact only one Purkinje cell, but at multiple sites. It is estimated that a parallel fiber that is 3mm long will synapse about 300 dendritic trees. Additionally, it takes about 50 active granule cells to activate a Purkinje cell (Ito, 2006).

Once climbing fibers and mossy (which turn into parallel) fibers have synapsed with the Purkinje cell, they activate the inhibitory nature of the Purkinje cells. However, as mentioned earlier, there are inhibitory interneurons in the molecular layer that interact with the Purkinje cells as well. The inhibitory interneurons, basket cells and stellate cells, are also activated by parallel fibers, and they inhibit the signal of Purkinje cells. Basket cells "supply inhibitory synapses to the bottleneck of the Purkinje cell soma" (Ito, 2006). Stellate cells inhibit Purkinje cells by synapsing onto the Purkinje dendrites (Ito, 2006).

Climbing fibers and mossy fibers provide afferent connections to the cerebellum. Climbing fibers originate only in the inferior olive, but mossy fibers can originate from numerous regions including the pons, spinal cord, and periphery neurons (Ito, 2006). The sole output of the cerebellar cortex is the Purkinje cell axon, which extends down to the core of the cerebellum into the deep nuclei.

The deep cerebellar nuclei are masses of gray matter that lie at the core of the cerebellum. Deep cerebellar nuclei have been identified in all mammalian species (Voogd & Glickstein, 1998). Three nuclei are commonly identified, although in some species one nuclei is further delineated, resulting in a count of four. The innermost nuclei are the fastigial nuclei. The fastigial nuclei are bilateral structures that lie in the middle of vermis of the cerebellum. They are the

smallest of the deep nuclei. They connect most extensively with the cerebellar cortex of the vermis. The interpositus nuclei lie in the intermediate zone of the cerebellar hemispheres, and thus connect mainly with the cortex there (Gould & Graybiel, 1976). They are situated just lateral of the fastigial nuclei. In some animals, the interpositus can be subdivided into the eboliform and globose nuclei, while in other species the distinction is less clear. For simplicity's sake, they are jointly referred to as the interpositus nuclei. The interpositus nuclei are also bilateral. The outermost nuclei are the dentate, or lateral, nuclei. They, like the fastigial and interpositus, are bilateral structures, and they lie in the cerebellar hemispheres. Their connections come from the lateral parts of the cerebellar hemispheres' cortex (Gould & Graybiel, 1976).

Connectivity

Until recently, psychology's general understanding of the cerebellum led to the assumption that it functions merely as a motor control system. It was established that the fastigial nuclei control reflexes, gross body movements like posture and balance, and were involved in saccadic eye movements. The interpositus nuclei controlled voluntary movements, and the dentate nuclei were involved in movement of the extremities as well as higher-order functioning (Ito, 2006). While the cerebellum does serve a significant role in motor control, more recent studies have attested to its numerous connections to brain regions other than the motor cortex, including structures responsible for higher cognition.

The cerebellum is connected to the rest of the brain by cerebellar peduncles, which are stems of connected neurons. Three peduncles attach the cerebellum to the brain stem, including the pons, which then in turn connects the cerebellum to the rest of the brain. These three peduncles are named according to their anatomical position in a vertical plane. The superior cerebellar peduncle connects the cerebellum to the brain stem above the pons, the middle

cerebellar peduncle connects the cerebellum to the pons, and the inferior cerebellar peduncle connects the cerebellum to the medulla, below the pons. The superior cerebellar peduncle is an efferent bundle of neurons, meaning it is largely responsible for carrying signals from the deep cerebellar nuclei to the rest of the brain. Conversely, the middle cerebellar peduncle carries the main afferent signals from the rest of the brain to the deep cerebellar nuclei. The pons is known to relay afferent information to the deep cerebellar nuclei, and since the medial cerebellar peduncle connects to the pons, this pathway easily carries afferent signals (Riva & Giorgi, 2000). The inferior cerebellar peduncle is involved in both efferent and afferent pathways (Akakin, et al., 2014).

Dentate Nuclei

There are three main fibers that innervate the cells of the dentate nuclei. The first type is the axons of the Purkinje cells, which enter the dentate nucleus from the dorsal and lateral directions. It has been suggested that each Purkinje axon innervates certain dentate nuclei cells that then correspond to specific areas in the brain. The other two types of fibers that innervate the dentate nuclei arrive from outside the cerebellum, probably the inferior olive. All the fiber types synapse on the neurons of the dentate nuclei either by crossing their dendrites, or by climbing through their dendritic tree (Matsushita & Iwahori, 1971b). Research has proposed that these fibers make up the majority of the cerebellum's white matter.

The connections the dentate nuclei make throughout the brain are the most extensively studied network of the cerebellum. Connections originating in the dentate nuclei make extensive contact with the thalamus, pons, inferior olive, and red nucleus. One major pathway of the dentate nuclei is the dentrorubrothalamic pathway. This pathway carries signals from the dentate nuclei, through the peduncles, and up the brain stem to the red nucleus (aka nucleus ruber). The

red nucleus is named for its high iron content, which makes it appear pink or red. It is located just below the thalamus. From the red nucleus, the signals travel to the thalamus. The thalamus relays the correct information to the appropriate regions of the brain. Since the red nucleus is heavily involved in motor coordination, this pathway is likely related to the dentate nuclei's involvement in planning and executing fine motor movements, namely the timing of voluntary movements (Akakin, et al., 2014).

Another pathway of the dentate nuclei is the spinocerebellar pathway. This pathway, as its name suggests, connects the cerebellum to the spinal cord, specifically the medulla. The inferior cerebellar peduncle is the main connection between the two areas. Compared to other cerebellar connections, this pathway is relatively short. This is advantageous, however, because it allows the dentate nuclei to exert control over and make adaptations for crucial movements about the body's position in space without taking time to send signals to the cerebrum (Akakin et al., 2014).

The dentate nuclei are also involved with the premotor cortex and the supplementary motor cortex. The pathway from the premotor cortex to the dentate nuclei is called the corticocerebellar pathway. Notice this pathway does not use the thalamus, as this is an important distinction. The supplementary motor cortex communicates with the dentate nuclei through the pontocerebellar tract (Akakin et al., 2014). Once again, this pathway does not run through the thalamus. Since these pathways do not connect to the thalamus, and therefore do not connect to any region of the cortex responsible for higher order functioning, it is likely that these movement-oriented pathways may function at somewhat of a subconscious, automatic level. The dentate nuclei also receive projections from the hypothalamus, parahippocampal gyrus, cingulate gyrus, superior temporal cortex, and posterior parietal cortex (Allen, et al., 2005).

A current topic of neuroscience research and arguably the most intriguing pathway of the dentate nuclei is the cerebellothalamocortical (CTC) pathway. This pathway runs from the dentate nuclei to the thalamus, and then the signals are spread to numerous regions of the cerebral cortex. This pathway is an efferent pathway for the dentate nucleus, and it is partnered with an afferent pathway that runs from the cerebral cortex through the pons and then to the dentate nuclei (Allen et al., 2005). The CTC pathway has been studied extensively, and scientists have shown the connections from the dentate nuclei and thalamus reach the skeletomotor and oculomotor cortices of the brain (Middleton & Strick, 1997b). These connections control muscle movements and eye movements, respectively. Perhaps more importantly, at least to recent research, is that the CTC pathway has been shown to contact areas of the prefrontal cortex involved in working memory (Middleton & Strick, 1997b). This CTC pathway also targets the inferior parietal lobe, which is involved in interpreting sensory signals (Allen et al., 2005).

As recent research shifted toward believing the cerebellum was involved in higher-order functioning, great leaps have been made in understanding the connections and role of the dentate nuclei. Middleton and Strick (1997b) used the transneuronal transport of the herpes simplex virus type 1 to study the CTC pathway in great detail. This technique tracks the path of the virus through strings of neurons, which reveals where the signal traveled in the brain. Middleton and Strick (1997b) injected the virus into the area in the motor cortex that controls arm movements, a movement known to rely heavily on the dentate nuclei. Upon following the virus's path, they concluded that the arm area of the motor cortex connected to dorsal regions of the contralateral dentate nuclei. They reported the dorsal part of the dentate nuclei projects to skeletomotor regions and the rostrocaudal portion projects to oculomotor regions of the brain. This finding confirms the original conclusion of the field of psychology that the cerebellum is involved in

motor control. However, continued research showed the dentate nuclei's connections to regions in the dorsolateral prefrontal cortex that were influenced by the CTC pathway. Middleton and Strick (1997b) found that specific areas of the dorsolateral prefrontal cortex involved in working memory, areas 46 and 9 of the cerebral cortex, connect with the pontine nuclei, which pass the signals on to the dentate nuclei. The neuronal pathway that connects the dentate nuclei to the dorsolateral prefrontal cortex originates (and terminates) in the ventral portions of the dentate nuclei. Because different parts of the dentate nuclei were observed connecting to different parts of the cerebrum, Middleton and Strick concluded there are distinct outputs of the dentate nuclei specified to project to different areas of the brain (Middleton and Strick, 1997b).

Various studies referencing the connections of the dentate nuclei have used lesions and behavioral tasks to provide information about the function of the dentate nuclei. An experiment performed by Joyal, Strazielle, and Lalonde (2001) showed that lesions to the dentate nuclei impair spatial learning tasks. In their study, rats received electrolytic lesions to the dentate nuclei, and their performances on motor and spatial learning tasks were compared to sham-lesioned control rats. Upon testing in the Morris Water Maze (MWM), lesioned rats swam for significantly longer times and longer distances before finding the hidden platform than the sham-lesioned controls. During the relocation phase, in which the researchers moved the hidden platform to the opposite quadrant, the lesioned rats were significantly slower in finding the hidden platform than controls. These results show that lesions to the dentate nuclei impaired acquisition of the spatial task in the MWM. The lesioned rats also displayed diminished spatial ability in the reversal task in the MWM.

A study by Kim, Uğurbil, and Strick (2001) used magnetic resonance imaging (MRI) of seven healthy human participants to show activation of the dentate nuclei. While in the MRI

scanning device, participants were asked to complete a task of rearranging small pegs to open peg holes. During this task, activation in the dentate nuclei was low. Then the participants were asked to complete what the researchers called the Insanity Task. The Insanity Task used the same equipment, but this time, peg movements had to follow restrictive rules. None of the seven participants were able to solve this task in the time allotted. The dentate nuclei were heavily involved in this task, recorded as being three to four times more active than in the previous, easier task. The researchers concluded that the additional cognitive demands of the Insanity Task required more dentate nuclei activation, thus suggesting the dentate nuclei's role in cognitive processing. These finding also supported Middleton and Strick's (1997b) original idea that the dentate nuclei have separate pathways for cognitive and motor information. Differences in dentate activation here were attributed to a cognitive demand rather than a motor function since the same limb movements were required for both tasks, yet the Insanity Task required significantly more dentate activation.

Klene, Bauer, and Swain (2005) conducted research on the CTC pathway and suggested that damage to the dentate nuclei may alter social interaction. They lesioned the dentate nuclei for one group of rats, and sham lesioned another group. Rats were placed in an open field with a stimulus rat, and the number and duration of subject-initiated contacts were recorded. They found that lesioned rats spent more time engaged in social interaction than their sham-lesioned counterparts. The researchers concluded that the CTC pathway was involved in social interaction, and its disruption via dentate nuclei lesions resulted in decreased social inhibition.

A similar experiment showed that dentate nuclei lesions also impact social anxiety, in addition to effects on social inhibition (Bauer, Brown, & Swain, 2004). The dentate nuclei were lesioned for animals in the experimental group, and control rats received sham lesions. Two

behavioral tasks were used to assess social tendencies: the social discrimination test and the social interaction test. In the social discrimination test, two cages were connected by a tube. To begin, the tube was blocked off. The experimental rat was placed in one cage, and a confederate rat was placed in the other cage. After 24 hours, the confederate rat was removed, the tube was opened, and the experimental rat was free to explore both sides. Results showed that rats with dentate nuclei lesions spent significantly more time in the unfamiliar territory – that is, the territory previously occupied by the confederate rat. In the social interaction test, an experimental rat was placed in an open field with a confederate rat. Animals with dentate nuclei lesions initiated significantly more contacts with the confederate rat than the sham control rats. These results suggest that rats with lesioned dentate nuclei exhibit decreased social anxiety as seen by their increased entries into unfamiliar territories, and by initiating more contacts with the confederate rat. The researchers suggest the reason for more initiated contacts is a decreased ability to interpret social cues.

In another important study, rats were trained to lever press to receive food on a fixed ratio schedule, and then on a progressive ratio schedule (Bauer, Kerr, & Swain, 2011). Training continued until the rat reached consistency on a progressive ratio of 20 and a breaking point was established. A breaking point is the amount of lever presses required before the rat deems that the effort to complete the task exceeds the pleasure of the reinforcer. After this training, the rats underwent surgery to lesion the dentate nuclei, and control animals underwent a sham procedure. Consistent responding on a progressive ratio of 20 was again established. The results showed the breaking points for rats in the lesion group decreased 38%. A decreased breaking point means the rat is willing to do less work for the reward, in this case, the same reward that it had previously worked much harder to obtain. The researchers concluded that the disruption of the

CTC pathway via dentate nuclei lesions negatively affects motivation. Taken together, these studies show the dentate nuclei's involvement in spatial learning, complex cognitive processing, social inhibition, social anxiety, social interaction, social discrimination, and motivation.

Interpositus Nuclei

The interpositus nuclei, like the other deep cerebellar nuclei, have extensive projections to the thalamus. In a study using anterograde labeling to mark efferent fibers of the interpositus nuclei, researchers found that these fibers reached the thalamus and were oriented rostrocaudally (Aumann, Rawson, Pichitpornchai, & Horne, 1996). This same study also used anterograde labeling to establish that the projections to the thalamus from the interpositus nuclei did not overlap with the projections to the thalamus from the dorsal column nuclei (which are located in the brain stem) (Aumann et al., 1996). Multiple researchers have suggested that the interpositus nuclei function by a similar pathway as the dentate nuclei. That is, connections from the interpositus nuclei project to the motor cortex, specifically the arm area (Middleton and Strick, 1997b), via the ventrolateral nucleus of the thalamus (Ito, 2006). This neural loop circles from the interpositus nucleus to the thalamus to the motor cortex and then makes its way back to the interpositus nuclei via the pons (Ito, 2006).

The interpositus nuclei also have a unique neural circuit involved in eyeblink conditioning. The function of this pathway will be detailed later, but its connectivity is relevant here. In this pathway, the interpositus nuclei project via the superior cerebellar peduncle to the contralateral red nucleus. From there, the red nucleus projects the signal to the appropriate motor nuclei controlling the eyeblink response (Thompson, 2005).

To further detail the connections of the interpositus nuclei, Gonzalo-Ruiz and Leichnetz (1990) injected horseradish peroxidase into the caudal part of both the anterior and posterior

areas of the interpositus nuclei. They found that many of the interpositus nuclei's fibers exit via the superior cerebellar peduncle and connect with the dorsal portion of the pons, also called the pontine tegmentum. Other connections were seen from the interpositus through the superior cerebellar peduncle to the red nucleus. The interpositus nuclei also project, although minimally, to the superior colliculus, periaqueductal gray, and the nucleus of the posterior commissure. Extensive connections with numerous nuclei of the thalamus were observed, as well as multiple sites in the pons, various olivary nuclei, and nuclei in the medulla, namely the lateral cuneate nucleus and lateral reticular nucleus (Gonzalo-Ruiz & Leichnetz, 1990).

Gonzalo-Ruiz and Leichnetz (1990) also studied the connectivity of the interpositus nuclei using retrograde labeling, and found that the olivary nuclei project to send information back to the interpositus nuclei. Numerous brain regions known to receive information from the somatomotor cortex were labeled as providing afferent connections to the interpositus nuclei. These include the basilar (ventral) pons, the lateral cuneate nuclei, and the lateral reticular nuclei (Gonzalo-Ruiz & Leichnetz, 1990). As can be concluded, the interpositus nuclei have efferent and afferent connections to many of the same regions.

The most extensively studied function of the interpositus nuclei is its role in eyeblink conditioning. Often using rabbits, researchers present a puff of air to the animal's eye, and it blinks. Here, the air is the unconditioned stimulus (US), and the blink because of the air is the unconditioned response (UR). Then, researchers pair the puff of air with another stimuli, often an auditory tone. After these pairings, the animal learns to blink at the sound of the tone regardless of whether a puff of air is presented. The tone is the conditioned stimulus (CS), and the blink because of the tone is the conditioned response (CR). It has been established that the US pathway projects from the interior olive to the cerebellum. The UR pathway does not use the cerebellum

at all, but projects directly onto the motor nuclei needed for the reflex. The CR pathway specifically involves the interpositus nuclei. It runs from the interpositus nuclei through the superior cerebellar peduncle to the red nucleus, where it is then separated into paths that connect to the relevant motor regions. The CS pathway involves sensory projections that come from the pontine nuclei and reach the cerebellum via mossy fibers (Robleto, Poulos, & Thompson, 2004).

Researchers have been able to inactivate certain steps in the eyeblink conditioning process to determine the exact function of the interpositus nuclei. Christian and Thompson (2003) found that lesioning the interpositus nuclei completely eliminated the eyeblink conditioning. They concluded that the interpositus nuclei were involved in the CS-CR pathway, but not the US-UR pathway because their lesion to the interpositus nuclei did not affect the UR. Additionally, Christian and Thompson (2003) lesioned various other parts of the cerebellum, but none of those lesions eliminated eyeblink responding, as was the case for interpositus nuclei lesions. Upon further analysis, they also hypothesized that there are specific sites in the interpositus nuclei where conditioning occurs (Christian & Thompson, 2003).

Robleto et al. (2004) continued the study of the function of the interpositus nuclei. They reiterated their laboratory's previous findings that inactivating the interpositus nuclei prevents acquisition of a learned response. In this study, they inactivated certain output pathways of the interpositus nuclei in an attempt to pinpoint the exact site of learning. They found that inactivating certain interpositus nuclei outputs did not have an effect on acquiring the learned response, but did have an effect in expressing this response.

Multiple studies performed in Richard Thompson's laboratory have shown that the interpositus nuclei are assisted during conditioning by the cerebellar cortex adjacent to them. The cerebellar cortex, however, is dependent on the interpositus nuclei to complete the conditioning,

whereas the opposite is not true. Upon reinstating function of previously inactivated interpositus nuclei, animals show no saving of the memory of conditioning. Christian and Thompson (2003) explained this to mean that no part of the memory was developed in the cortex itself. A later study agreed that the cortex may be involved, but that the interpositus nuclei are mainly responsible for the memory trace needed in conditioning (Robleto et al., 2004). Additionally, Purkinje cell deficient animals are able to learn, albeit at a slower rate, so learning and memory cannot be mediated by the cerebellar cortex alone (Thompson, 2005). These results give strong support to the hypothesis that the interpositus nuclei may be the memory loci in the brain.

If the memory loci truly are located in the interpositus nuclei, then it must be established how the nuclei carry out that function. Christian and Thompson (2003) suggested that GABA and GABA receptors may be important for learning and producing the CR. Additionally, NMDA receptors likely play a role in the acquisition of the interpositus nuclei's memory, but probably are not as important in the performance of the CR. The researchers concluded that the modulating process of making a memory is protein synthesis in the interpositus nuclei (Christian & Thompson, 2003; Thompson, 2005). Data exists that shows an increase in excitatory synapses in the interpositus nuclei after eyeblink conditioning (Kleim et al., 2002). This increase could also serve as a mediating process of memory. Studies suggest a maintenance of this excitement might delay extinction (Kleim et al., 2002; Robleto et al., 2004).

As its projections to the red nucleus and other motor areas suggest, the interpositus nuclei do have a role in movement. However, it has been well established that the interpositus nuclei control movements of limbs, so recent literature has focused mainly on the cognitive benefits the interpositus nucleus provides. Gonzalo-Ruiz and Leichnetz (1990) argue that somatomotor

connections are the primary focus of the interpositus nuclei, while eye and head movements are secondary.

Fastigial Nuclei

While the fastigial nuclei are the smallest of the deep cerebellar nuclei, their connectivity is not lacking in comparison to the interpositus and dentate nuclei. The fastigial nuclei's best known connections project to certain areas of the brain stem, and extensive projections to the reticular formation and vestibular nuclei have been reported (Angaut & Bowsher, 1970; Berntson & Schumacher, 1980; Matsushita & Iwahori, 1971a; Thomas, 1956). The fastigial nuclei are also responsible for the motor control of the body's core, which includes whole-body movements and balance (Coffman et al., 2011). Further, the fastigial nuclei connect to the superior colliculus, suggesting their role in eye movement and gaze shift (Angaut & Bowsher, 1970). Since midbrain structures like the superior colliculus and substantia nigra are involved in movement, Angaut and Bowsher (1970) suggested a tecto-cerebellar-tectum loop as a fastigial nuclei pathway for motor control. Additionally, fastigial nuclei connections to the inferior olive support their role in movement (Matsushita & Iwahori, 1971a).

The fastigial nuclei also connect to various regions of the brain known to have primary functions other than motor control. Connections to the periaqueductal gray, pons, and more specifically the locus coeruleus indicate roles of the fastigial nuclei in pain control, facial sensations, and cognition and behavioral flexibility, respectively (Berntson & Schumacher, 1980; Matsushita & Iwahori, 1971a).

More recently, research has provided support for the claim that the fastigial nuclei are connected to regions in the forebrain, although this research is still primitive. However, it led researchers to believe the fastigial nuclei played a role in higher cognitive functioning. Direct

connections from the fastigial nuclei to limbic structures have become a key area of interest. The fastigial nuclei connect to the hippocampus, and the connections are especially strong in the CA3 region (Berntson & Schumacher, 1980; Heath & Dempsey, 1980; Heath & Harper, 1974). Extensive connections have been reported between the fastigial nuclei and the amygdala (Berntson & Schumacher, 1980; Heath & Harper, 1974). Both the lateral nucleus and the basal nucleus of the amygdala showed substantial connections from the fastigial nuclei (Heath & Harper, 1974). Additionally, the septum and hypothalamus receive efferent connections from the fastigial nuclei (Berntson & Schumacher; Heath & Dempsey, 1980). The thalamus, however, is the limbic area most associated with cerebellar connections. The fastigial nuclei have been shown to project extensively to the thalamus, targeting its various nuclei (Angaut & Bowsher, 1970; Heath & Harper, 1974; Matsushita & Iwahori, 1971a). Since the thalamus is the relay center of the brain, fastigial nuclei signals can travel to various regions of the forebrain upon reaching the thalamus. Fastigiothalamic signals have been shown to project to the neocortex, secondary sensory areas, and other cortical sites in the temporal lobe (Angaut & Bowsher, 1970; Harper & Heath, 1974). It is postulated that other areas of the cerebral cortex are also targeted, although more research on these projections is needed.

The function of the fastigial nuclei in controlling whole-body movements, muscle tone, and posture and balance has been widely accepted (Coffman et al., 2011; Heath & Harper, 1974). However, with more extensive research showing vast fastigial nuclei connections, other functions can now be attributed to the fastigial nuclei. Stimulation of the fastigial nuclei elicits instinctive behaviors in the rat such as eating, grooming, gnawing, and self-stimulatory behavior (Berntson & Schumacher, 1980). Animals with lesions to the cerebellar midline including the fastigial nuclei exhibit abnormal visuomotor patterns as tested on the MWM (Bobèe et al., 2000).

These behaviors are likely attributable to the connections the fastigial nuclei have with midbrain, hindbrain, and brain stem structures, as they do not require higher cognitive processing. However, studies have demonstrated numerous functions of the fastigial nuclei in higher cognitive processes, especially emotionality, as suggested by their connections to limbic and cortical areas.

While there is conflicting evidence whether fastigial nuclei stimulation elicits attack behaviors or taming behaviors, further research shows fastigial nuclei stimulation attenuates septal rage and controls outburst of anger in humans (Berntson & Schumacher, 1980). Most studies examining the functions of the fastigial nuclei, however, have been performed using behavioral measures of activity, anxiety, and social interaction in nonhuman animals. In a strain of guinea pigs that were genetically altered to have a defective cerebellar vermis, the most remarkable difference between these guinea pigs and control animals was their lack of exploratory behaviors. Motor control was assessed as normal, meaning they physically could explore, but had no motivation to engage in that behavior (Lev-Ram, Valsamis, Masliah, Levine, & Godfrey, 1993). Lev-Ram et al. (1993) concluded that the fastigial nuclei are likely involved in clinical disorders or neuropsychiatric diseases not related to motor control.

In this line of research, two experiments are especially relevant. First, Berntson and Schumacher (1980) performed surgery to lesion the fastigial nuclei and measured numerous instinctive and cognitive behaviors. General body condition, locomotor ability, righting reflexes, stepping reflexes, and visual and tactile orientation were examined. As a measure of higher-order functions, exploratory behavior and social interaction were measured in an open field. Exploration was quantified by assessing the number of gridlines on the floor of the apparatus the animal crossed. During social interaction, an additional rat was placed in the open field. The

researchers measured number and duration of contacts the subject rat initiated with the confederate rat, as well as total time spent in close proximity to the confederate rat, and any instances of defensive postures or fighting. The animals with fastigial nuclei lesions exhibited less activity, exploration, and social interaction when compared to control animals. This suggests the fastigial nuclei play a facilitating role in these three behaviors. Additionally, the dentate nuclei and the vermal cortex of the cerebellum were lesioned to compare the results with the outcomes of the fastigial nuclei lesions. The results reported were true for fastigial nuclei lesions, but other lesions did not show the same effects.

Berntson and Schumacher (1980) did not find any alterations in the instinctive, lower-order behaviors of the lesioned animals. To account for this, the authors suggest two distinct pathways originate from the fastigial nuclei. A fastigiobulbar pathway extends down to the midbrain, hindbrain, and brain stem and controls instinctive behaviors and primitive movement commands. Ascending projections reaching up to the forebrain, namely the limbic system, constitute a separate pathway responsible for the fastigial nuclei's role in higher-order functions. Other researchers agree with this distinction of pathways, and some have suggested the caudal portion of the fastigial nuclei gives rise to the descending pathway, and the rostral part of the nuclei generate ascending projections (Angaut & Bowsher, 1970; Matsushita & Iwahori, 1971a).

In a second crucial experiment, Bobèe, Mariette, Tremblay-Leveau, and Caston (2000) ablated the entire cerebellar vermis in 10-day-old rats. The experiment began when the animals were 12 weeks old. Motor activity was assessed, as were attentional abilities, anxiety, burying behaviors, and social interaction. The researchers found greater motor activity in the lesioned animals. They reported lesioned animals showed difficulty switching attention among environmental stimuli. Anxiety was measured in the elevated plus maze, and the lesioned

animals entered more open arms than controls and spent more time in open spaces. Lesioned animals exhibited more burying behavior, perhaps modeling human escape behavior. Finally, the social interaction test showed lesioned animals spent more time in an area previously occupied by a confederate rat than they did their own familiar territory. Overall, this study reported animals with vermal lesions demonstrated more activity and less anxiety than control animals.

While the results reported by Bobèe et al. (2000) are supportive of findings in the dentate nuclei (Bauer et al., 2004), they are in contrast with the findings of Berntson and Schumacher (1980). Berntson and Schumacher's (1980) report of decreased activity in lesioned animals directly contrasts the report of Bobèe et al. (2000) that showed increased activity in lesioned animals. Bobèe et al. (2000) reported less anxiety, which conflicts with Berntson and Schumacher's (1980) report of less social interaction and exploration, which indicates increased anxiety. These differences may be because of the specific region that was lesioned, since Berntson and Schumacher (1980) lesioned only the fastigial nuclei, and Bobèe et al. (2000) destroyed the entire vermis. The differences could also be influenced by the time at which the lesion was performed. Berntson and Schumacher (1980) lesioned adult rats, where Bobèe et al. (2000) lesioned 10-day-old rats. Lesioning rat pups could allow compensatory functions from other brain regions to develop as the rats mature, which would result in the opposite outcome as an adult. Regardless of the reasons, it is important to resolve this drastic disagreement. Further research is needed to understand how the fastigial nuclei contribute to higher-order behaviors such as social interaction.

Autism

Autism is a disease characterized by irregular behavior as well as abnormal brain development. Atypical social interaction is the most ubiquitous behavioral symptom of autism

(Ciesielski, Harris, Hart, & Pabst, 1997; Chevallier, Kohls, Troiani, Brodkin, & Schultz, 2012; Minshew & Williams, 2007), and cerebellar deficiencies are the most commonly reported abnormality in the autistic brain (Acosta & Pearl, 2003; Bailey et al., 1998; Bauer et al., 2011; Carper & Courchesne, 2000; Critchley, 2000). Ninety-five percent of individuals with autism exhibit cerebellar abnormalities, which is a far higher percentage than any other brain abnormality reported in autism (Allen & Courchesne, 2003).

Since brain abnormalities in autism often involve the cerebellum, research has been especially focused on Purkinje cells. Autistic cerebella exhibit fewer total neurons than a normal brain (Bauman & Kemper, 1985), and diminished cell count and cell size have been reported in the deep cerebellar nuclei (Acosta & Pearl, 2003). It has also been reported that individuals with autism have a smaller glial cell to neuron ratio (Bailey et al., 1998). Autistic brains often show a remarkably diminished Purkinje cell count (Acosta & Pearl, 2003; Bailey et al., 1998; Ciesielski et al., 1997; Ritvo et al., 1986). Allen and Courchesne (2003) suggest an early loss of Purkinje cells may lead to abnormally activated deep cerebellar nuclei because of their extensive reciprocal connections. However, more evidence on whether the Purkinje cells are the cause or result of abnormal cerebellar nuclei is needed.

Perhaps the most widely reported cerebellar abnormality in autism is hypoplasia of vermal lobules VI and VII. The vermis is divided into 10 lobules, named numerically in a rostral-caudal plane. The first five lobules constitute the anterior lobe, and are separated from the rest of the vermis by the primary fissure. Lobules VI-IX make up the posterior lobe, which is bordered on its caudal edge by the posterolateral fissure. Lobule X makes up the flocculondular lobe and lies posterior to the posterolateral fissure (Stoodley & Schmahmann, 2010). Research on the functions of the vermal lobules implicate lobules I-V and VIII in sensorimotor processes and

lobules VI, VII, IX, and X in nonmotor functions related to higher cognitive areas (Stoodley & Schmahmann, 2010). Specifically, research has pointed to hypoplasia in lobules VI and VII as an underlying contribution to the deficits in cognitive processes seen in autism (Akshoomoff, 2005; Allen & Courchesne, 2003; Ciesielski et al., 1997; Critchley, 2000; Lev-Ram et al., 1993; Riva & Giorgi, 2000; Saitoh, Courchesne, Egaas, Lincoln, & Schreibman, 1995; Stoodley & Schmahmann, 2010). The deep cerebellar nuclei have been known to project to the portions of the cerebellum adjacent to them, thus the fastigial nuclei connects heavily with the vermis. Using horseradish peroxidase as a tracer, injections into vermal lobules VI and VII showed extensive labeling in the fastigial nuclei (Gould & Graybiel, 1976). Additionally, lobule VII has been shown to connect to the prefrontal cortex, further confirming its cognitive role. The connection of the vermal cortex to the fastigial nuclei and the base of knowledge of abnormalities in lobules VI and VII of the vermis in autism suggest a fastigial nuclei contribution to the higher cognitive functions of these lobules.

In addition to the abnormalities seen in the cerebellum, other brain regions known to connect with the cerebellum also exhibit deficiencies in autism. Carper and Courchesne (2000) explain a negative correlation between cerebellar deficiency and frontal cortex volume. A brain with a more severely damaged or underdeveloped cerebellum will have a larger frontal cortex than a brain with a more intact cerebellum. Since both the frontal cortex and the cerebellum contribute to higher cognitive functions, this information suggests a compensatory function of the frontal lobe. Cognitive functions affected by abnormalities in the cerebellum may be possible because of the frontal lobe overtaking responsibility for that function (i.e., compensation). Reciprocally, frontal lobe abnormalities may not always be caused by direct damage to the frontal lobe. For example, individuals with autism display an “absence of a frontally localized

neurophysiological response in relation to attention" (Carper & Courchesne, 2000, p. 836). This same abnormality, however, is seen in people who have suffered from a stroke or tumor that affected the cerebellum, suggesting abnormalities in the frontal lobe may be a response to or compensation for damage to the cerebellum. Bauer, Peterson, and Swain (2013) provide directional evidence that the cerebellum deficiencies cause frontal lobe abnormalities. They found that lesions to the dentate nuclei decreased the proportion of immature to mature dendritic spines in the prefrontal cortex. They concluded that damage to the dentate nuclei was responsible for the changes seen in the prefrontal cortex. This study supports the directional relationship that shows cerebellum damage influences frontal lobe architecture and function, but more research on this topic is needed.

Furthermore, autism presents brain abnormalities in the amygdala, hippocampus, and septal nuclei (Acosta & Pearl, 2003; Akshoomoff, 2005). The amygdala, which is known to receive efferent projections from the fastigial nuclei, has also been implicated in a social motivation network in the brain (Chevallier, et al., 2012). Deficiencies in the fastigial nuclei's stage of this circuit would cause a decrease in social interaction. Abnormalities in the hippocampus, which has extensive connections with the fastigial nuclei, have been reported (Akshoomoff, 2005) in autism. Further, autistic brains show an increase in cell-packing density, suggesting brain immaturity (Bauman & Kemper, 1985). Lack of or delayed synaptic pruning could account for this, but more research is needed to make a definitive claim. The septum, an area in connection with the fastigial nuclei, has also been shown to be abnormal in autism (Acosta & Pearl, 2003). This may account for inappropriate expression of affect, especially anger, seen in many individuals with autism (Ritvo et al., 1986).

Most symptoms of autism can be attributed to the dysfunction of the deep cerebellar nuclei. Social deficits are among the first manifested symptoms in autism and arguably the most ubiquitous abnormality of the disorder (Acosta & Pearl, 2003; Chevallier et al., 2012, Ciesielski et al., 1997; Minshew & Williams, 2007), but other common symptoms include impaired language (Acosta & Pearl, 2003; Minshew & Williams, 2007), attentional deficits (Allen & Courchesne, 2003; Bobèe et al., 2000), and lack of exploratory behaviors (Lev-Ram et al., 1993). Although not the focus of this study, it is important to note abnormal gait, and inflexible and repetitive movements are also common symptoms of autism (Acosta & Pearl, 2003; Ciesielski et al., 1997; Minshew & Williams, 2007). These motor abnormalities accredit the cerebellum's simplest function of motor coordination. Further, abnormal eye movements have been seen in autism, and this represents another main function of the fastigial nuclei, but this function will not be reviewed here. Overall, abnormalities seen in autism can be attributed to the functions of the fastigial nuclei or regions with which they have direct connections.

Social Interaction

Since social interaction is an outwardly manifested behavior, it is fairly simple to implement into a behavioral study. Many studies that have examined social interaction between two animals have used an open field as the setting (Adriani et al., 2005; Al-Afif, Staden, Krauss, Schwabe, & Hermann, 2013; Berntson & Schumacher, 1980). Two animals are placed in a large box together, and behaviors of contact between the two animals are then recorded.

Another way to measure social behaviors is through social discrimination, which uses an apparatus with two large boxes and a smaller box connecting them. In the experiment by Bobèe et al. (2000), one box housed an experimental rat for 24 hours, and the other box housed an unknown rat for the same duration. The small cage in the middle was closed off, so neither

animal could move out of their respective box. After 24 hours, the unknown rat was removed from the apparatus and the experimental rat was free to move about the three chambers. Social discrimination was measured by how much time the experimental rat spent in its own territory compared to the unfamiliar territory that previously housed the confederate. Other measures of social discrimination have followed similar procedures (Brown, Nawrocki, & Swain, 2003).

As per usual for social interaction experiments, the current study implemented the open field test and measured behaviors between the two animals. However, in an effort to more thoroughly examine social interaction, a three-chambered apparatus was also used, albeit slightly differently than previously reported. The social discrimination procedures do not introduce a confederate rat into the experimental rat's cage, and thus are not directly measuring social interaction. The present study used a three-chambered apparatus, but one of the sides contained a confederate rat so that social interaction, rather than social discrimination could be measured. This test required the experimental animal to cross the small area in the middle of the boxes to "visit" the confederate rat, who was restricted to its own side. Since autism has implications in abnormal social motivation (Caston et al., 1998; Chevallier et al, 2012), this test not only measured social interactions, but also provided information about how motivated the experimental rat was to seek out social contact from another animal.

Purpose

The purpose of the present experiment was to determine the role of the fastigial nuclei in social interaction. In addition to the fastigial nuclei's known role in motor behaviors, the fastigial nuclei have also been shown to connect to regions of the brain involved in higher cognitive functioning. Connections with the thalamus, amygdala, septum, hippocampus, and the cerebellar cortex make these nuclei an intriguing candidate for mediating the cognitive aspect of social

interaction. Numerous studies have implicated an abnormal cerebellum in disorders such as autism that exhibit deficient social behavior. Within the cerebellum, lobules VI and VII of the vermis and the fastigial nuclei have been suggested to contribute to these social deficiencies.

The dentate nuclei of the cerebellum have been implicated in autism, as the dentate nuclei are a critical part of the CTC pathway, which connects the dentate nuclei to the thalamus and cerebral cortex. Studies have reported that damage to the CTC pathway may affect motivational processes that contribute to symptoms of autism (Bauer et al., 2011; Peterson, et al., 2012). While this information should not be ignored, the present study focused on the role of the fastigial nuclei because autism research has provided increasing evidence toward an abnormal cerebellar vermis, which contains the fastigial nuclei, as a main contributor to symptoms of the disorder.

Methods

Subjects

Eighteen male Long-Evans hooded rats (*rattus norvegicus)* were ordered from Envigo (formerly Harlan) (Indianapolis, Indiana) and housed individually upon arrival. Three rats were randomly chosen as confederate rats, meaning they served as stranger rats to the experimental animals for the assessment of social interaction. These rats were housed in a separate vivarium to avoid exposure to and familiarity with the other experimental animals. The confederates did not undergo surgery or receive any microinfusions. Eight of the remaining fifteen rats were randomly assigned to the Drug Inactivation Group (DIG) and the other seven were assigned to the Saline Control Group (SCG). All animals weighed between 157 and 186 grams upon arrival. After a period of acclimation, the rats weighed between 240 and 278 grams, which approximately corresponded to the size of the rat brain mapped in the Paxinos and Watson

(1986) rat brain atlas. This ensured surgical coordinates were accurate. The animals were maintained on a 12hr/12hr light/dark schedule. Standard laboratory food and water was available *ad libitum* in the animals' home cages.

Materials

Fifteen of the 18 rats underwent cannula implantation surgery. This surgery was performed using a stereotax (Stoelting Co., Wood Dale, IL). Twenty-two gauge double guide cannulas (Plastics One C232G-2.0/SPC, cut to 5.0mm) were implanted into the rats' cerebella, straddling the midline. Twenty-eight gauge double internal infusion cannulas (Plastics One C232I-2.0/SPC, cut to 5.5mm) were placed down the guide cannulas during drug and saline administration. A microsyringe pump (New Era Pump Systems, Inc., Farmingdale, NY) was used to administer the drug or saline at the correct speed. A Hamilton 7000 series microsyringe attached to the pump guided the drug or saline through the infusion cannulas. The anesthetic drug bupivacaine (Sigma-Aldrich Corp., bupivacaine hydrochloride; pharmaceutical secondary standard) was administered to DIG animals at a ratio of 0.38μg in 0.5μl of saline.

A gait analysis procedure required an alley constructed with walls. The alley was 80cm long and 14cm wide and lined with white paper. The alley is shown in Figure 1. Blue nontoxic paint (Crayola washable Tempera Artista II) was applied to the animals' feet.

Figure 1. The gait analysis alley lined with white paper.

The first behavioral test was performed using a unique social interaction cage. The cage consisted of two standard shoebox rat cages connected by a polyvinyl chloride (PVC) tube. The tube had a diameter of 11cm and measured 10cm long. The tube connected the cages from the center of the sides of the cage. In one side, an area measuring 24cm by 12cm and extending to the top of the cage was blocked off in one part of the cage using a metal grid barrier. A clear acrylic cover was placed over each of the cages and was raised 1.5cm above the top of the cage to allow for ventilation but prevent escape. The cage setup is shown in Figure 2. A second behavioral test was performed in an open field. This large box measured 91.5cm by 91.5cm and had walls extending 57.5cm high. To facilitate collection and analysis of behavioral data, the floor of the apparatus was marked off with electrical tape into 36 equal squares. This apparatus is shown in Figure 3.

Figure 2. The social interaction chamber.

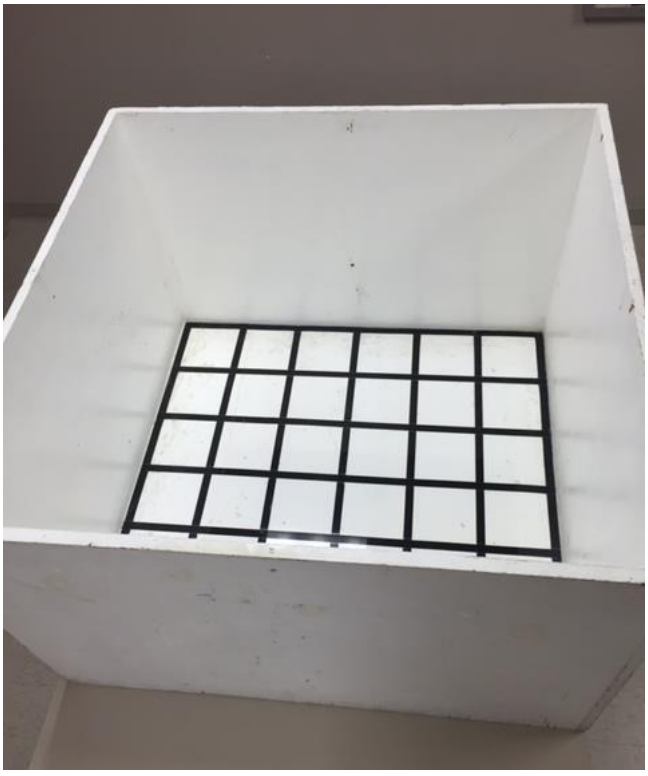


Figure 3. The open field.

Behavioral sessions were video recorded using a Canon PowerShot SD1200 IS digital camera positioned 186cm above the floor of the apparatuses. Neither apparatus had accessible food or water available during the behavioral task. Separate video recordings were taken for each animal's six behavioral phases. The video recordings were randomly coded for data collection on behavioral measures. This ensured the experimenter recording the data was blind to the animals' group and phase.

Procedure

Eight rats were in the Drug Inactivation Group (DIG), seven rats were in the Saline Control Group (SCG), and three additional rats were randomly assigned as confederate rats. The eight DIG animals received microinfusions of saline during the three baseline phases on experimental days 1, 3, and 5. These animals received microinfusions of bupivacaine during the three treatments phases on experimental days 2, 4, and 6. This group allowed for within-subjects analyses since the same animals were alternating between receiving saline and receiving bupivacaine. The animals in the SCG received saline on all six days of the experiment. This control group allowed for between-subjects analyses with the DIG animals in both baseline and treatments phases.

Surgery. For the 15 rats undergoing surgery, on the day prior to surgery, each animal received one ounce of Carpofen gel. Surgical procedures were performed according to UW-Milwaukee IACUC guidelines. On the day of surgery, the animal was anesthetized in a small chamber using 4% isoflurane in oxygen (4L/min). Upon ensuing unconsciousness, the animal was tested for reflexes using a tail pinch. The tail pinch test was used periodically throughout the procedure to ensure the rat stayed under an appropriate level of anesthesia. Conditional on a nonresponse to the tail pinch, the rat's head was placed on the stereotax using the teeth bar and

secured using a nose cone. The isoflurane was switched from flowing into the sedation chamber to flowing through the nose cone. The rat was set on a heating pad which was available to be switched on throughout the procedure, depending on the individual rat's body temperature. The rat's body was positioned on its ventral surface with its arms and legs assuming a natural position. Ear bars were used to secure the rat's skull from moving during surgery. Once the rat was secured, its anesthesia was changed to 1.5-3% isoflurane in oxygen (0.6-0.8L/min) and was maintained at this level until the surgery was completed. Eye ointment was put on the animals' eyes to protect them and prevent them from drying out during the procedure. Swabs to clean the animal's head were alternated between alcohol and betadine. Once the head was disinfected, the surgeon used cotton Q-tips wetted with saline to part the rat's hair down an anterior-posterior line on the middle of the head. A scalpel was used to make an incision from between the eyes to the neck muscles. Using Q-tips, the hair and skin were pulled back from the incision to expose the skull. Coordinates for Bregma and Lambda were taken using the stereotax. The roll, pitch, and yaw of the animal's skull were adjusted until Bregma and Lambda measurements were within 0.1mm of each other. Then, coordinates derived from the Paxinos and Watson rat atlas (1986) were added and subtracted from Bregma to find the target region. From Bregma, the fastigial nuclei lie 11.6mm posterior, 1.0mm lateral on each side, and 5.5mm ventral. Using an electric Dremel tool, one large hole was drilled into the animal's skull directly superior to the fastigial nuclei. Additionally, a small, single hole was partially drilled posterior to Bregma, but anterior to Lambda, and just lateral of the midline in order to place an anchoring screw. After the holes were drilled, Bregma coordinates were again measured and the coordinates of the target structures were recalculated to ensure accuracy despite possible head movement from the vibration of the drilling procedure. A small anchoring screw was screwed into the partially

drilled hole anterior of Lambda. Bone wax was applied to the surface of the large drilled hole above the fastigial nuclei. A double 22-gauge guide cannula was slowly lowered into this hole near the back of the head. It was lowered the calculated distance through the brain using the stereotax, allowing time for the brain to compensate for the compression caused by the cannula. The guide cannulas were measured exactly 0.5mm above the center of the fastigial nuclei. See Figure 4. Bleeding was absorbed using Q-tips as the cannula was lowered, or Gelfoam if bleeding was excessive. Dental cement was mixed and immediately applied to the area surrounding the cannula. After the dental cement dried, the stereotax was lifted, leaving the cannula in place. Any areas of the incision not covered with dental acrylic were closed using nylon sutures. The borders of the dental acrylic and the incision were washed using hydrogen peroxide to clean any dried blood or damaged tissue. A topical antibiotic and analgesic, Neosporin, was applied to the wound to prevent infection. At this time, the isoflurane was cut off, and oxygen was delivered through the nose cone. The ear bars were then removed, and as the rat regained consciousness, it was placed in its home cage to recover. The animal was closely monitored for at least 30 minutes in its home cage. Once the animal was sufficiently recovered, it was returned to the vivarium. One ounce of Carpofen was given to the animal immediately after surgery, and another ounce was given to the animal the following day. One DIG animal died during surgery. Thus, the count of experimental animals was reduced to 14: seven in the DIG and seven in the SCG.

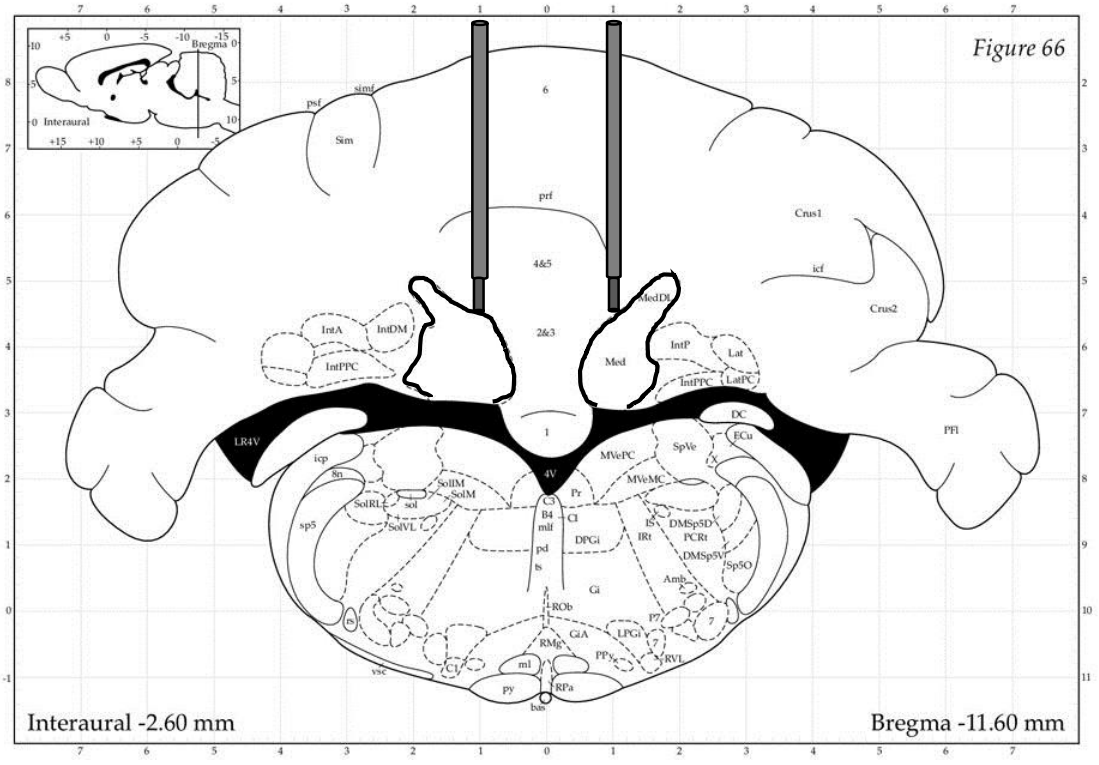


Figure 4. Diagram of guide cannula and infusion cannula placement directly dorsal to the fastigial nuclei, which are outlined. Shown in a coronal plane. Adapted from Paxinos and Watson (1986) Figure 66.

Habituation. Habituation procedures were performed for the injection procedure, the social interaction chamber, the open field apparatus, and the gait analysis test. These were performed on post-surgery day five, after the animals had sufficiently recovered. The habituation was conducted on the day prior to the first experimental day. Two habituation sessions were conducted for each animal, one in the morning and one in the afternoon.

To acclimate to the injection procedure, the animals were tightly wrapped in a cloth to restrict movement of the limbs and core. Only the head protruded from the wrapping. The researcher held the rat inside of the cloth and placed the animal next to the microinfusion pump system for 90 seconds. This allowed the animal to acclimate to the small humming noise emitted

by the pump and to the restriction of the burrito wrap. This was performed twice on the day before the first baseline.

The habituation to the social interaction chamber consisted of placing the experimental rat in the apparatus with no confederate and allowing the rat free access to both sides of the chamber. This occurred for 10 minutes in the morning, one day before behavioral testing began. In the afternoon of the day before testing, the experimental rat was placed in the social interaction chamber along with each of the confederate rats, one at a time. The confederate was restricted to one corner of the confederate side of the apparatus, and a metal grid was placed between the confederate rat and the rest of the apparatus. The confederate was not able to move around the cage. The first session lasted five minutes. After five minutes, the first confederate was removed, and a second confederate replaced it. The experimental animal was able to acclimate to the chamber with the second confederate rat in it for five more minutes. Finally, the second confederate rat was replaced with the third confederate, and five additional minutes ensued. The session lasted for 15 minutes. This apparatus habituation was implemented to lessen the effects of a novel environment, which rats are known to actively explore, and a novel confederate rat, thus making the results of the experiment more honest about social interaction.

Habituation to the open field consisted of a ten minute session where the experimental rat was alone and free to explore the apparatus. This occurred in the morning session on the day before testing. The afternoon habituation session lasted 15 minutes, and the experimental animal was exposed to each of the three confederates, one at a time, for five minutes each. Both animals were free to explore the entire cage and one another.

For the gait analysis, the animal was placed at one end of a straight alley measuring 80cm long and 14cm wide, which is similar in size to previous gait analysis procedures (Scali et al.,

2013; Yu, Matloub, Sanger, & Narini, 2001). A small paper plate with blue paint on it was placed at the entry of the alley. The animal's feet were dipped in the paint, and then the animals walked through the alley, which was lined with white paper to record each footprint. After walking, the animal was removed from the alley, and it was returned to its home cage. This habituation was performed twice on the day before behavioral testing.

Behavioral testing. Behavioral testing began five days after surgery to give the animals ample time to recover. Each of the 14 experimental animals were restrained and placed next to the microsyringe pump. All of the animals received a microinfusion of saline through infusion cannulas measured to extend exactly 0.5mm beyond the end of the guide cannulas to reach the center of the fastigial nuclei. Saline was administered at 0.5µl/90 seconds. One animal died before testing on Experimental Day 1, which resulted in 13 animals participating in behavioral testing: seven animals in the Drug Inactivation Group (DIG) and seven in the Saline Control Group (SCG).

A baseline measure for each animal for each of the social interaction apparatuses was conducted. For the social interaction cage, the confederate rat was placed in the closed-off grid in one side of the apparatus. The experimental rat was placed in the other side of the apparatus, but allowed to move freely between both sides of the cage. The session lasted 10 minutes. A video recording was taken, and after the session was over, an observer recorded 30 dependent variables from the video recordings. The dependent variables were be number of times the experimental rat entered the confederate's side of the apparatus (defined by entry of all four paws), total time spent on each side (defined by all four paws in a side), total time spent in the tube (defined by at least one paw in the tube), number and length of nose to nose contacts with the confederate rat initiated by the experimental rat (defined by the experimental rat's nose touching the confederate

rat's nose), number and length of nose to body contacts with the confederate rat initiated by the experimental rat (defined by the experimental rat's nose touching the confederate rat's body other than the nose), and number and length of touch contacts with the confederate rat initiated by the experimental rat (defined by the experimental rat's body other than the nose touching the confederate rat's body other than the nose). The six variables involving specific contacts were also recorded for contacts that the confederate rat initiated. The frequency of defecations, urinations, bites, and vocalizations were recorded for both the confederate and the experimental animals. Additionally, frequency and duration of peeking (defined by the experimental rat's head but not body protruding from the tube), and frequency and duration of rearing and grooming were measured in the experimental animal.

For the open field, the experimental rat and the confederate rat were placed in the apparatus simultaneously. They were placed in the apparatus in diagonally opposite corners. The session lasted 10 minutes. A video recording was taken, and after the session was over, observers recorded 32 dependent variables. The dependent variables were number and length of nose to nose contacts with the confederate rat initiated by the experimental rat, number and length of nose to body contacts with the confederate rat initiated by the experimental rat, number and length of touch contacts with the confederate rat initiated by the experimental rat, and number and length of on top contacts initiated by the experimental rat (defined by having at least two paws on top of the other animal). Each of these measures was recorded for contacts that the confederate rat initiated as well. Additionally, the frequency of defecations, urinations, bites, and vocalizations were recorded for both animals. Total number of contacts, total time spent in social interaction, the average length of contacts, and time spent in close proximity were measured. In the experimental rat only, frequency and duration of rearing and grooming were also measured.

The gait analysis test was administered as it was in the habituation procedure. A small paper plate with blue paint on it was placed at the entry of the alley. The animal's feet were placed in the blue paint, and then the animal walked down an alley of white paper, leaving footprints to be analyzed. The animal was assessed visually for postural deficits. Coordination was assessed by calculating the distances between each of the animal's feet (i.e., the distance between the left front paw on stride 1 and the left front paw on stride 2, etc.) as well as the width between the left and right strides of the animal. These quantification measures were adapted from Parkkinen et al. (2013). If needed, the animal was coaxed down the alley via gentle prodding.

The previously described events explains the procedure for the A phase of the experiment, which happened on days 1, 3, and 5. All animals received saline on these days. Each day after the A phase, a B phase was conducted. This B phase occurred on days 2, 4, and 6 of the experiment. During the B phase, the Drug Inactivation Group (DIG) animals received microinfusions of bupivacaine, and Saline Control Group (SCG) animals received microinjections of saline. For the SCG animals, the same procedure was carried out in the A phases and the B phases. For the DIG animals, however, the infusions in the B phase differed because they received bupivacaine. For the B phases, all 13 experimental animals were again restrained and placed next to the microsyringe pump. The DIG animals received bupivacaine and the SCG animals received saline through infusion cannulas measured to extend exactly 0.5mm beyond the guide cannulas. Bupivacaine was administered at 0.38µg in 0.5µl of saline/90 seconds, and saline was administered at 0.5µl/90 seconds. Bupivacaine has been shown to penetrate the brain regions within 1mm of the injection site, and its effects last between 75 and 90 minutes (Peterson et al., 2012). The testing session began immediately after the

administration of the saline or drug. The gait analysis procedure was conducted identically on the B phase days as it was on the A phase days.

Sessions in the social interaction apparatus and the open field again lasted 10 minutes and observers recorded the same behaviors. After the 10 minute testing sessions, the animal was returned to its home cage where the effects of the drug wore off.

The A phase was repeated on Experimental Day 3. On Experimental Day 4, a second B phase was conducted identically to the first B phase. Experimental Day 5 repeated the A phase for a third time, and Experimental Day 6 repeated the B phase for a third time. One animal died before testing on Experimental Day 6, resulting in a count of 13 animals on the first five days and a count of 12 animals on the sixth day: six DIG animals and six SCG animals.

Seven of the experimental animals chronologically followed the procedure previously described, meaning they were infused, tested in the social interaction chamber, tested in the open field, and then performed the gait analysis. To avoid confounds of social satiation the experimental rat might have developed to the confederate rat, the other seven rats (randomly determined) were infused, tested in the open field first, then tested in the social interaction chamber, and then performed the gait analysis. Each experimental animal underwent six gait analyses and was tested for a cumulative 120 minutes (60 minutes total for each apparatus), spread out for six sessions over six days: baseline, treatment, baseline, treatment, baseline, treatment (ABABAB).

The three confederate rats were counterbalanced among the experimental animals to minimize any effects of familiarity of the confederate. Each confederate interacted with each experimental animal only twice in each apparatus and never on the same day or consecutive days. See Table 1.

Table 1

Counterbalanced Assignment of Confederate Animals

Animal	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
	CH/OF	CH/OF	CH/OF	CH/OF	CH/OF	CH/OF
DIG 1	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1
DIG 2	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1
DIG 3	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1
DIG 4	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1
DIG 5	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1
DIG 6	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2
DIG 7	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2
SCG 1	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2
SCG 2	C2/C3	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2
SCG 3	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3
SCG 4	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3
SCG 5	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3
SCG 6	C3/C1	C1/C2	C2/C3	C3/C1	C1/C2	C2/C3

Note. Counterbalanced assignment of the three confederate animals (C) in the social interaction chamber (CH) and the open field (OF) for each experimental animal.

Interobserver Agreement. Data was recorded from video recordings using a continuous recording method on a trial-by-trial basis. Each 10-minute behavioral testing session was a separate trial. A second observer analyzed 5% of trials, which was eight out of the 154 total trials: 13 animals on 2 apparatuses with 6 phases (accounting for the death of an animal before the last day of testing). The eight sessions were randomly chosen, as were the variables scored. Because of how many behaviors occurred during the trials, and to account for any differences in observer reaction time starting the stopwatch, it was designated that if the two observers' scores were within three frequency measures or 10 seconds, that trial would be counted as an agreement. The number of trials agreed upon was then divided by the total number of trials and converted to a percentage. Interobserver agreement was 87.5%. Additionally, the first observer rescored eight randomly chosen trials out of the 154 trials (5%) to demonstrate intraobserver

reliability. Agreements were calculated the same way as interobserver agreements. Intraobserver agreement was 87.5%.

Inactivation verification. The extent of the inactivation was analyzed using traditional histology methods to verify the placement of the cannulas. At the end of the behavioral measures, the animals were sacrificed in a CO2 chamber. The infusion cannula was inserted into the guide cannula, and an electrolytic lesion was performed using 100μA for 10 seconds. This lesion was performed to make the location of the cannula tip more obvious during histology. After the electrolytic lesion, animals were perfused using 200mL of phosphate buffer and 400mL of paraformaldehyde. Brains were removed and stored in paraformaldehyde for 24 hours, after which they were moved to a 30% sucrose solution for cryoprotection. Tissue was collected in coronal slices at 40 microns using a cryostat. Tissue was stained using the Nissl stain cresyl violet, and iron deposits from the lesions were detected via the histochemical reaction produced by Prussian blue. Brain slices were examined under a microscope to determine the extent of the lesion in relation to the fastigial nuclei. Microinfusions of 0.5μl of lidocaine have been shown to spread no more than 1mm (Boeijinga, Mulder, Pennartz, Manshanden, & Lopez da Silva, 1993). Since bupivacaine is a lidocaine derivative, it is believed to diffuse over an area of similar size. Bupivacaine was preferred to lidocaine in the study because it has longer lasting effects, which allowed for all behavioral tests to be completed before its effects wore off. If the placement of the cannula was within 1mm of the fastigial nuclei, it was considered a successful placement and sufficient inactivation was assumed. Successful placements and therefore successful inactivations were observed for all 13 experimental animals. An example lesion is shown in Figure 5.

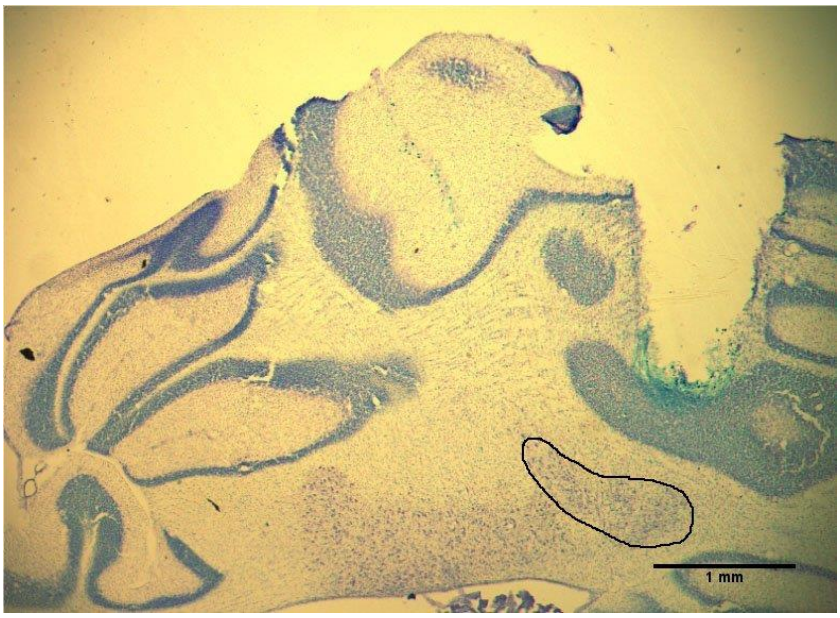


Figure 5. Verification of cannula placement above the fastigial nuclei in a coronal plane. This figure shows a cannula lowered down through the dorsal portion of the cerebellum to reach within 1mm of the fastigial nucleus, which is outlined in black. One side of the lesion is depicted here, however, each animal received bilateral cannulae and bilateral microinfusions.

Results

Gait Analysis

For the gait analysis, the independent variable was the phase of the experiment, either baseline (A) or treatment (B). The dependent variables were falls, back right paw stride length, back left paw stride length, front right paw stride length, front left paw stride length, back feet stride width, and front feet stride width. Each animal's footprints from the A phases were visually and statistically compared to its own footprints in the B phases. If a significant difference was present, that animal would have been removed from the day's testing to ensure motor deficits did not prevent activity in the social interaction chamber or the open field. No animals needed to be removed from this study. An example of the outcome of a gait analysis is shown in Figure 6. Results of the gait analysis showed no falls occurred on any of the trials.

Statistical and visual analysis revealed no significant differences, meaning the animals did not suffer from motor deficits. Insignificant results of the gait analysis are shown in Table 2.

Figure 6. The gait analysis. This figure shows the results of one gait analysis. The starting point is the bottom of the figure.

Table 2
Within-subjects ANOVA results for the gait analysis

Stride Measured	Mean	*df*	*F*	*p*
Front Right	A, *M*=13.17 B, *M*=12.71	6	.656	.449
Front Left	A, *M*=13.68 B, *M*=13.13	6	.222	.654
Back Right	A, *M*=12.67 B, *M*=12.65	6	.000	.988
Back Left	A, M=12.72 B, *M*=13.15	6	.411	.545
Width Back Paws	A, *M*=3.91 B, *M*=3.98	6	.051	.829
Width Front Paws	A, *M*=3.89 B, *M*=3.82	6	.113	.748

Note. No values showed significance at the $p<0.05$ level.

Social Interaction Chamber

The experimental design followed a within-subjects ABABAB reversal design for the 14 experimental animals, but it also allowed for between-subject analyses among the animals in the Drug Inactivation Group (DIG) and the animals in the Saline Control Group (SCG). The first A phases were baselines in which every animal received a saline microinfusion, and the B phases included microinfusions of bupivacaine for DIG animals and microinfusions of saline for SCG animals. For each statistical test, a *p*-value of <.05 was considered significant, and a *p*-value of <.15 was considered a trend toward significance.

Results for the social interaction chamber were analyzed using a one-way ANOVA for each dependent variable. The independent variable was the phase of the experiment. There were 30 dependent variables. Social interaction variables included number of times the experimental rat entered the confederate's side of the apparatus, total time spent in social interaction, total number of contacts, average length of contacts, frequency and duration of nose to nose contacts with the confederate rat initiated by the experimental rat, frequency and duration of nose to body contacts with the confederate rat initiated by the experimental rat, and frequency and duration of

touch contacts with the confederate rat initiated by the experimental rat. These contact variables were also measured for instances that the confederate rat initiated contact with the experimental animal. Anxiety measures including defecations, urinations, bites, and vocalizations were also recorded and assessed for both the experimental animal and the confederate rat. Variables evaluated specifically for the experimental rat included frequency and duration of peeking, frequency and duration of rearing, and frequency and duration of grooming.

Upon recording data from the video recordings, numerous variables failed to occur often enough to be included in the analyses. For this reason 12 variables were dropped: frequency and duration of touch contacts, defecations, urinations, bites, and vocalizations for both the experimental animals and the confederates.

One-way ANOVAs were run separately for each of the 18 remaining dependent variables. First, between-subjects one-way ANOVAs compared the average A phase data of the DIG animals against the average A phase data of the SCG animals. This analysis was run to establish whether differences existed between the animals during the baseline phases. No significant differences were found for any of the 18 variables. Statistical results are shown in Table 3. Next, between-subjects one-way ANOVAs compared the average B phase data of the DIG animals to the average B phase data of the SCG animals. This test was run to examine differences between behaviors of animals under the influence of bupivacaine to behaviors of animals that received saline. No significant differences resulted here either, however the frequency and duration of grooms were trending to be higher in SCG animals. Results are shown in Table 4. Third, within-subjects repeated-measures ANOVAs were run to compare the average A phases of the DIG animals with the average B phases of those same experimental animals. This test detailed how the behavior of the animals changed when they received saline compared

to when those same animals received bupivacaine on the other experimental days. No significant differences were found, but the frequency of rears and number of entries were trending to be higher in the B phases. These results are shown in Table 5.

Table 3

Between-Subjects ANOVA for average baseline: DIG animals compared to SCG animals in the social interaction chamber

Dependent Variable	Mean	*df*	*F*	*p*
Number of times the experimental rat enters the confederate's side of the apparatus	DIG, *M*=6.76 SCG, *M*=8.06	1,11	2.192	.167
Total time spent in social interaction	DIG, *M*=33.59 SCG, *M*=31.48	1,11	.069	.798
Total number of contacts	DIG, *M*=12.19 SCG, *M*=11.11	1,11	.196	.667
Average length of contacts	DIG, *M*=2.66 SCG, *M*=2.78	1,11	.111	.745
Frequency of nose to body contacts initiated by experimental animal	DIG, *M*=4.57 SCG, *M*=4.44	1,11	.008	.932
Duration of nose to body contacts initiated by experimental animal	DIG, *M*=14.63 SCG, *M*=16.14	1,11	.076	.787
Frequency of nose to body contacts initiated by confederate animal	DIG, *M*=1.33 SCG, *M*=.95	1,11	.267	.615
Duration of nose to body contacts initiated by confederate animal	DIG, *M*=5.07 SCG, *M*=3.30	1,11	.301	.594
Frequency of nose to nose contacts initiated by experimental animal	DIG, *M*=4.43 SCG, *M*=3.89	1,11	.527	.483
Duration of nose to nose contacts initiated by experimental animal	DIG, *M*=9.66 SCG, *M*=7.98	1,11	1.570	.236
Frequency of nose to nose contacts initiated by confederate animal	DIG, *M*=1.71 SCG, *M*=1.78	1,11	.014	.908
Duration of nose to nose contacts initiated by confederate animal	DIG, *M*=3.17 SCG, *M*=3.80	1,11	.205	.660
Frequency of rears	DIG, *M*=26.10 SCG, *M*=28.33	1,11	.301	.594
Duration of rears	DIG, *M*=146.96 SCG, *M*=145.87	1,11	.002	.967
Frequency of grooms	DIG, *M*=5.14 SCG, *M*=6.67	1,11	1.231	.291
Duration of grooms	DIG, *M*=172.03 SCG, *M*=170.93	1,11	.002	.962
Frequency of peeks	DIG, *M*=7.24 SCG, *M*=8.00	1,11	.287	.603
Duration of peeks	DIG, *M*=38.03 SCG, *M*=33.64	1,11	.091	.769

Note. No values showed significance at the $p<0.05$ level.

Table 4

Between-Subjects ANOVA for average treatment: DIG animals compared to SCG animals in the social interaction chamber

Dependent Variable	Mean	*df*	*F*	*p*
Number of times the experimental rat enters the confederate's side of the apparatus	DIG, *M*=7.50 SCG, *M*=8.33	1,11	.539	.478
Total time spent in social interaction	DIG, *M*=29.46 SCG, *M*=25.44	1,11	.326	.580
Total number of contacts	DIG, *M*=11.83 SCG, *M*=11.16	1,11	.084	.778
Average length of contacts	DIG, *M*=2.38 SCG, *M*=2.23	1,11	.316	.585
Frequency of nose to nose contacts initiated by experimental animal	DIG, *M*=3.93 SCG, *M*=4.44	1,11	.219	.649
Duration of nose to nose contacts initiated by experimental animal	DIG, *M*=8.51 SCG, *M*=8.38	1,11	.002	.962
Frequency of nose to nose contacts initiated by confederate animal	DIG, *M*=2.34 SCG, *M*=1.50	1,11	1.902	.195
Duration of nose to nose contacts initiated by confederate animal	DIG, *M*=3.26 SCG, *M*=2.93	1,11	.048	.830
Frequency of nose to body contacts initiated by experimental animal	DIG, *M*=4.69 SCG, *M*=4.45	1,11	.057	.815
Duration of nose to body contacts initiated by experimental animal	DIG, *M*=12.86 SCG, *M*=11.42	1,11	.162	.695
Frequency of nose to body contacts initiated by confederate animal	DIG, *M*=1.05 SCG, *M*=.72	1,11	.175	.684
Duration of nose to body contacts initiated by confederate animal	DIG, *M*=4.27 SCG, *M*=2.64	1,11	.196	.667
Frequency of rears	DIG, *M*=29.31 SCG, *M*=27.56	1,11	.176	.683
Duration of rears	DIG, *M*=156.18 SCG, *M*=130.69	1,11	1.980	.187
Frequency of grooms	DIG, *M*=4.88 SCG, *M*=7.61	1,11	2.685	.130*
Duration of grooms	DIG, *M*=173.33 SCG, *M*=209.98	1,11	2.355	.153*
Frequency of peeks	DIG, *M*=7.40 SCG, *M*=8.33	1,11	.217	.650
Duration of peeks	DIG, *M*=29.36 SCG, *M*=25.56	1,11	.159	.698

Note. No values showed significance at the $p<0.05$ level. * Two values significantly trending at *p*-value < .15.

Table 5

Within-Subjects ANOVA for average baseline compared to average treatment: DIG animals in the social interaction chamber

Dependent Variable	Mean	*df*	*F*	*p*
Number of times the experimental rat enters the confederate's side of the apparatus	A, *M*=6.76 B, *M*=7.5	1,6	2.910	.139*
Total time spent in social interaction	A, *M*=33.59 B, *M*=29.46	1,6	.304	.601
Total number of contacts	A, *M*=12.19 B, *M*=11.83	1,6	.064	.809
Average length of contacts	A, *M*=2.66 B, *M*=2.38	1,6	.534	.493
Frequency of nose to body contacts initiated by experimental animal	A, *M*=4.57 B, *M*=4.69	1,6	.024	.882
Duration of nose to body contacts initiated by experimental animal	A, *M*=14.63 B, *M*=12.86	1,6	.224	.652
Frequency of nose to body contacts initiated by confederate animal	A, *M*=1.33 B, *M*=1.05	1,6	.095	.769
Duration of nose to body contacts initiated by confederate animal	A, *M*=5.07 B, *M*=4.27	1,6	.034	.860
Frequency of nose to nose contacts initiated by experimental animal	A, *M*=4.23 B, *M*=3.93	1,6	.801	.405
Duration of nose to nose contacts initiated by experimental animal	A, *M*=9.66 B, *M*=8.51	1,6	.572	.478
Frequency of nose to nose contacts initiated by confederate animal	A, *M*=1.71 B, *M*=2.34	1,6	1.807	.227
Duration of nose to nose contacts initiated by confederate animal	A, *M*=3.17 B, *M*=3.26	1,6	.007	.938
Frequency of rears	A, *M*=26.10 B, *M*=29.31	1,6	2.996	.134*
Duration of rears	A, *M*=146.96 B, *M*=145.08	1,6	.563	.481
Frequency of grooms	A, *M*=5.14 B, *M*=4.88	1,6	1.247	.307
Duration of grooms	A, *M*=172.03 B, *M*=173.33	1,6	.028	.872
Frequency of peeks	A, *M*=7.24 B, *M*=7.40	1,6	.056	.816
Duration of peeks	A, *M*=38.03 B, *M*=29.36	1,6	1.201	.315

Note. No values showed significance at the $p<0.05$ level. * Two values significantly trending at *p*-value < .15.

The same data was further delineated and each specific experimental day was compared separately. These tests were run to understand if there were differences between the DIG animals and the SCG animals during each specific day. Rather than averaging all three A phases, just the A1 data from the DIG animals were compared to the A1 data of the SCG animals using one-way ANOVAs. This was also carried out for days A2, A3, B1, B2, and B3. There were no meaningful significant findings relating to social interaction.

Additionally, statistical analyses were run after omitting data from which the confederate animal was not actively participating. Rodents typically sleep during the light cycle of the day, which is when behavioral testing took place. Because testing lasted most of the day, some of the confederates became fatigued and fell asleep during testing sessions. In an effort to evaluate whether this affected the data, analyses were run again excluding sessions when the confederate slept. One-way ANOVAs were run to examine the between-subjects effects of both the average A phase data between the two groups of experimental animals as well as the average B phase data between the two groups of experimental animals. Repeated measures ANOVAs were also run to compare within-subjects effects between the DIG animals' average A phase data with the average B phase data of those same animals. Although eliminating the data when the confederates were sleeping resulted in some variables approaching significance, the data still showed no meaningful effects.

Open Field

Results for the open field provide better information about social interaction. Similar tests were run on the open field data as were on the social interaction chamber data. As in the social interaction chamber, numerous variables failed to occur often enough to be included in the

analyses. In the open field, eight variables were dropped: defecations, urinations, bites, and vocalizations for both the experimental and confederate rats.

Between-Subjects Analyses. Between-subjects data was analyzed first. One-way ANOVAs were run on the remaining 24 variables. The first statistical analysis compared the average A phases of the Drug Inactivation Group (DIG) animals to the average A phases of the Saline Control Group (SCG) animals. This test was run to show whether the behaviors in baseline, when the DIG and SCG animals all received saline, were similar between the groups. No significant differences were found. See Table 6 for ANOVA outcomes.

Table 6

Between Subjects ANOVA Results: Average DIG Baseline Compared to Average SCG Baseline in the Open Field

Dependent Variable	Mean	*df*	*F*	*p*
Time in close proximity	DIG, *M*=396.02 SCG, *M*=396.75	1,11	.000	.985
Total time spent in social interaction	DIG, *M*=296.46 SCG, *M*=299.21	1,11	.006	.940
Total contacts	DIG, *M*=68.71 SCG, *M*=62.5	1,11	.697	.422
Average length of contacts	DIG, *M*=4.81 SCG, *M*=5.01	1,11	.101	.757
Frequency of nose to body contacts initiated by the experimental animal	DIG, *M*=21.33 SCG, *M*=20.17	1,11	.229	.641
Duration of nose to body contacts initiated by the experimental animal	DIG, *M*=79.82 SCG, *M*=83.36	1,11	.074	.791
Frequency of nose to body contacts initiated by the confederate animal	DIG, *M*=11.33 SCG, *M*=9.78	1,11	.306	.591
Duration of nose to body contacts initiated by the confederate animal	DIG, *M*=37.47 SCG, *M*=39.21	1,11	.017	.900
Frequency of nose to nose contacts initiated by the experimental animal	DIG, *M*=2.95 SCG, *M*=3.17	1,11	.111	.745
Duration of nose to nose contacts initiated by the experimental animal	DIG, *M*=4.61 SCG, *M*=6.22	1,11	.675	.429
Frequency of nose to nose contacts initiated by the confederate animal	DIG, *M*=1.43 SCG, *M*=2.06	1,11	.604	.454
Duration of nose to nose contacts initiated by the confederate animal	DIG, *M*=1.87 SCG, *M*=3.11	1,11	.610	.451
Frequency of touch contacts initiated by the experimental animal	DIG, *M*=8.67 SCG, *M*=9.83	1,11	.566	.468
Duration of touch contacts initiated by the experimental animal	DIG, *M*=56.40 SCG, *M*=87.19	1,11	1.912	.194
Frequency of touch contacts initiated by the confederate animal	DIG, *M*=3.76 SCG, *M*=2.94	1,11	.580	.462
Duration of touch contacts initiated by the confederate animal	DIG, *M*=12.99 SCG, *M*=13.97	1,11	.037	.850
Frequency of on top contacts initiated by the experimental animal	DIG, *M*=13.14 SCG, *M*=9.39	1,11	1.536	.241
Duration of on top contacts initiated by the experimental animal	DIG, *M*=74.29 SCG, *M*=43.54	1,11	1.836	.203
Frequency of on top contacts initiated by the confederate animal	DIG, *M*=6.10 SCG, *M*=5.17	1,11	.396	.542
Duration of on top contacts initiated by the confederate animal	DIG, *M*=29.01 SCG, *M*=22.62	1,11	.907	.361
Frequency of rears	DIG, *M*=22.38 SCG, *M*=18.44	1,11	.835	.381
Duration of rears	DIG, *M*=50.46 SCG, *M*=38.01	1,11	1.095	.318
Frequency of grooms	DIG, *M*=5.62 SCG, *M*=6.33	1,11	.228	.642
Duration of grooms	DIG, *M*=106.25 SCG, *M*=97.93	1,11	.207	.658

Note. No values were significant at the $p<0.05$ level.

One-way repeated measures ANOVAs were also run to check for differences between the SCG animals' behaviors. Since they received saline each of the six days, a comparison across these days offers knowledge of the stability of the control group. No significant differences were found between the SCG animals' days. Significance values can be seen in Table 7. Additionally, to better understand how the DIG animals were behaving in their baseline phases, one-way between-subjects ANOVAs were run comparing the six saline days from the SCG group with the three saline days of the DIG group. Each animal's baseline phase data was plotted, and a line of best fit was established. The slopes of each animal's best fit lines were statistically compared. No significant differences were found between the DIG animals' baselines and the SCG animals' baselines. Data are shown in Table 8.

Table 7

Within-Subjects ANOVA Results: Comparison of SCG Animal Data When Given Saline (all six experimental days)

Dependent Variable	*Mean*	*df*	*F*	*p*
Time in close proximity	A1, *M*=383.98 A2, *M*=429.24 A3, *M*=361.28 B1, *M*=432.24 B2, *M*=405.07 B3, *M*=447.00	1,5	1.304	.317
Total time spent in social interaction	A1, *M*=291.99 A2, *M*=322.13 A3, *M*=283.51 B1, *M*=310.46 B2, *M*=284.90 B3, *M*=305.39	1,5	.305	.604
Total contacts	A1, *M*=66.17 A2, *M*=59.17 A3, *M*=62.17 B1, *M*=79.17 B2, *M*=71.17 B3, *M*=79.11	1,5	1.410	.288
Average length of contacts	A1, *M*=4.60 A2, *M*=5.75 A3, *M*=4.67 B1, *M*=4.44 B2, *M*=4.13 B3, *M*=4.30	1,5	.716	.436
Frequency of nose to body contacts initiated by the experimental animal	A1, *M*=23.67 A2, *M*=19.50 A3, *M*=17.33 B1, *M*=31.83 B2, *M*=27.83 B3, *M*=29.50	1,5	3.243	.132
Duration of nose to body contacts initiated by the experimental animal	A1, *M*=102.58 A2, *M*=79.90 A3, *M*=67.60 B1, *M*=125.97 B2, *M*=104.74 B3, *M*=97.22	1,5	2.360	.185
Frequency of nose to body contacts initiated by the confederate animal	A1, *M*=10.33 A2, *M*=10.50 A3, *M*=8.50 B1, *M*=15.00 B2, *M*=12.67 B3, *M*=13.83	1,5	.520	.503
Duration of nose to body contacts initiated by the confederate animal	A1, *M*=36.80 A2, *M*=45.07 A3, *M*=35.77 B1, *M*=55.83 B2, *M*=42.75 B3, *M*=53.88	1,5	.572	.483
Frequency of nose to nose contacts initiated by the experimental animal	A1, *M*=3.51 A2, *M*=3.57 A3, *M*=11.57 B1, *M*=4.67 B2, *M*=2.32 B3, *M*=8.88	1,5	1.545	.269
Duration of nose to nose contacts initiated by the experimental animal	A1, *M*=3.51 A2, *M*=3.57	1,5	1.545	.269

	A3, *M*=11.57 B1, *M*=4.67 B2, *M*=2.32 B3, *M*=8.88			
Frequency of nose to nose contacts initiated by the confederate animal	A1, *M*=2.67 A2, *M*=1.17 A3, *M*=2.33 B1, *M*=1.67 B2, *M*=1.17 B3, *M*=2.17	1,5	.673	.449
Duration of nose to nose contacts initiated by the confederate animal	A1, *M*=3.40 A2, *M*=1.14 A3, *M*=4.78 B1, *M*=1.36 B2, *M*=1.32 B3, *M*=2.50	1,5	.858	.397
Frequency of touch contacts initiated by the experimental animal	A1, *M*=7.00 A2, *M*=9.67 A3, *M*=12.83 B1, *M*=7.50 B2, *M*=7.67 B3, *M*=7.17	1,5	1.289	.308
Duration of touch contacts initiated by the experimental animal	A1, *M*=46.54 A2, *M*=131.26 A3, *M*=83.78 B1, *M*=61.13 B2, *M*=50.30 B3, *M*=50.30	1,5	1.630	.258
Frequency of touch contacts initiated by the confederate animal	A1, *M*=3.50 A2, *M*=1.33 A3, *M*=4.00 B1, *M*=2.67 B2, *M*=4.50 B3, *M*=2.50	1,5	1.460	.281
Duration of touch contacts initiated by the confederate animal	A1, *M*=10.38 A2, *M*=3.96 A3, *M*=27.56 B1, *M*=14.31 B2, *M*=19.51 B3, *M*=14.64	1,5	1.699	.249
Frequency of on top contacts initiated by the experimental animal	A1, *M*=6.65 A2, *M*=4.72 A3, *M*=7.65 B1, *M*=8.00 B2, *M*=9.00 B3, *M*=8.17	1,5	.147	.717
Duration of on top contacts initiated by the experimental animal	A1, *M*=52.61 A2, *M*=40.34 A3, *M*=37.66 B1, *M*=28.16 B2, *M*=31.45 B3, *M*=29.03	1,5	.990	.365
Frequency of on top contacts initiated by the confederate animal	A1, *M*=6.50 A2, *M*=4.83 A3, *M*=4.17 B1, *M*=5.33 B2, *M*=6.67 B3, *M*=5.50	1,5	.312	.601
Duration of on top contacts initiated by the confederate animal	A1, *M*=36.17 A2, *M*=16.90 A3, *M*=14.80 B1, *M*=19.04	1,5	1.420	.287

	B2, *M*=32.53 B3, *M*=71.04			
Frequency of rears	A1, *M*=17.60 A2, *M*=22.20 A3, *M*=20.00 B1, *M*=25.60 B2, *M*=27.40 B3, *M*=20.20	1,5	1.551	.281
Duration of rears	A1, *M*=36.39 A2, *M*=43.11 A3, *M*=42.57 B1, *M*=44.91 B2, *M*=55.30 B3, *M*=34.63	1,5	1.095	.354
Frequency of grooms	A1, *M*=6.67 A2, *M*=5.67 A3, *M*=6.67 B1, *M*=6.17 B2, *M*=8.00 B3, *M*=5.17	1,5	.570	.484
Duration of grooms	A1, *M*=62.18 A2, *M*=112.94 A3, *M*=118.66 B1, *M*=61.31 B2, *M*=84.92 B3, *M*=68.70	1,5	1.338	.300

Note. No values were significant at the $p<0.05$ level.

Table 8

Between-Subjects ANOVA Results: Average DIG Baseline Compared to Average SCG Baseline and Treatment in the Open Field

Dependent Variable	Mean	*df*	*F*	*p*
Time in close proximity	DIG, *M*= -1.00 SCG, *M*=5.39	1,11	.181	.679
Total time spent in social interaction	DIG, *M*=23.17 SCG, *M*= -.51	1,11	.616	.449
Total contacts	DIG, *M*= -.21 SCG, *M*=3.32	1,11	.301	.594
Average length of contacts	DIG, *M*=.41 SCG, *M*= -.19	1,11	.832	.381
Frequency of nose to body contacts initiated by the experimental animal	DIG, *M*= -.36 SCG, *M*=1.96	1,11	.865	.372
Duration of nose to body contacts initiated by the experimental animal	DIG, *M*=2.32 SCG, *M*=3.85	1,11	.023	.883
Frequency of nose to body contacts initiated by the confederate animal	DIG, *M*= -.27 SCG, *M*=.87	1,11	.173	.686
Duration of nose to body contacts initiated by the confederate animal	DIG, *M*= -10.03 SCG, *M*=2.81	1,11	.967	.347
Frequency of nose to nose contacts initiated by the experimental animal	DIG, *M*=1.03 SCG, *M*=.08	1,11	.947	.351
Duration of nose to nose contacts initiated by the experimental animal	DIG, *M*=1.32 SCG, *M*=.46	1,11	.215	.652
Frequency of nose to nose contacts initiated by the confederate animal	DIG, *M*=.45 SCG, *M*= -.09	1,11	1.093	.318
Duration of nose to nose contacts initiated by the confederate animal	DIG, *M*=.30 SCG, *M*= -.21	1,11	.704	.419
Frequency of touch contacts initiated by the experimental animal	DIG, *M*=.50 SCG, *M*= -.15	1,11	.331	.577
Duration of touch contacts initiated by the experimental animal	DIG, *M*=12.33 SCG, *M*= -9.92	1,11	1.477	.250
Frequency of touch contacts initiated by the confederate animal	DIG, *M*= -.62 SCG, *M*=.09	1,11	.817	.385
Duration of touch contacts initiated by the confederate animal	DIG, *M*= -2.05 SCG, *M*=2.57	1,11	2.068	.178
Frequency of on top contacts initiated by the experimental animal	DIG, *M*=1.07 SCG, *M*= -.17	1,11	.542	.477
Duration of on top contacts initiated by the experimental animal	DIG, *M*= -10.27 SCG, *M*= -4.40	1,11	.145	.711
Frequency of on top contacts initiated by the confederate animal	DIG, *M*=1.14 SCG, *M*=.05	1,11	.584	.461
Duration of on top contacts initiated by the confederate animal	DIG, *M*=12.19 SCG, *M*=5.36	1,11	.471	.507
Frequency of rears	DIG, *M*=2.36 SCG, *M*=.94	1,11	.631	.444
Duration of rears	DIG, *M*=4.23 SCG, *M*=1.35	1,11	.488	.499
Frequency of grooms	DIG, *M*=2.02 SCG, *M*=.03	1,11	1.701	.219
Duration of grooms	DIG, *M*= -9.52 SCG, *M*= -3.10	1,11	.327	.579

Note: Analyses were performed using slope values from lines of best fit showing baseline data.

No values were significant at the $p<0.05$ level.

Next, one-way ANOVAs were run on the average B phase data of the DIG animals compared to the average B phase data of the SCG animals. This test compared the differences between the animals under the influence of bupivacaine and animals under the influence of saline. This analysis revealed multiple significant differences. Results for each dependent variable for this statistical test are shown in Table 9. Frequency of nose to body contacts initiated by the experimental animal was higher in the SCG animals compared to the DIG animals, $F(1,11)=7.40$, $p=.02$. Results are shown in Figure 7. The duration of these contacts was also significantly higher in SCG animals than DIG animals, $F(1,11)=14.22$, $p=.03$. Results are shown in Figure 8. The frequency of touch contacts initiated by the confederate rat was significantly higher in DIG animals, $F(1,11)=11.20$, $p=.01$. See Figure 9. Additionally, both frequency and duration of on top contacts with the confederate were significantly higher in DIG animals, $F(1,11)=5.97$, $p=.03$; $F(1,11)=6.69$, $p=.03$, respectively. These results are depicted in Figures 10 and 11.

Table 9

Between-Subjects ANOVA Results: Average DIG Treatment Compared to Average SCG Treatment in the Open Field

Dependent Variable	Mean	*df*	*F*	*p*
Time in close proximity	DIG, *M*=423.95 SCG, *M*=420.04	1,11	.017	.899
Total time spent in social interaction	DIG, *M*=319.09 SCG, *M*=300.25	1,11	.479	.503
Total contacts	DIG, *M*=78.24 SCG, *M*=75.65	1,11	.159	.698
Average length of contacts	DIG, *M*=4.17 SCG, *M*=4.29	1,11	.039	.847
Frequency of nose to body contacts initiated by the experimental animal	DIG, *M*=22.07 SCG, *M*=29.72	1,11	7.404	.020*
Duration of nose to body contacts initiated by the experimental animal	DIG, *M*=69.59 SCG, *M*=109.31	1,11	14.220	.003*
Frequency of nose to body contacts initiated by the confederate animal	DIG, *M*=14.52 SCG, *M*=13.83	1,11	.086	.774
Duration of nose to body contacts initiated by the confederate animal	DIG, *M*=66.10 SCG, *M*=50.82	1,11	.595	.457
Frequency of nose to nose contacts initiated by the experimental animal	DIG, *M*=2.11 SCG, *M*=3.44	1,11	2.718	.127**
Duration of nose to nose contacts initiated by the experimental animal	DIG, *M*=2.92 SCG, *M*=5.29	1,11	1.365	.267
Frequency of nose to nose contacts initiated by the confederate animal	DIG, *M*=1.48 SCG, *M*=1.67	1,11	.157	.699
Duration of nose to nose contacts initiated by the confederate animal	DIG, *M*=2.24 SCG, *M*=1.72	1,11	.518	.487
Frequency of touch contacts initiated by the experimental animal	DIG, *M*=10.14 SCG, *M*=7.44	1,11	2.491	.143**
Duration of touch contacts initiated by the experimental animal	DIG, *M*=54.55 SCG, *M*=49.30	1,11	.142	.714
Frequency of touch contacts initiated by the confederate animal	DIG, *M*=5.47 SCG, *M*=3.22	1,11	11.203	.007*
Duration of touch contacts initiated by the confederate animal	DIG, *M*=27.32 SCG, *M*=16.15	1,11	3.350	.094**
Frequency of on top contacts initiated by the experimental animal	DIG, *M*=13.79 SCG, *M*=8.39	1,11	5.971	.033*
Duration of on top contacts initiated by the experimental animal	DIG, *M*=63.79 SCG, *M*=29.55	1,11	6.689	.025**
Frequency of on top contacts initiated by the confederate animal	DIG, *M*=7.67 SCG, *M*=5.83	1,11	1.805	.206
Duration of on top contacts initiated by the confederate animal	DIG, *M*=32.58 SCG, *M*=40.87	1,11	.536	.479
Frequency of rears	DIG, *M*=24.57 SCG, *M*=22.61	1,11	.115	.741
Duration of rears	DIG, *M*=64.40 SCG, *M*=42.12	1,11	1.433	.256
Frequency of grooms	DIG, *M*=5.86 SCG, *M*=6.44	1,11	.120	.736
Duration of grooms	DIG, *M*=100.04 SCG, *M*=71.65	1,11	1.738	.214

Note. *significant at $p<.05$; **significant trend at $p<.15$

While not meeting the stringent criteria of significance at a p-value of <0.05, some variables showed trending significance, where the criteria is established at a *p*-value of <0.15. The frequency of nose to nose contacts initiated by the experimental rat was trending towards being higher in the SCG animals, $F(1,11)=2.72$, $p=.13$. The frequency of touch contacts initiated by the experimental rat was trending toward a higher number in the DIG animals, $F(1,11)=2.49$, $p=.14$. Finally, the duration of touch contacts initiated by the confederate rat was trending towards significance, showing longer times with the DIG animals, $F(1,11)=3.35$, $p=.09$.

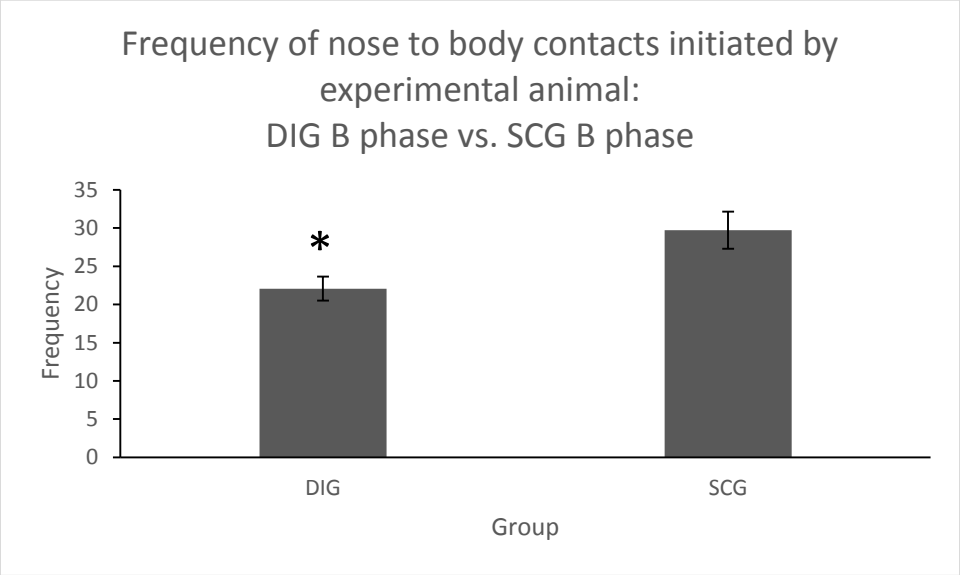


Figure 7. Frequency of nose to body contacts initiated by the experimental animal. This figure shows the difference between frequency of nose to body contacts initiated by the experimental animal in animals with inactivated fastigial nuclei (DIG) and animals with intact fastigial nuclei (SCG). Results are significant at p<0.05 level. * $p=0.02$.

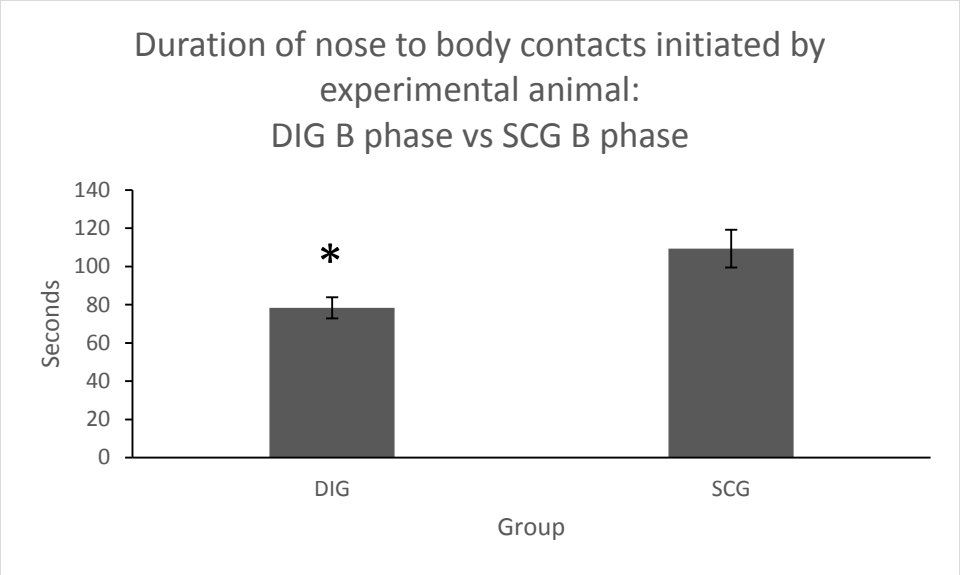


Figure 8. Duration of nose to body contacts initiated by the experimental animal. This figure shows the difference between duration of nose to body contacts initiated by the experimental animal in animals with inactivated fastigial nuclei (DIG) and animals with intact fastigial nuclei (SCG). Results are significant at $p<0.05$ level. * $p=0.03$.

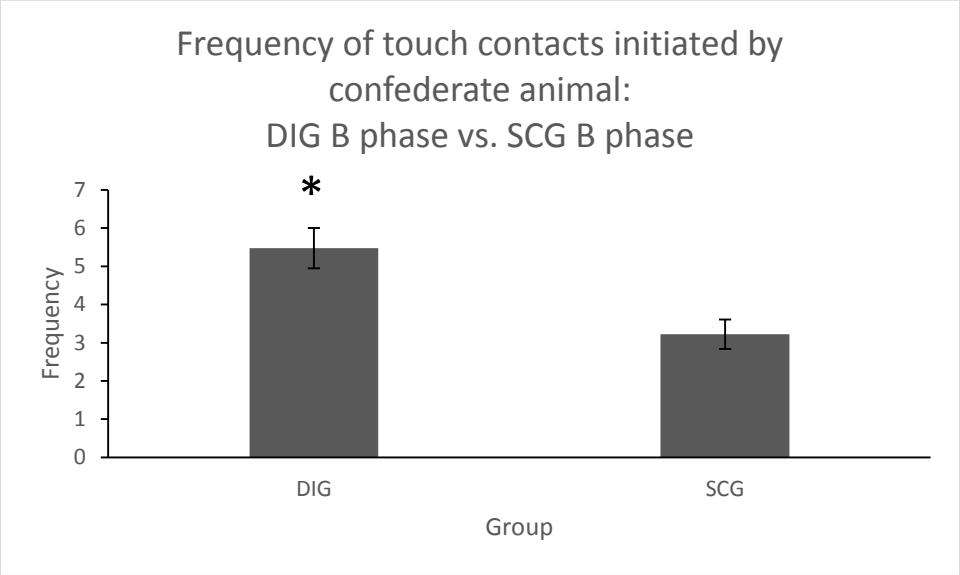


Figure 9. Frequency of touch contacts initiated by the confederate animal. This figure shows the difference between frequency of touch contacts initiated by the confederate animal when paired with animals with inactivated fastigial nuclei (DIG) or animals with intact fastigial nuclei (SCG). Results are significant at $p<0.05$ level. * $p=0.01$.

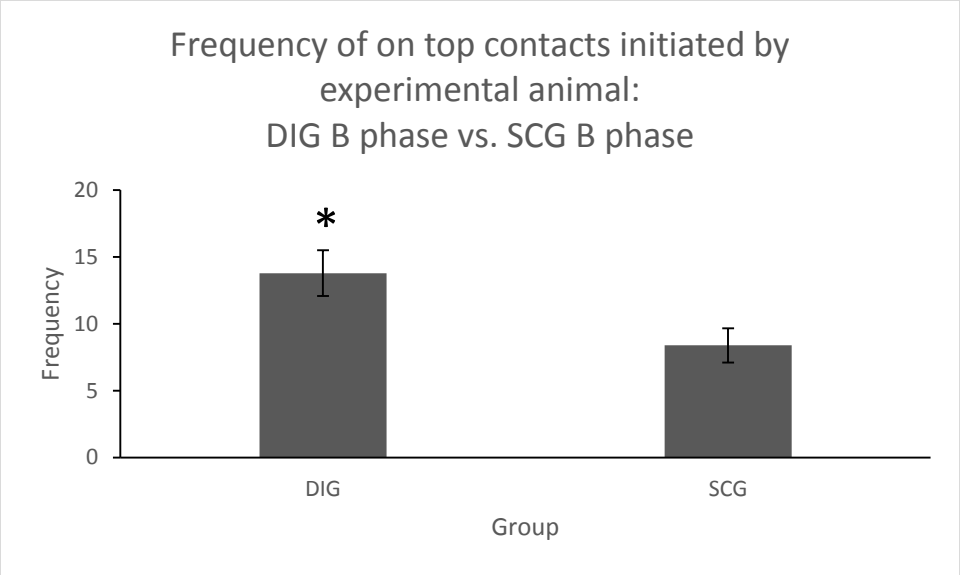


Figure 10. Frequency of on top contacts initiated by the experimental animal. This figure shows the difference between frequency of on top contacts initiated by the experimental animal for animals with inactivated fastigial nuclei (DIG) or animals with intact fastigial nuclei (SCG). Results are significant at $p<0.05$ level. * $p=0.03$.

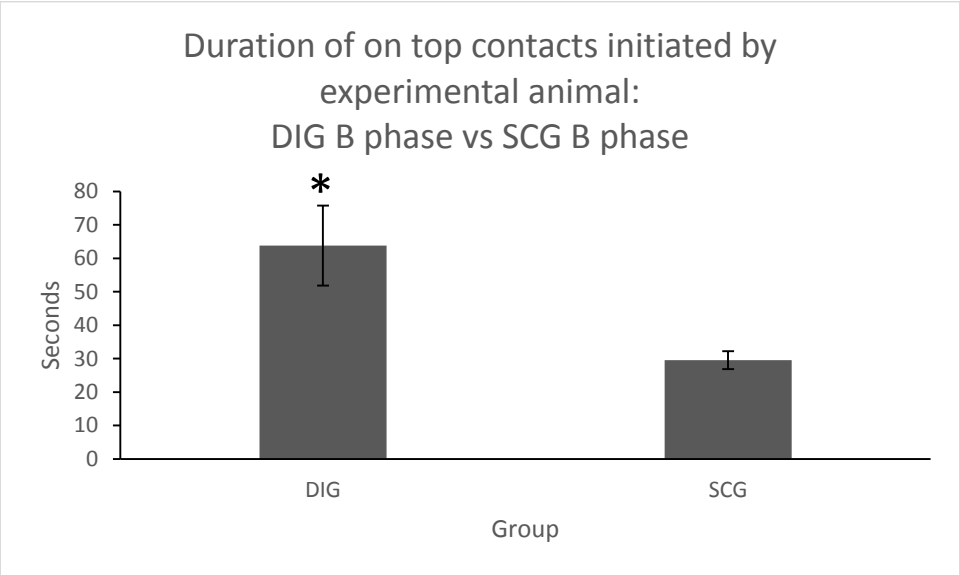


Figure 11. Duration of on top contacts initiated by the experimental animal. This figure shows the difference between duration of on top contacts initiated by the experimental animal for animals with inactivated fastigial nuclei (DIG) or animals with intact fastigial nuclei (SCG). Results are significant at $p<0.05$ level. * $p=0.01$.

Within-Subjects Analyses. To evaluate the level of consistency among different days in the same phase, the DIG animals' data was analyzed as A1 averages compared to A2 averages compared to A3 averages. Time in close proximity was significantly different between A1 averages and A2 averages, $F(1,6)=16.31$, $p=.01$, and also between A2 averages and A3 averages $F(1,6)=7.53$, $p=03$. The differences between A1 averages and A3 averages was trending toward significance, $F(1,6)=0.054$, $p=.054$. A2 showed the longest time in close proximity, followed by A3, and A1 showed the least amount of time in close proximity. The duration of touch contacts initiated by the confederate was significantly lower in the A1 averages than the A3 averages, $F(1,6)=7.01$, $p=.04$. Frequency and duration of rears were both lower in A1 than in A2, $F(1,6)=8.90$, $p=.03$; $F(1,6)=9.30$, p=.02, respectively. Additionally, seven other social interaction variables were trending toward significance. A similar repeated measures ANOVA was run on the averages of the DIG animals' B phases, comparing B1 averages to B2 averages to B3 averages. No significant differences were found in this analysis.

With repeated measures ANOVAs, the averages of both the A and B phases of the DIG animals were compared. These tests detailed the differences that occurred within each DIG animal. They compared the behaviors the animal exhibited while it was infused with saline and the behaviors of that same animal when it was infused with bupivacaine on the other experimental days. Statistical results are shown in Table 10. Duration of touch contacts initiated by the confederate was significantly higher in the B phase $F(1,6)=9.13$, $p=.02$. Results shown in Figure 12. The amount of total contacts was significantly higher in the B phase as well $F(1,6)=7.53$, $p=.03$. See Figure 13. Additionally, the average length of contacts was trending toward significance, with the longer contacts happening in the A phase $F(1,6)=3.30$, $p=.12$. Results depicted in Figure 14. Nose to nose frequency $F(1,6)=2.97$, $p=.14$, and duration,

$F(1,6)=5.46$, $p=.06$, initiated by the experimental animal were both trending toward significance with more and longer contacts in the A phase. These results are shown in Figures 15 and 16. Frequency of touch contacts initiated by the confederate were trending toward being higher in the B phase $F(1,6)=5.82$, $p=.052$. Finally, the duration of rears was trending toward being significantly higher in the A phases $F(1,6)=2.92$, $p=.10$.

Table 10

Within-Subjects ANOVA Results: Average A phases of DIG animals compared to average B phases of DIG animals in the Open Field

Dependent Variable	Mean	*df*	*F*	*p*
Time in close proximity	A, *M*=396.02 B, *M*=423.95	1,6	2.517	.164
Total time spent in social interaction	A, *M*=296.46 B, *M*=319.09	1,6	.564	.481
Total contacts	A, *M*=68.71 B, *M*=78.24	1,6	7.528	.034*
Average length of contacts	A, *M*=4.81 B, *M*=4.17	1,6	3.299	.119**
Frequency of nose to body contacts initiated by the experimental animal	A, *M*=21.33 B, *M*=20.07	1,6	.334	.584
Duration of nose to body contacts initiated by the experimental animal	A, *M*=79.82 B, *M*=69.58	1,6	1.721	.238
Frequency of nose to body contacts initiated by the confederate animal	A, *M*=11.33 B, *M*=14.52	1,6	1.978	.209
Duration of nose to body contacts initiated by the confederate animal	A, *M*=37.47 B, *M*=66.10	1,6	1.923	.215
Frequency of nose to nose contacts initiated by the experimental animal	A, *M*=2.95 B, *M*=2.12	1,6	2.966	.136**
Duration of nose to nose contacts initiated by the experimental animal	A, *M*=4.61 B, *M*=2.92	1,6	5.464	.058**
Frequency of nose to nose contacts initiated by the confederate animal	A, *M*=1.43 B, *M*=1.48	1,6	.010	.922
Duration of nose to nose contacts initiated by the confederate animal	A, *M*=1.87 B, *M*=2.24	1,6	.212	.662
Frequency of touch contacts initiated by the experimental animal	A, *M*=8.67 B, *M*=10.14	1,6	1.815	.227
Duration of touch contacts initiated by the experimental animal	A, *M*=56.40 B, *M*=54.55	1,6	.035	.858
Frequency of touch contacts initiated by the confederate animal	A, *M*=3.76 B, *M*=5.48	1,6	5.820	.052**
Duration of touch contacts initiated by the confederate animal	A, *M*=12.99 B, *M*=27.32	1,6	9.127	.023*
Frequency of on top contacts initiated by the experimental animal	A, *M*=13.14 B, *M*=13.79	1,6	.268	.623
Duration of on top contacts initiated by the experimental animal	A, *M*=74.29 B, *M*=63.79	1,6	1.238	.308
Frequency of on top contacts initiated by the confederate animal	A, *M*=6.10 B, *M*=7.67	1,6	1.628	.249
Duration of on top contacts initiated by the confederate animal	A, *M*=29.01 B, *M*=32.58	1,6	.481	.514
Frequency of rears	A, *M*=22.10 B, *M*=24.40	1,6	1.419	.248
Duration of rears	A, *M*=49.35 B, *M*=64.25	1,6	2.916	.104**
Frequency of grooms	A, *M*=5.50 B, *M*=5.80	1,6	.173	.683
Duration of grooms	A, *M*=107.42 B, *M*=101.03	1,6	.176	.680

Note. *significant at $p<.05$; **significant trend at $p<.15$

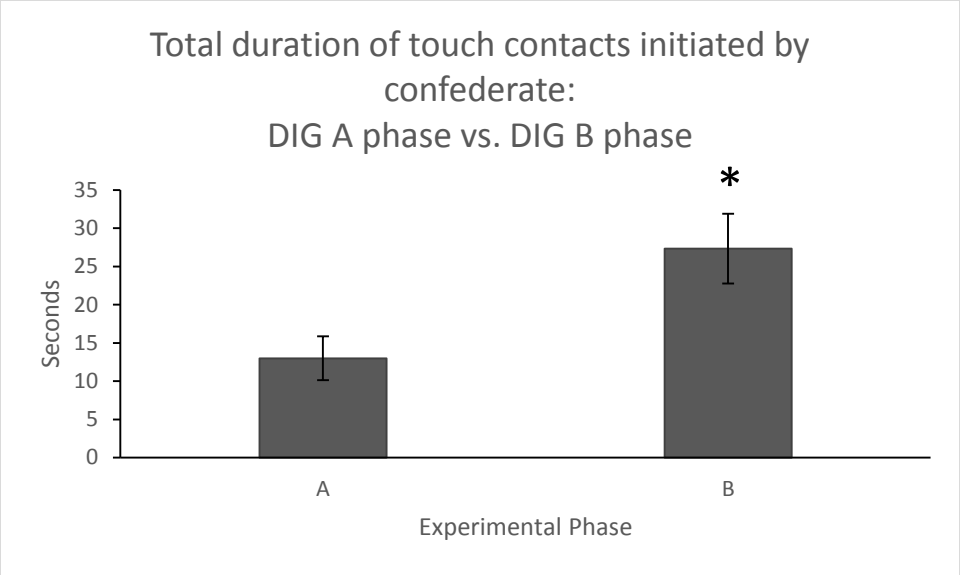


Figure 12. Duration of touch contacts initiated by the confederate animal. This figure shows the difference between duration of touch contacts initiated by the confederate animal when paired with animals with intact fastigial nuclei (A) or paired with that same animal when its fastigial nuclei were inactivated (B). Results are significant at $p<0.05$ level. * $p=0.02$.

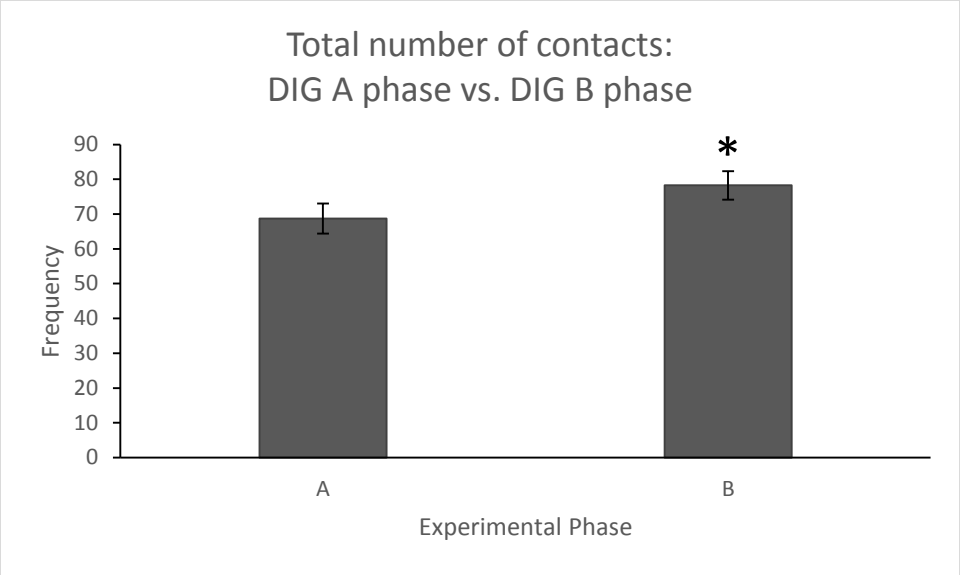


Figure 13. Total number of contacts. This figure shows cumulative total number of contacts in which the two animals participated both when the experimental animal had intact fastigial nuclei (A) and when that same animal's fastigial nuclei were inactivated. This measure is the sum of nose to body, nose to nose, touch, and on top contacts. This measure disregards which animal initiated the contacts and what body parts were involved. Results are significant at $p<0.05$ level. * $p=0.03$.

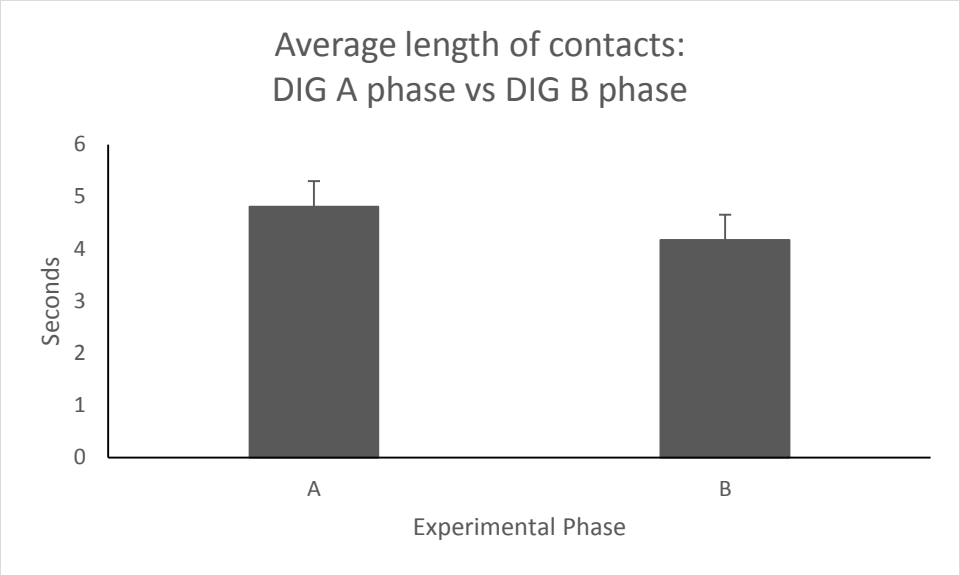


Figure 14. Average length of contacts. This figure shows average length of the contacts the experimental and confederate animal engaged in. This measure was calculated by dividing the total time spent in interaction by the total number of contacts. This measure disregards which animal initiated the contacts and what body parts were involved. Results are trending at p<0.15 level. *p*=0.12.

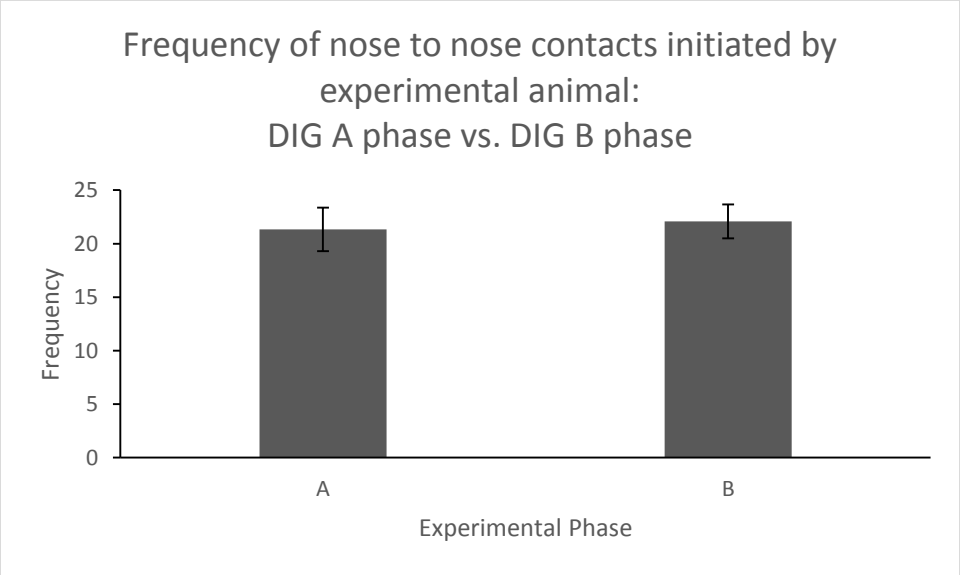


Figure 15. Frequency of nose to nose contacts initiated by the experimental animal. This figure shows the difference between frequency of nose to body contacts initiated by the experimental animal when it had intact fastigial nuclei (A) compared to when it had inactivated fastigial nuclei (B). Results are trending at p<0.15 level. * *p*=0.14.

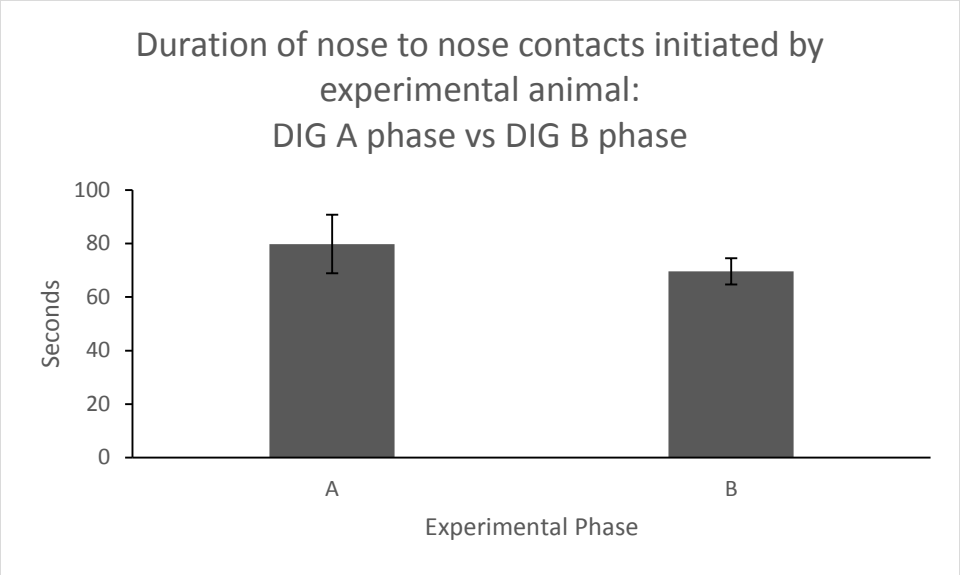


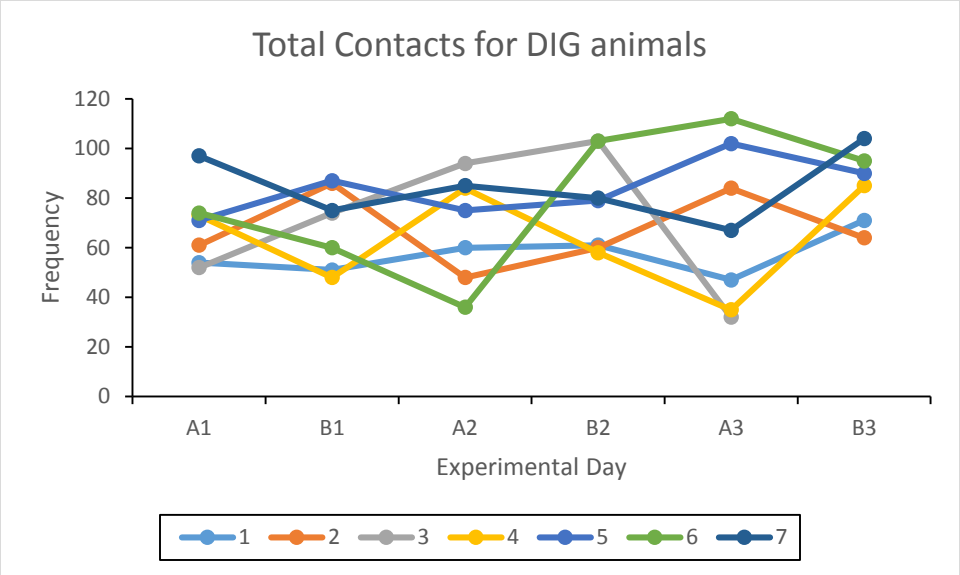
Figure 16. Duration of nose to nose contacts initiated by the experimental animal. This figure shows the difference between duration of nose to body contacts initiated by the experimental animal when it had intact fastigial nuclei (A) compared to when it had inactivated fastigial nuclei (B). Results are trending at $p<0.15$ level. * $p=0.06$.

As in the social interaction chamber, some of the confederates showed fatigue or completely fell asleep during the testing session. Additional analyses were run on data excluding the trials with an inactive confederate. The DIG animals' average A phase data was compared to the SCG animals' average A phase data, the DIG animals' average B phase data was compared to the SCG animals' average B phase data, and the average A phase data of the DIG animals was compared to their own average B phase data. These analyses slightly changed the various *p*-values of the data, but no meaningful differences were shown compared to the analyses with the complete data set.

Visual Analysis

Additionally, data from the social interaction chamber and the open field were graphically represented for the seven DIG animals and examined by visual analysis. This allowed for visual representations of the within-subjects data, both averages and specific data

from individual animals. Visually drastic changes in animal behavior among the baseline and treatment phases in both apparatuses were regarded as a significant effect of the fastigial nuclei's mediation of social interaction. Figure 17 shows the total contacts Drug Inactivation Group (DIG) animals engaged in as well as the average values of the same measure. The graph suggests total contacts were higher when the animals were under the influence of the drug, in the B phase. Figure 18 shows the average length of the contacts made by the DIG animals. Average length of contacts appear higher in the A phase of the experiment, when the animals had fully functioning fastigial nuclei. Figure 19 and 20 show the frequency and duration of nose to nose contacts initiated by DIG animals, respectively. Both frequency and duration are higher in the A phase of the experiment. Finally, Figure 21 shows the duration of touch contacts the confederate animal initiated with the DIG animals. Contacts are longer in the B phase of the experiment, when the DIG animals were under the influence of bupivacaine.



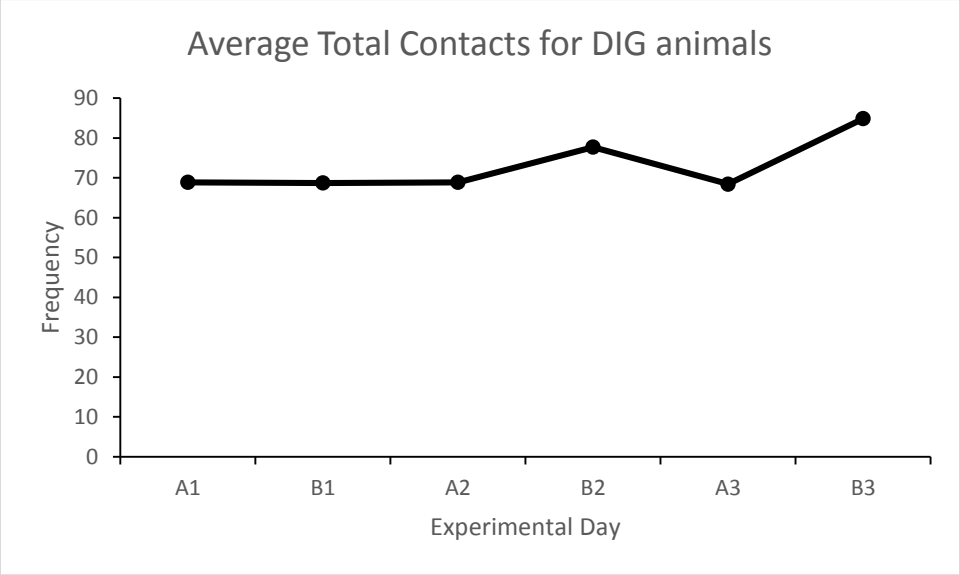
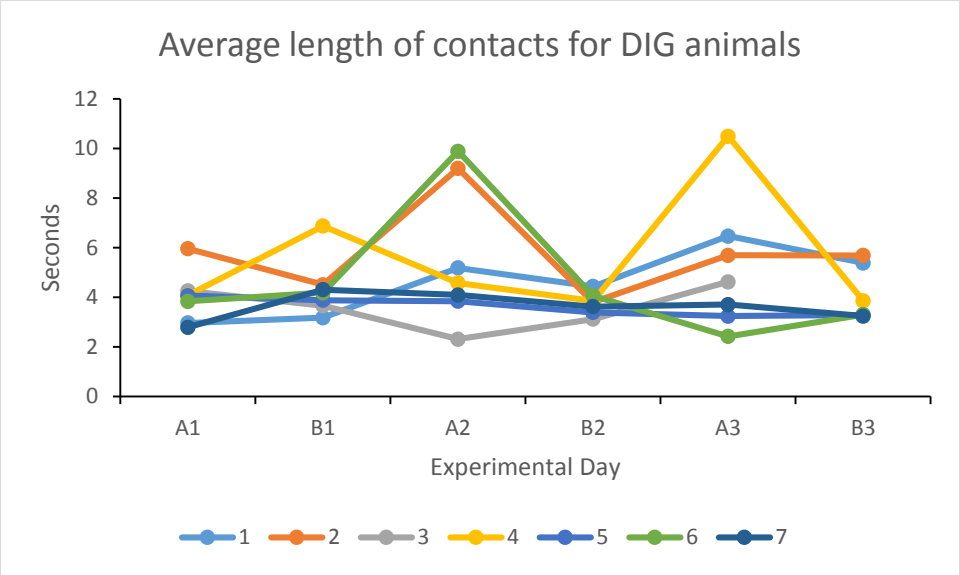


Figure 17. Total contacts for DIG animals and average contacts for DIG animals. The top graph shows each DIG's animal's individual scores for each experimental day. The bottom graph shows the average of the animals' scores to show an overall directional effect. The alternating nature of the lines suggest more contacts in the B phases compared to the A phases.



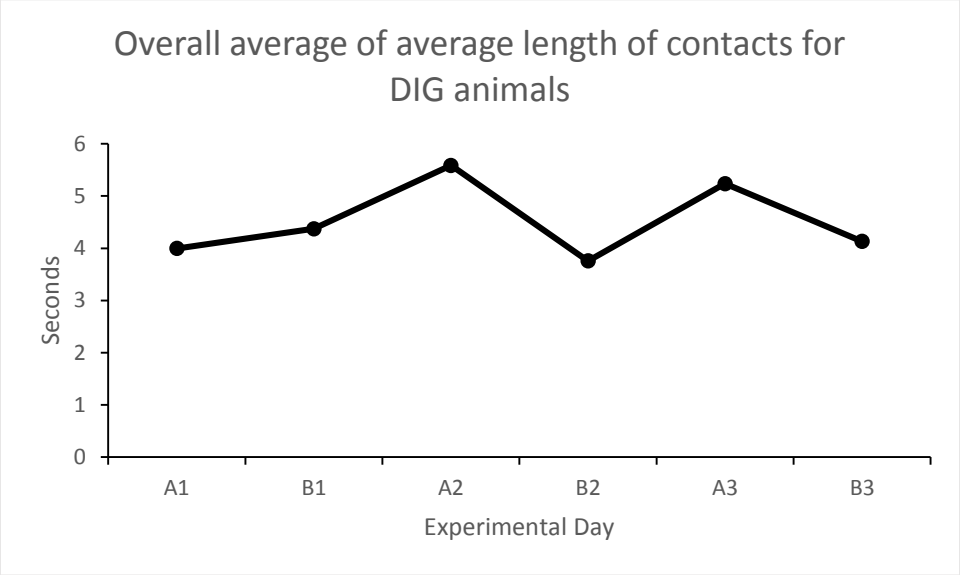
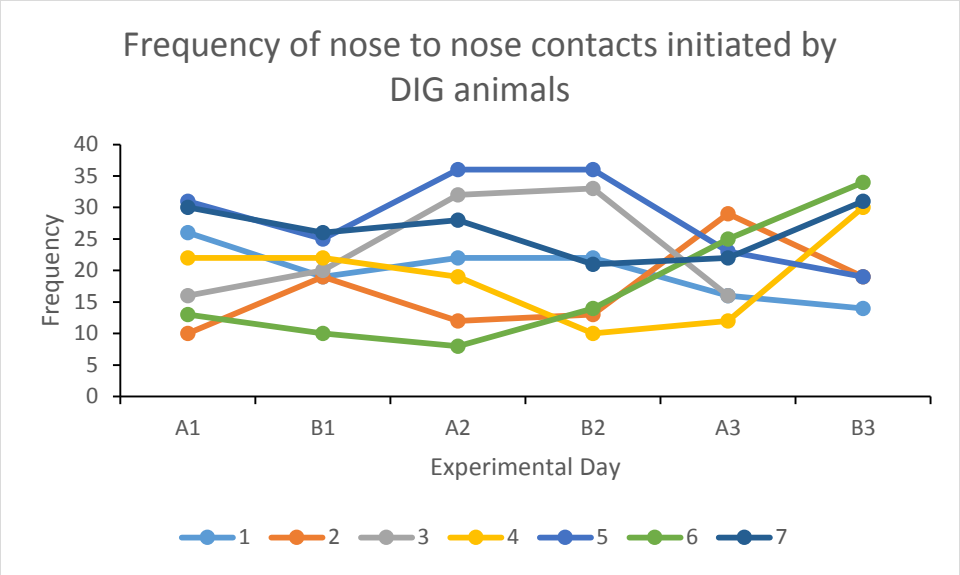


Figure 18. Average length of contacts for individual DIG animals and average length of contacts for DIG animals calculated together. The top graph shows each DIG's animal's individual scores for each experimental day. The bottom graph shows the average of the animals' scores to show an overall directional effect. The alternating nature of the lines suggest longer contacts occurred in the A phases compared to the B phases.



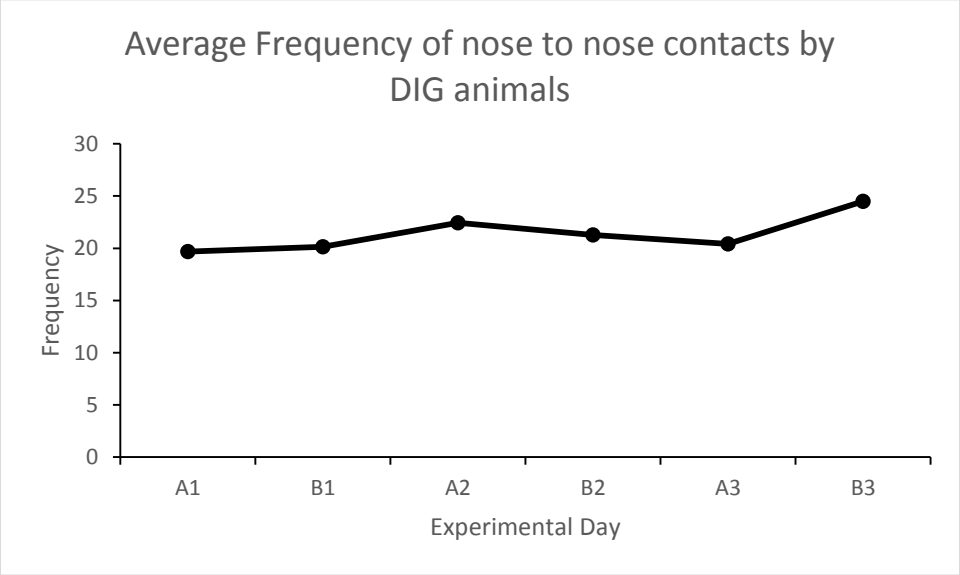
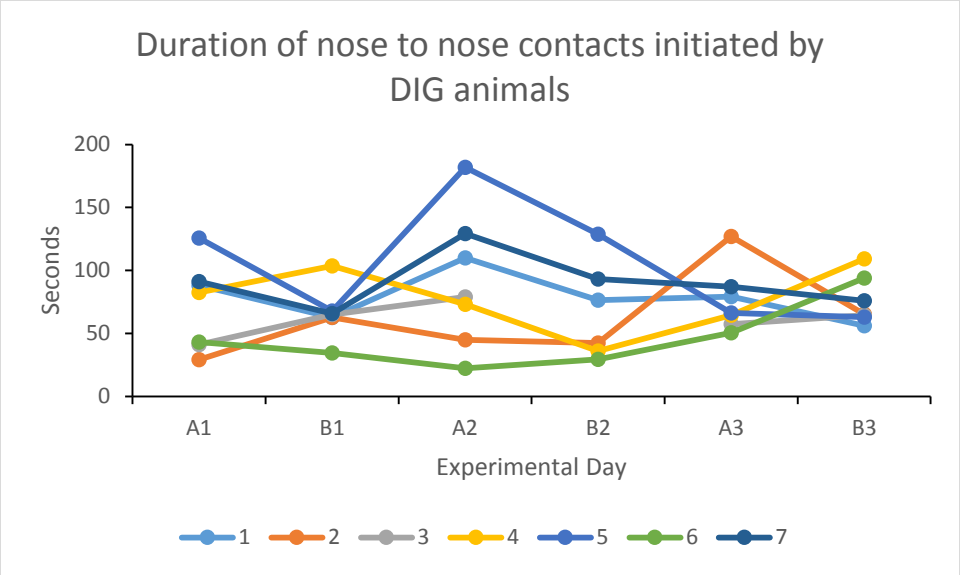


Figure 19. Frequency of nose to nose contacts initiated by DIG animals. The top graph shows each DIG's animal's individual scores for each experimental day. The bottom graph shows the average of the animals' scores to show an overall directional effect. The alternating nature of the lines suggest more nose to nose contacts occurred in the A phases compared to the B phases.



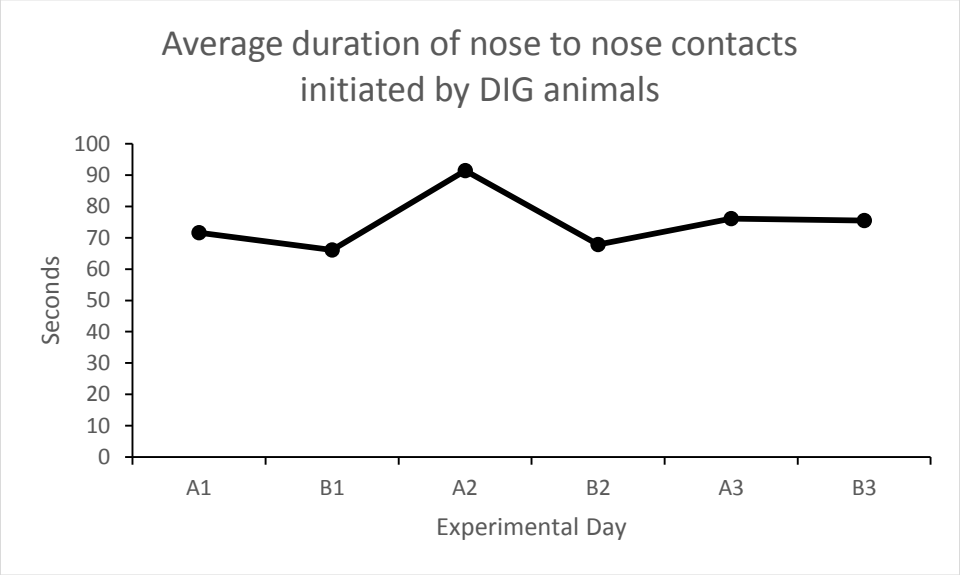
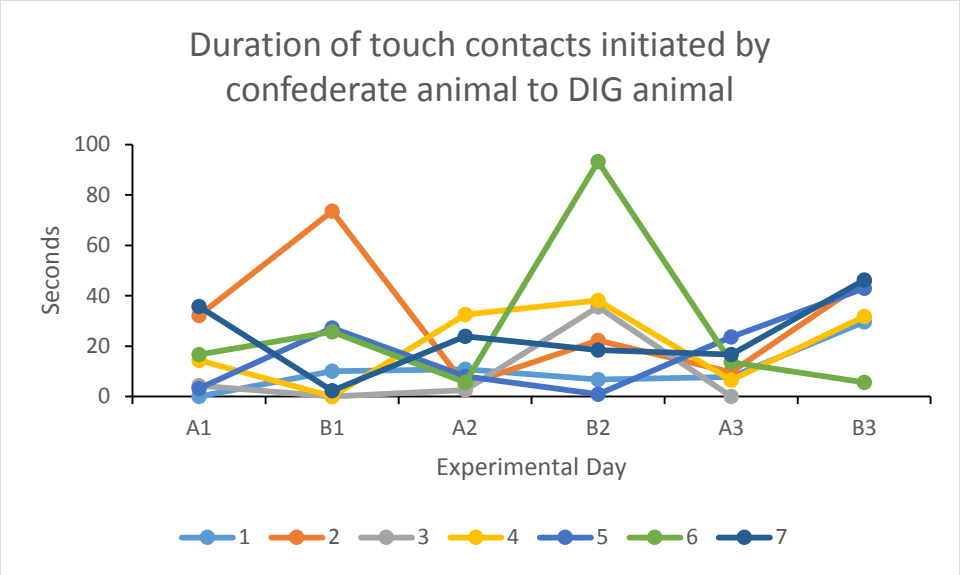


Figure 20. Duration of nose to nose contacts initiated by DIG animals. The top graph shows each DIG's animal's individual scores for each experimental day. The bottom graph shows the average of the animals' scores to show an overall directional effect. The alternating nature of the lines suggest longer nose to nose contacts occurred in the A phases compared to the B phases.



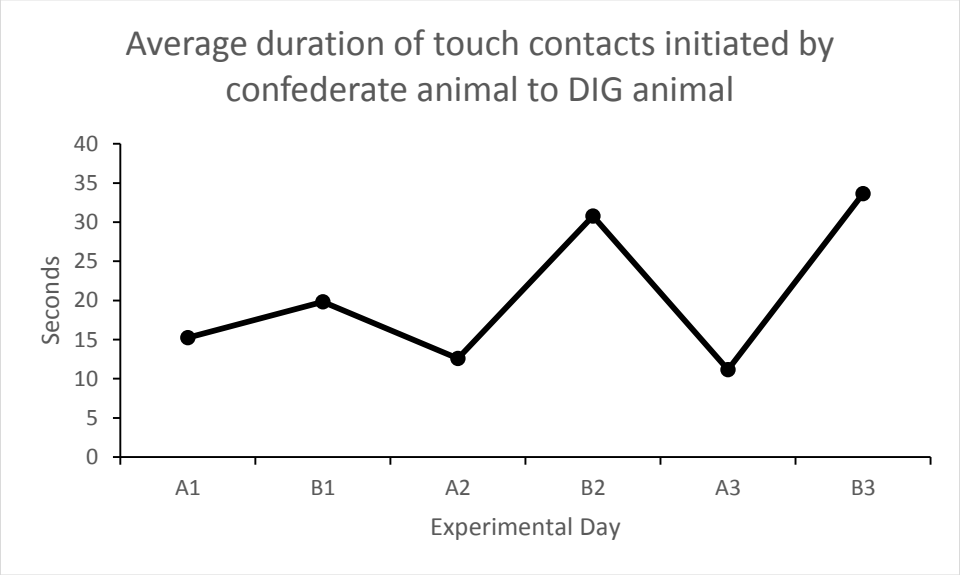


Figure 21. Duration touch contacts initiated by confederate animals to DIG animals. The top graph shows each DIG's animal's individual scores for each experimental day. The bottom graph shows the average of the animals' scores to show an overall directional effect. The alternating nature of the lines suggest longer touch contacts from the confederate occurred in the B phases compared to the A phases.

Discussion

Although the social interaction chamber did not show significant effects among social interaction behaviors, the data from the open field follows the original hypothesis. It was hypothesized that the animals with inactivated fastigial nuclei because of the bupivacaine would exhibit deficient social behaviors similar to social abnormalities seen in people with autism and other similar disorders.

The lack of significant differences in the comparison that examined the average A phases of the Drug Inactivation Group (DIG) animals against the average A phases of the Saline Control Group (SCG) animals supports the hypothesis that the animals in the two groups behaved similarly when not under the influence of bupivacaine, that is, when the fastigial nuclei were functioning normally. This also helped validate the effectiveness of the drug. Since no behaviors were different between groups during the three baseline phases, the differences seen in the B phases can be attributed to the effect of the bupivacaine.

The analysis comparing the average B phases of the DIG animals with the average B phases of the SCG animals posits more substantial conclusions. Significance was found among both the frequency and duration of the nose to body contacts initiated by the experimental animal. There were more and longer contacts initiated with the confederate by the SCG animals than the DIG animals. Contacts involving the animal's nose are likely intense contacts. During the nose to body contacts, the experimental animal is actively sniffing and exploring the confederate animal, which suggests a strong social interest. Because the DIG animals engaged in this behavior fewer times and for shorter durations, it can be concluded that the DIG animals, with inactivated fastigial nuclei, were less likely to participate in intense social contacts than the SCG animals whose fastigial nuclei were fully functioning.

Another finding shows that DIG animals initiated on top contacts with the confederate more frequently and for longer durations than the SCG animals. At face value, this finding seems contradictory of the hypothesis because it indicates more social contact. However, upon further investigation, it can also support the idea that DIG animals are less social. While contacts involving the nose are intense, contacts involving the body are more passive. Thus, the DIG animals are more passively engaging in social contact. Climbing on top of the confederate animal can also be seen as an attempt to stop the confederate rat from initiating more contact. Engaging in on top contacts prevents the confederate animal from generating interactions because he is pinned down. This interpretation suggests the DIG animals are engaging in more and longer attempts to stop social interaction.

The final significant finding of the between-subjects analysis of the B phase data is that the confederate animals engaged in touch contacts more frequently with the DIG animals than they did with SCG animals. While the confederate animals' behaviors are not as telling as the experimental animals' behaviors, this finding provides important information. It suggests the DIG animals were often oriented away from the confederate. If the experimental animal is not facing the confederate, intense contacts involving the nose are impossible. However, the confederate can still initiate touch contacts, as was the case here.

Although demonstrated slightly differently than originally hypothesized, the findings in this between-subjects analysis among B phase data show the DIG animals, who had inactivated fastigial nuclei because of bupivacaine administration, engaged in diminished social behaviors. The intensity of contacts was much lower in the DIG animals, and behaviors to prohibit the confederate from pursuing contact were seen more frequently and for longer duration in the DIG

animals. The additional variables that were approaching significance also support these conclusions.

The within-subjects analysis also provides important information about the behaviors of the experimental animals. The total number of contacts was significantly higher during DIG animals' B phases when compared to those same animals' A phases, meaning they engaged in more contacts while under the influence of bupivacaine compared to when those same animals were given saline. While this seems to oppose the original hypothesis, the average length of those contacts may provide more telling information. The average length of contacts was trending toward significance in that the length of the contacts was longer during the A phase than in the B phase. Taken together, these findings show that with inactivated fastigial nuclei, animals engage in more contacts than when under the influence of saline, but those contacts are shorter. One possible explanation for more but shorter contacts is that the animals with inactivated fastigial nuclei experience social satiation significantly quicker than when the fastigial nuclei are functioning normally. Another interpretation suggests animals with inactivated fastigial nuclei find social contact less reinforcing. Therefore, the quality of the contacts they engage in is poor, and the interaction is broken off more quickly.

Another finding in the within-subjects analysis is that the confederate rat initiated longer touch contacts in the B phase compared to the A phase. In other words, the confederate animal initiated longer touch contacts with the animals when they were under the influence of bupivacaine than with those same animals when they were under the influence of saline. Similar to the interpretation of the previous statistical analysis of the B phase between the DIG and SCG, this may suggest that with inactivated fastigial nuclei, DIG animals are less often oriented toward

the confederate. This may promote the confederate to engage in touch contacts rather than intense contact involving the nose of the experimental animal.

The variables found to be trending towards significance also support the hypothesis. Both frequency and duration of nose to nose contacts initiated by the experimental animal were trending lower in the B phase, supporting the idea that those animals engage in less intense contacts while bupivacaine was actively inactivating the fastigial nuclei. The frequency of touch contacts initiated by the confederate was also trending, which suggests the same idea as the duration of those contacts: the experimental animals' orientation may promote touch contacts from the confederate.

These findings support the hypothesis of the within-subjects analysis that the animals would demonstrate diminished social behaviors while under the influence of bupivacaine than when under the influence of saline. Although the higher total number of contacts found in the B phase does not obviously support the original hypothesis, when taken together with the average length of those contacts, it suggests either a social satiation effect or a lack of quality interactions for the DIG animals.

Contrary to the hypothesis, the within-subjects comparison of the three A phases (A1 compared to A2 compared to A3) in DIG animals showed significant differences in frequency of rears, duration of rears, time in close proximity, total time spent in interaction, and the duration of touch contacts initiated by the confederate. Between which two phases those differences occurred, however, was inconsistent, and only time in close proximity was different among all three days. This data was also run omitting the sessions with a sleeping confederate, and while some values of significance changed, this analysis did not produce a more stable baseline. These baseline inconsistencies may have affected some of the significance values of the within-subjects

data. With a more stable baseline, the effects of inactivated fastigial nuclei may have been more robust and more widespread. Reasons for this inconsistency are unclear, but it is possible that using a different confederate rat during each of the A phases caused this instability. While it is likely that more than three A phases would have established a consistent and stable baseline, the attempt to keep the confederates as much a stranger animal as possible prohibited exposing the confederate to the experimental animal for numerous A phases. This experiment focused on short-term interactions with a stranger animal. Conducting enough baselines to reach a reliably stable baseline would have introduced a strong sense a familiarity between the experimental animals and the confederate, and interactions would need to be analyzed in the long-term.

In the comparison of the three B phases (B1 compared to B2 compared to B3) among the DIG animals, there were no significant findings. This shows the bupivacaine had similar effects each time it was administered. It also suggests the effect of the bupivacaine was robust enough to override any individual differences among the confederate animals.

Overall, regardless of whether the data was analyzed using a between-subjects design or a within-subjects design, the DIG animals engaged in social behaviors thought to repress social interactions when the fastigial nuclei were inactivated. Under the influence of the drug, these animals engaged in less intense contacts, were less often oriented toward the confederate rat, and exhibited more behaviors to stop the confederate from initiating social contacts.

The findings of this experiment support similar previous findings that damage to the fastigial nuclei results in diminished social interaction. Berntson and Schumacher's (1980) study included electrolytic lesions of the fastigial nuclei and subsequent social interaction testing in an open field. They found that animals that had received fastigial nuclei lesions engaged in significantly less social behavior. Their three measures of social interaction included number of

contacts, cumulative duration of contacts, and time spent in close proximity. Each of these variables showed lower scores for the lesioned animals than the sham control animals.

The current study also analyzed total number of contacts, duration of contacts (total time spent in interaction), and time in close proximity, however, the results were not identical to those reported by Berntson and Schumacher (1980). The current study found significantly lower scores in total contacts, but did not find significant differences in total time spent in social interaction or time in close proximity. There are several possible explanations for the differences. First, Berntson and Schumacher (1980) used 40 animals in their study, where the current study used only 14. A larger number of animals can increase the significance of findings. Additionally, it is unclear how long the testing sessions were and whether or not the confederates were stranger animals in Berntson and Schumacher's (1980) study. These potential inconsistencies could have accounted for the varying results. The overall idea, however, is supported: inactivated or lesioned fastigial nuclei result in diminished social behavior.

Additionally, the current study adds important information to the existing literature. Berntson and Schumacher (1980) did not examine specific types of contacts between the experimental animals and the confederate animals. The information reported here describes new findings about the nature of these contacts. Animals with inactivated fastigial nuclei engage in fewer interactions involving their nose. They engage in passive social contacts and attempt to prevent social interaction more often. Intense contacts involving active sniffing and pursuing were usually initiated by the confederate animals. The data suggest the experimental animals showed deficits similar to those seen in people with autism. The experimental rat avoided orientation toward the confederate rat, and people with autism often avoid social contacts with others. The experimental rat sat on top of the confederate in an attempt to stop contacts, just as a

person with autism might engage in behaviors to stop interaction. The experimental rat showed a general disinterest for the intensity of nose to nose and nose to body contacts, just as a person with autism might act toward contacts with the eyes and face.

Another relevant study to this experiment was performed by Bobèe et al. (2000). Bobèe et al. (2000) performed surgery in young rats to completely remove the cerebellar vermis, which houses the fastigial nuclei. Then animals were then allowed to recover and live to adulthood before testing occurred. Once adults, the rats were tested in a social discrimination chamber. This was not a direct measure of social interaction, but rather a measure of whether the animal prefers to visit a familiar territory or a novel territory. The experimental animal occupied the familiar territory before the testing session, and a confederate rat occupied the other side. The confederate rat was then removed, and the experimental animal could spend time in either side. Bobèe et al. (2000) reported that the animals without their cerebellar vermis occupied the unfamiliar territory more often than the control animals, so they had less social inhibition. The idea that the animals without fastigial nuclei were more actively visiting a territory previously occupied by another animal contrasts the previous discussion that damaged or missing fastigial nuclei would induce less social behavior. The contrasting results between the current study and the data discussed in Bobèe et al. (2000) could be explained by the age of the animals at the time of surgery. Bobèe et al. (2000) lesioned very young animals and allowed them to mature, leaving time for developing brain regions to establish compensatory connections and functions. Thus, the results reported may not be directly attributable to the lack of fastigial nuclei function. In addition, the surgical procedure removed the entire cerebellar vermis, which may have allowed the function of the cerebellar cortex to affect the results. In the current study, animals were already adults upon

undergoing surgery, and the pharmacological inactivation specifically targeted the fastigial nuclei.

The social discrimination chamber used by Bobèe et al. (2000), however, sparked the idea for the unique social interaction chamber used in the current study. The unique social interaction chamber was built much like the social discrimination chamber. The biggest difference was that the current study included a confederate rat during the time of testing to allow examination of social interaction. The confederate was restricted by a metal grid barrier to allow the experimental animal to explore unprompted and unthreatened. Instead of testing whether the experimental animal visited an unfamiliar or familiar territory, the experimental animals were observed to see if they spent more time with another animal or by themselves in their own side. The results of the current study's social interaction chamber did not produce significant effects, so a conclusion about whether an animal with inactivated fastigial nuclei is more or less likely to visit a confederate cannot be confirmed or denied. Limitations to the unique social interaction chamber may account for the lack of significance. First, because no bedding – familiar or unfamiliar – was placed in the chamber, it may not have been appropriately obvious to the experimental animal which side was meant to be familiar and which side was meant to be the confederate's area. Also, the confederate was restricted by a metal grid barrier, but the grid was only present on the confederate's side of the chamber. This may have introduced a novel object that distracted the experimental animal and caused it to spend more time by the grid, regardless the confederate animal. This confound could be eliminated by adding an identical grid to the other side of the chamber.

Although numerous studies in the literature have connected damaged or underdeveloped fastigial nuclei of the cerebellum to symptoms of autism, this study reports more exact details

about the specific social abnormalities the fastigial nuclei may mediate. Animals with inactivated fastigial nuclei engaged in significantly fewer and shorter interactions involving their nose than when the fastigial nuclei were intact. This can be generalized to the population with autism. Research has established that people with autism often avoid eye contact if another person initiates it (Akshoomoff, 2005), and they exhibit reduced attention to the eyes of people they look at (Dawson, Webb, & McPartland, 2005). Additionally, people with autism show abnormalities in attention and attentional facial processing (Allen & Courchesne, 2005). Each of these ideas suggests people with autism shy away from and infrequently initiate eye contact, which can be defined as an intense social contact. Similarly, the animals with inactivated fastigial nuclei in this study avoided intense social contacts.

This study's results also showed animals with inactivated fastigial nuclei were less often oriented toward the confederate rat, prompting the confederate rat to make touch contacts rather than nose to nose or nose to body contacts. People with autism also show deficiencies in social orientation. This symptom of autism is exhibited as a lack of visual orientation toward social stimuli. A study by Dawson, Meltzoff, Osterling, Rinaldi, and Brown (1998) showed that people with autism exhibited an inferior ability to orient themselves or react to social stimuli – such as their name being called or someone clapping their hands – than they did toward nonsocial stimuli, like a rattle or jack-in-the-box.

The final interpretation of the results of this study pertains to preventative behaviors. The Drug Inactivation Group (DIG) animals engaged in more and longer on top contacts, which suggests those animals were attempting to stop social interactions with the confederate animal. Individuals with autism sometimes engage in preventative behaviors when confronted with social interactions. Although reasons for engaging in these behaviors vary drastically among

people with autism, one possible explanation is an attempt to escape demands of the situation (Butler & Luiselli, 2007). If the situation proves uncomfortable or aversive to the individual because it demands social contact, behaviors to stop the interactions may occur.

Results from this study suggest animals with inactivated fastigial nuclei can serve as a model for some of the abnormal social behaviors seen autism. The findings demonstrate similar social deficits to those seen in autism, such as avoiding intense social contacts and prohibiting further social contacts from stranger animals. In conclusion, the fastigial nuclei mediate the quality and intensity of social interaction and play an important role in normal social functioning.

Future research should examine whether the fastigial nuclei themselves or their connections and pathways are responsible for these social deficits. Because the fastigial nuclei are highly connected to other brain regions through various pathways, it is possible an entire circuit or another brain region in conjunction with the fastigial nuclei contributes to the social deficits reported here. Studies inactivating various parts of the fastigial nuclei's circuitry would add important information to the literature on how the cerebellum functions in relation to the rest of the brain.

The new knowledge provided from this study that the fastigial nuclei mediate intense social interactions can also prompt research in the autism realm. The understanding that fastigial nuclei abnormalities may alter social behaviors can lead to more focused diagnostic imaging, giving researchers and diagnosticians a specific brain region to examine for damage. Additionally, identifying a specific function of the fastigial nuclei can shine light on which other brain regions may have a similar function and could compensate for atypical development or damage to the fastigial nuclei if that damage is identified early in a child's development. Finding

a way to promote these compensatory functions of other brain regions may become a key component to autism treatments, but more research must be conducted.

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