THE ANTINOCICEPTIVE ROLE OF THE ANTERIOR INTERPOSED NUCLEUS OF THE CEREBELLUM

by

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ABSTRACT

THE ANTINOCICEPTIVE ROLE OF THE

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The cerebellum has been extensively studied for its role in pain. It is safe to say that the cerebellum has a modulatory role in pain. It is active during various types of pain, but it also plays a role in inhibiting pain. Its overall contribution to pain has yet to be defined. This study was performed to contribute to knowledge pertaining to the cerebellum's role in pain. Specifically, the role of the interposed nucleus in descending inhibition was of interest.

To test the role of the interposed nucleus in descending inhibition, single-unit extracellular electrophysiological recordings were collected to observe dorsal horn neuronal responses to mechanical stimuli (brush, pressure, pinch) with and without electrical stimulation first in the left and then in the right hemisphere of the intermediate cerebellar cortex. This area inhibits nociceptive spinal neuronal responses to mechanical stimuli; therefore, recordings of neuronal responses were taken before and after 1 of 4 drugs was microinjected into the left interposed nucleus. Drugs used in the study were ACSF, GABA, lidocaine, and bicucilline. The results suggest that the interposed nucleus may have a nociceptive contribution to pain modulation.

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CHAPTER 1

INTRODUCTION

The cerebellum has been continuously found to be involved in pain. In spite of this consistent observation, the role the cerebellum plays in pain has remained elusive. Several studies have suggested the cerebellum to have a pronociceptive role, while others have implied an antinociceptive role. Pain is a complex event that can lead to tremendous suffering. Uncovering the physiological mechanisms that lead to pain will aid in discovering more effective tools for its treatment. Descending inhibition is a necessary function of life that gates nociceptive spinal signals allowing organisms to function normally. The better we understand descending inhibitory mechanisms, the better equipped we will be treating and coping with pain. The goal of this study was to further explore the cerebellum's role in pain and to provide some definition to the neurophysiology of its involvement.

1.1 Descending Inhibition is Adaptive

Pain is a multidimensional experience with three major components: sensory, affective, and cognitive appraisals (Melzack & Casey, 1968). Both pain and its inhibition are necessary adaptive components of survival. Without pain, there is no perception of noxious stimuli, leaving an organism vulnerable to all forms of harm. Congenital insensitivity to pain is a rare disease that is extremely maladaptive, due to self-mutilating tendencies from no sensation of pain (Gatchel et al., 2007; Chatrian et al., 1975). Without pain, there is no protective signal warning an organism that there is a tissue insult. Congenital insensitivity to pain often results in death unless constant intervention is present to ensure that an individual is protected against his or herself. This disease illustrates just how important the perception of pain is to human survival.

On the other hand, many people suffer from chronic pain. Chronic pain is considered to be a disease, since there is pain in the absence of noxious stimulation. The pain persists and is often very debilitating. It is a huge economical drain, and pain is the primary reason for seeking medical care (Gatchel et al., 2007). There is currently no cure for the many chronic pain syndromes that exist. Even treatments are often ineffective, which means that there is a desperate need for improvement. In order to improve treatments, research is done to broaden our basic understanding of both acute and chronic pain. Understanding normal pain gives a better understanding of how chronic pain develops and how to reverse the disease state. The ultimate goal for treating or curing chronic pain is finding a way to inhibit the unnecessary pain.

Regular inhibition of pain then is also a necessary adaptive component of survival because without it, we would be more susceptible to chronic pain. To date, many antinociceptive brain sites have been discovered. Electrical stimulation in each of the following brain regions leads to inhibition of nociception in dorsal horn spinal projection neurons: anterior cingulate cortex (Senapati et al., 2005a), primary motor cortex (Senapati et al., 2005b); primary somatosensory cortex (Senapati et al., 2005c); medial septum diagonal band of Broca (Hagains et al., 2011a); and the cerebellum (Hagains et al., 2011b). Activity in these brain regions occurs to produce some function other than analgesia, but analgesia appears to be a secondary function related to each site. Having an inhibitory function would assist an organism in performing regular and necessary functions in spite of pain. This would help to minimize the suffering associated with an injury so that an organism is not so impaired that it cannot continue to gather food or perform any essential daily behavior.

It is hypothesized that the aforementioned brain regions have their antinociceptive effect through their connections to the brain stem, more specifically the periaqueductal grey (PAG). The PAG is a key component in the phenomenon descending inhibition (Millan, 2002; Basbaum & Fields, 1984). Descending inhibition occurs when supraspinal structures exert inhibitory control over spinal nociception, as mentioned above. It is possible that chronic pain

may be a product of the malfunction of descending inhibition. Without descending inhibition, there would be a greater amount of nociceptive activity. For example, there is tonic descending inhibition of many nociceptive neurons in the spinal cord (Laird & Cervero, 1990; Millan, 2002). Without tonic descending inhibition or evoked descending inhibition, nociception is left unchecked and may result in a chronic pain state. During an injury, injury-related spinal neurons are sensitized by a neuroprotective mechanism to guide behavior to guard the injury. This mechanism is known as central sensitization (Millan, 2002). Long-term potentiation (LTP) is the phenomenon responsible for the development of central sensitization. LTP occurs when calcium enters the cell and changes the gene expression of the cell. The new phenotype leads to a more potentiated or sensitive cell. All of this happens when there is an abundance of cell activity (increased membrane current), which happens in the spinal cord when there is noxious stimulation. Descending inhibition could be a countermeasure that returns cells to a non-potentiated/normal state by reducing the membrane current and preventing the influx of calcium. Without descending inhibition, cells would be easily sensitized; they would also never be inhibited following sensitization, which would likely lead to a pain syndrome. Therefore, it is imperative that descending controls are better understood to try to look for new solutions to treating chronic pain and understanding pain in general.

1.2 Functions of the Cerebellum

The cerebellum has a clear role in motor activity. A large amount of evidence of the cerebellum's importance in motor activity comes from clinical lesion studies that have demonstrated impairments in keeping time (Ivry & Keele, 1989), rhythmic motor sequences (Bengtsson, Ehrsson, Forssberg, & Ullen, 2004), posture and gait/medial and intermediate damage (Timmann, Brandauer, Hermsdorfer, Ilg, Konczak, Gerwig, Gizewski, & Schoch, 2008), limb movements/lateral and intermediate damage, and occulomotor ability/medial damage (Timmann et al., 2008).

Traditionally the cerebellum has been considered a region of the brain responsible for processing a wealth of sensory information for the purpose of aiding movement via connections with the motor cortex; while there is some truth to this idea, it does not appear to be the entire story of the cerebellum (Strick, Dum, & Fiez, 2009). It apparently has many functional roles: motor learning (Doyon et al., 2002; Doyon & Benali, 2005; Mason & Iversen, 1977), pure cognitive function (Fiez, 1996; Glickstein & Doron, 2008), and antinociception (Dey & Ray, 1982; Siegel & Wepsic, 1974), as well as pronociception (Chambers & Sprague, 1955; Chambers & Sprague, 1975; Saab et al. 2001; Saab & Willis, 2003). Stimulation of the medial nucleus or superior peduncle in cats elicits feeding and grooming behaviors (Berntson, Potolicchio, & Miller, 1973; Reis, Doba, & Nathan, 1973). Stimulation of the medial nucleus can also produce predatory attack, whereas bilateral lesions of the medial nuclei do not produce changes in motor output (Reis, Doba, & Nathan, 1973). The cerebellum is important for the production of speech as evidenced by imaging studies (Petersen, Fox, Posner, Mintun, & Raichle, 1988) and deficits in speech associated with cerebellar damage (Holmes, 1939). Damage to the cerebellum has resulted in cognitive deficits known as cerebellar cognitive affective syndrome (Schmahmann & Sherman, 1998); however, clinical studies of patients with cerebellar damage have been inconsistent, probably due to the nature of brain damage, and need to be explored with more experimentally controlled approaches (Strick, Dum, & Fiez, 2009). More scrutiny has also been encouraged to try to rule out motor confounds when studying the cerebellum, since most any or all tasks measuring cognitive functions will require some type of motor activity (Strick, Dum, & Fiez, 2009). So then the cerebellum's contribution to these activities is still being studied to develop a more comprehensive understanding of its non-movement functions.

1.3 Anatomy of the Cerebellum

The basic components of the cerebellum consist of the cerebellar cortex and the deep cerebellar nuclei (DCN) (Figure 1). Incoming signals project to their respective sites in both of

these regions. These signals first activate the DCN and then continue into the cortex where ultimately a Purkinje cell is activated. The Purkinje cells are the only output of the cerebellar cortex. They have inhibitory projections to their respective DCN. There are four other neurons that make up the cerebellar cortex: stellate cells, basket cells, Golgi cells, and Granule cells. The majority of cerebellar output comes from the DCN, which includes the dentate, fastigial, and interpositus nuclei. The only other cerebellar efferents are those that project from the cerebellar

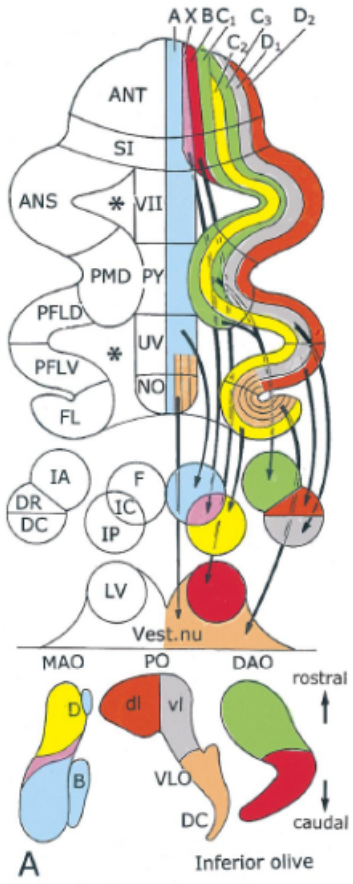

Figure 1. Diagram of the organization of the connections of the cerebellar cortex and recpective DCN (Voogd & Glickstein, 1998, reproduced without permission). The cerebellar cortex is represented by A and B (vermis), C1-C3 (intermediate hemisphere), and D1 and D2 (lateral hemisphere). Abbreviations: ANS, ansiform lobule; ANT, anterior lobe; bc, brachium conjunctivum; cr, restiform body; D dorsomedial cell column; DC, dorsal cap; dl, dorsal leaf; DR,rostromedial dentate nucleus; F, fastigial nucleus; FL, flocculus; IA,anterior interposed nucleus; IC, interstitial cell group; IP, posterior interposed nucleus; LV, lateral vestibular nucleus; MAO, medial accessory olive; PFL (D/V), (dorsal/ventral) paraflocculus; PMD, paramedian lobule; PO, principal olive; SI, lobules simplex; vl, ventral leaf; VLO, ventrolateral outgrowth; X, X zone.

cortex to the vestibular nuclei in the medulla, which are functionally similar to the DCN.

The cytological arrangement of the cerebellar cortex begins with incoming projections from climbing fibers and mossy fibers, both of which are excitatory (Eccles, Ito, & Szentagothai, 1967). These cells are not considered to be part of the cerebellar cortex; rather they are the main source of input. The climbing fibers project directly to Purkinje cells making many synapses by wrapping around the arborous dendritic branches; each cell's terminations reach no more than 10 Purkinje cells (Shinoda, Sugihara, Wu, & Sugiuchi, 2000; Scheibel & Scheibel, 1954) and in many cases wrap only one Purkinje cell (Cajal, 1911) giving each climbing fiber a strong and narrow input to their targets producing a complex spike (Eccles, Llinas, & Sasaki, 1966a). The mossy fibers on the other hand project to granule cells, the only excitatory neurons of the cerebellar cortex. Granule cells bifurcate along the surface of the cortex to form axons known as parallel fibers; parallel fibers travel long distances making synapse with multiple Purkinje cells spreading a smaller activation, known as simple spikes, across a greater range of cells with combinations of EPSPs and IPSPs (Eccles, Llinas, & Sasaki, 1966b). It is important to note that each of these incoming pathways ultimately excites Purkinje cells, which are the only output of the cerebellar cortex. Purkinje cells are GABAergic interneurons that descend on and inhibit their respective DCN. Here the focus will be on the intermediate hemisphere of the anterior cerebellar cortex. It is the DCN that is connected to the anterior interposed nucleus.

1.4 Pain & the Cerebellum

When discussing the activation of various brain sites during the perception of pain, it is difficult to isolate the way that each individual site is contributing to that experience; this is in part because it is possible that a brain area is active to antagonize or reduce the pain, even during pain perception. It is therefore necessary to carefully isolate each area's contribution.

The cerebellum consists of multiple sub-regions that may contribute to the different components of pain and may also have analgesic properties. It is well established that the cerebellum is active during nociception and the perception of pain (Saab & Willis, 2003;

Moulton, Schmahmann, Becerra, & Borsook, 2010); on the other hand, the role it plays in the modulation of pain has been very difficult to clarify.

Early studies of pain involving the cerebellum demonstrated that the intermediate hemisphere of the anterior lobe was active during nociceptive stimulation of A-delta fibers and C fibers (Ekerot, Gustavsson, Oscarsson, & Schouenborg, 1987) and that firing in climbing fibers synchronized in response to similar cutaneous stimulation (Ekerot, Oscarsson, & Schouenborg, 1987b). Electrical stimulation of the saphenous nerve at C-fiber intensity can evoke cerebellar cortical potentials bilaterally (Ekerot et al., 1987a; Ekerot et al., 1987b; Ekerot et al., 1991a; Ekerot et al., 1991b; Wu & Chen, 1990). Cerebellar cortical neuronal activity can be triggered by both visceral and somatic stimulation (Saab & Willis, 2001). Imaging studies too have consistently reported the cerebellum to be active during the perception of pain for humans (Casey et al., 1996; Casey et al., 1994; Svensson et al., 1997; Ingvar & Hsieh, 1999). An fMRI study observed an activation of the ipsilateral posterior portion of the cerebellar cortex in anticipation of painful heat stimulus versus bilateral activation of the anterior cerebellar cortex in response to pain itself (Ploghaus, Tracey, Gati, Clare, Menon, Matthews, & Rawlins, 1999). Although it was not specified, it appears that the activity was limited to the vermis and intermediate areas, which are mostly responsive to sensory processing. The cerebellum plays some role in empathic responses to pain. In one study hemodynamic responses were recorded while painful shock was applied to the hands of females' romantic partners. The lateral cerebellum ipsilateral to the shock was singled out as part of the empathic neural response (Singer, Seymour, O'Doherty, Kaube, Dolan, & Frith, 2004). Given the connection of the lateral cerebellum with the cortex and forebrain, it may play a role in the cognitive/emotional processes of pain; however, the specifics of this role have remained undefined. The cerebellum has also been suggested to be involved in the empathic response to pictures of depictions of people accidentally hurting themselves, although there is no indication of where the activity in the

cerebellum occurs (Jackson, Meltzoff, & Decety, 2005). These studies taken together illustrate the need to consider further whether or not the cerebellum has a cognitive contribution to pain.

Many imaging studies have found cerebellar activation in response to noxious stimuli. A PET study using a noxious heat stimulus to the forearm observed cerebral blood flow changes in the ipsilateral vermis, as well as the contralateral thalamus, cingulate cortex, primary and secondary somatosensory cortices, and insula and also the ipsilateral secondary cortex, thalamus, and medial dorsal midbrain (Casey, Minoshima, Berger, Koeppe, Morrow, & Frey, 1994). A common inference made concerning such an observation is that the cerebellar activation was due to a motor preparation as would be expected in a situation involving pain; however, other premotor sites, especially the premotor and supplementary motor cortices, showed no change in the aforementioned study. It may not be reasonable in that case then to assume such a contribution. Another study imaged brain responses to a "moderately hot" stimulus (43.5°-49.5° C) to produce moderate pain i n awake, mildly sedated, moderately sedated, and anesthetized participants. They observed similar activations to that of the previously described study plus activation in the primary motor cortex; after the subjects entered into a state of unconsciousness, the cerebellum continued to respond to the heat stimulus (Hofbauer, Fiset, Plourde, Backman, & Bushnell, 2004). Some subjects in this study moved in spite of being unconscious, which makes it difficult to say what the cerebellum's role was; additionally, absence of movement does not necessarily mean absence of all neural motor activity. And although it has been concluded that this finding is evidence that the cerebellum is not necessary for conscious perception of pain (Moulton, Schmahmann, Becerra, & Borsook, 2010), this type of assertion may be overstated since perception of an event does not always come with a report. For example, anterograde amnesia patients could experience pain and fail to report it five minutes later; likewise, anesthesia inhibits the formation of memory and presumably the ability to report perception. A PET study with an innocuous/noxious discrimination element evoked activation in the vermis with warm (43° C), hot (50° C), and cold

(6° C) stimuli; however, this study observed bilate ral activation of the premotor cortex making the contribution of the cerebellum appear as if it could be motor again (Casey, Minoshima, Morrow, & Koeppe, 1996). Another two noxious stimuli of interest have been muscle pain and acute cutaneous pain from stimulation by a laser (Svensson, Minoshima, Beydoun, Morrow, & Casey, 1997). Similar patterns of activation were recorded for these types of noxious stimuli that also included the ipsilateral vermis. Cerebellar activity from the ipsilateral vermis, lateral anterior lobe, and bilateral DCN was recorded by PET in response to capsaicin injection, but no cerebellar activity was detected in response to a noxious brush stimulus (allodynia) except in the ipsilateral lateral nucleus (Iadarola, Berman, Zeffiro, Byas-Smith, Gracely, Max, & Bennet, 1998). The difference between the cerebellar responses to capsaicin versus allodynia raises interest. If cerebellar activity is a motor response, allodynia should produce similar withdrawal and/or motor urge regardless of the type of pain. This further complicates the cerebellum's role in pain.

Many studies suggest that the cerebellum has an antinociceptive influence. Electrical stimulation in the intermediate anterior lobe areas and related rostral lateral-interpositus nuclear-brachium conjunctivum regions in the cerebellum produces a higher pain threshold for tail shock in squirrel monkeys (Siegel & Wepsic, 1974). A reduction of the excitatory effects of dorsal root stimulation was also found in cats when the anterior cerebellar vermis or lateral nucleus was stimulated (Hagbarth & Kerr, 1954). In an interesting rat study, either electrical stimulation or microinjecting morphine into the anterior cerebellar cortex induced analgesic effects in rats (Dey & Ray, 1982). Intraperitoneal injection of naloxone attenuated the analgesic effect of cerebellar cortex morphine injections; additionally, lesions of the culmen area of the anterior cerebellar cortex attenuated the analgesic effects of systemic morphine (Dey & Ray, 1982). Many of these cerebellar sites overlap with the aforementioned sites that respond to noxious input.

Part of the analgesic affects appears to be due at least in part to a descending spinal mechanism. We too found that stimulation of either the vermis or the intermediate hemisphere of the anterior cerebellar cortex in rats produces inhibition of nociceptive spinal activity (Hagains, Senapati, Huntington, He, & Peng, 2011). The intermediate cerebellar cortex descends to the anterior interposed nucleus via Purkinje cells, which inhibits the interposed nucleus by releasing GABA. The interposed nucleus is rich with $GABA_A$ receptors (Ito, 1984) and has mostly glutamatergic projections (Nieuwenhuys, Voogd, & Huijzen, 2008). Therefore the release of GABA into the interposed nucleus would inhibit its output. The current study was designed with these facts in mind and was modeled after our previous study in order to investigate the pathway of descending inhibition produced by electrical stimulation of intermediate cerebellar cortex.

Understanding the antinociceptive mechanism involved in cerebellar stimulation would help unravel the mystery of the cerebellum's role in pain. The intermediate hemisphere of the cerebellar cortex contributes to sensory processing from the limbs. Stimulation of the intermediate hemisphere leads to direct and indirect excitement of Purkinje cells, which project to the interposed nucleus (Voogd & Glickstein, 1998). In turn, the interposed nucleus sends projections to brainstem structures (Teune et al., 2000) that are related to descending spinal antinociception. It is expected that the analgesic effect by cerebellar activation is through midbrain structures that activate the descending inhibitory system. In fact, the ipsilateral ventral PAG projects to the inferior olive (Rutherford, Anderson, & Gwyn, 1984), so there may be a reciprocal or looped relationship with the descending system.

The anterior interposed nucleus also has projections to the spinal trigeminal nucleus, parvocellular reticular nucleus, anterior pretectal nucleus, magnocellular red nucleus, and a minor projection to the PAG (Teune et al., 2000). The projections from the anterior interposed nucleus to various structures in the brainstem were demonstrated by injection of anterograde tracers, *Phaseolus vulgaris Leucoagglutinin* (PhaL) or biotinylated dextran amine (BDA). These

targeted brainstem structures are widespread, including structures that are involved in the descending inhibitory system (Fields & Basbaum 1984), such as (1) lateral, medullary, parvocellular, lateral paragigantocellular, and giantocelluar reticular nuclei in the medulla oblongata (Fields et al., 1975); (2) red nucleus (Basbaum & Fields, 1979; Huisman et al., 1981; Liu et al., 1991), parabrachial nucleus (Chiang et al., 1994; Chiang et al., 1995; Meng et al., 2000; Terenzi et al., 1992), dorsal raphe nucleus (Fields et al., 1977; Follett & Gebhart, 1992), periaqueductal gray (Reynolds 1969), and pretectal nucleus (Rees et al., 1995; Rees & Roberts, 1993; Villarreal et al., 2004; Wilson et al., 1991) in the mesencephalon; (3) lateral and dorsal hypothalamus area (Condés-Lara et al., 2008; Condés-Lara et al., 2009; Cox & Valenstein, 1965; Holden et al., 2005; Holden et al., 2009; Holden & Pizzi, 2008; Lopez et al., 1991), and thalamic nuclei (Yang & Follett, 2003) (Duncan et al., 1998; Gybels, 2001; Hosobuchi et al., 1975; Marchand et al., 2003; Owen et al., 2006; Owen et al., 2007) in the diencephalon.

As illustrated above, the cerebellum's role in pain has remained very elusive. The cerebellum is clearly an important structure to producing movement. Movement can be counterproductive to healing and tends to produce pain with injury-related movements. It would make sense then if the cerebellum contained a facilitative nociception mechanism in order to promote healing by discouraging movement. It is possible then that stimulating the cerebellar

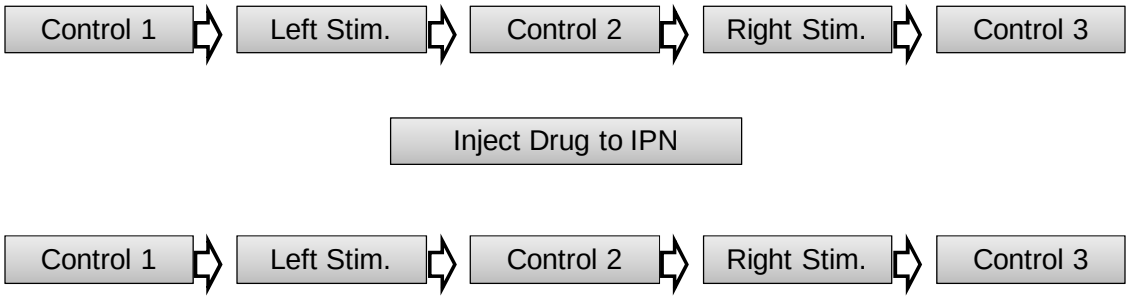

Figure 2. Flowchart summarizing the order of treatments. Each block on the top and bottom rows represent *brush, pressure, and pinch* events with (Left or Right Stim.) or without (Control) electrical stimulation to the indicated side of the intermediate hemisphere of the cerebellar cortex. The top row is the first series of *brush, pressure, pinch.* A drug was injected following the completion of the first series indicated as *Inject Drug into IPN*. The bottom row is the second series (same as the first), which occurred following drug injection.

cortex is inhibiting nociception because it is inhibiting cerebellar output. It was ***hypothesized*** that inhibiting the anterior interposed nucleus would inhibit dorsal horn nociceptive activity. To test this hypothesis, the following *specific aim* was addressed.

Specific aim: Determine the circuitry and neurotransmitters involved in antinociception produced by stimulation of the anterior cerebellar cortex. This was achieved by microinjecting lidocaine, GABA, or bicucilline into the anterior interposed nucleus; dorsal horn neuronal responses to mechanical stimulation with and without electrical stimulation of the left and right intermediate cerebellar cortex were recorded before and after a drug injection (Figures 2 & 3). It was expected that the temporary lesion produced by lidocaine would enhance the inhibitory effect produced by electrical stimulation of the cerebellar cortex from the right side but not the left. GABA was also expected to enhance the inhibitory effect of electrical stimulation of the cerebellar cortex for stimulation on either side; however, bicucilline was expected to attenuate descending inhibition by antagonizing $GABA_A$ receptors in the anterior interposed nucleus.

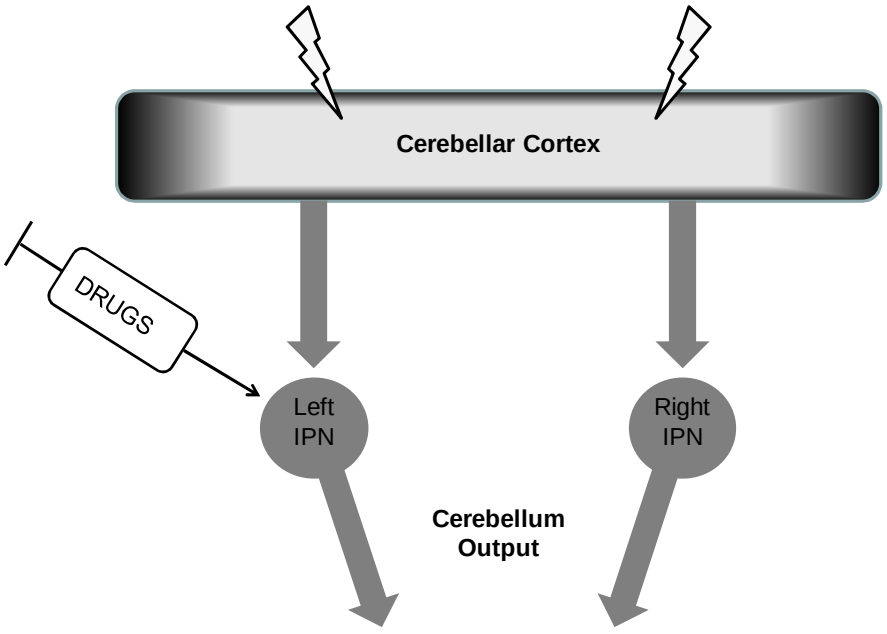

Figure 3. Anatomical representation of experimental design. This diagram illustrates how the left side of the cerebellum is manipulated while leaving the right side unchanged. IPN: Interposed Nucleus

CHAPTER 2

METHODS

Twenty-three rats were used in this experiment. All surgical procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee. The procedures were in accordance with the guidelines published by the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmerman, 1983).

2.1 Animal Preparation

Animals were anesthetized with an injection of sodium pentobarbital (50 mg/kg, ip). A laminectomy was performed to expose 3-4 cm of the spinal cord over the lumbosacral enlargement. A tracheotomy was performed in case of a need for artificial. A jugular vein cannulation was used to provide a consistent and constant administration of pentobarbital at a rate of 1.2 ml/hr (50 mg/kg). A craniotomy was performed to expose the skull for stereotaxic procedures. Following surgeries the spinal cord was fixed in a stereotaxic frame, the dura was removed, and mineral oil was poured over the spinal cord to preserve its moisture. The head too was fixed in the stereotaxic frame. Body temperature was monitored and maintained at 37℃ with a feedback controlled heating pad and a r ectal thermal sensor probe.

2.2 Stereotaxic Procedures

First bipolar electrodes were placed into both the left and right (one electrode for each side; Figure 3) intermediate hemispheres of the cerebellar cortex (12 mm caudal from Bregma; 2 mm lateral; and 1 mm deep). These two electrodes were used to deliver electrical stimulation via a Grass Stimulator at 5 volts, 300 Hz, and 0.5 ms duration, according to previous results (Hagains et al., 2011).

Next a 23 gauge guide cannula equipped with a dummy-cannula was inserted into the interposed nucleus (11.5 mm caudal from Bregma; 2.5 mm lateral left; 5.5 mm deep). Once a rat was ready for data collection, the dummy cannula was replaced by a 30 gauge injection cannula that extended past the guide cannula 6 mm deep. The cannula was used to microinject one drug into each rat depending on the group. One group received artificial cerebrospinal fluid (ACSF) to serve as a control. The next group received lidocaine to serve as a temporary lesion or to inhibit all activity in the interposed nucleus. The third group received GABA to inhibit the

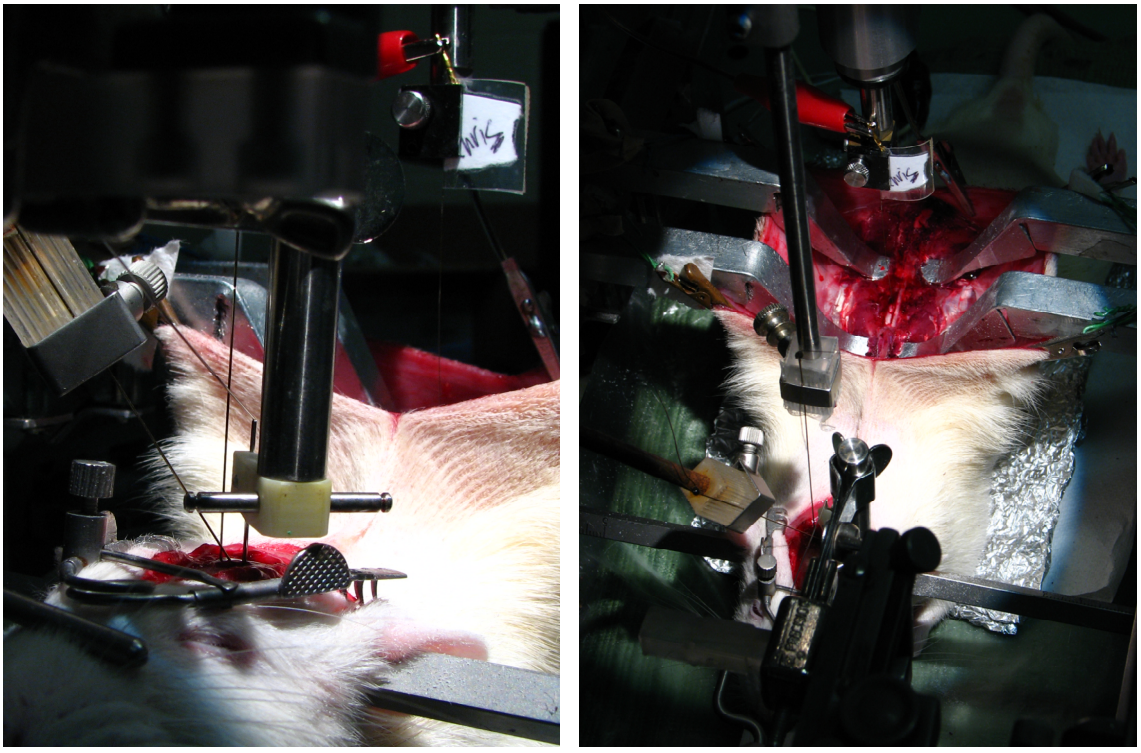

Figure 4. Experimental setup. The picture on the left is of a rat's head (nose in bottom left corner). The guide cannula (with a dummy cannula coming out of the top) is attached to the large bar on the right (center of picture). Coming in at an angle from the far left is the stimulating electrode that was placed in the left cerebellar cortex. Sort of in between those two is the stimulating electrode (arranged vertically) that was placed in the right cerebellar cortex. In the upper right corner, the top of the recording electrode can be seen (with *Chris* flag); it continues downward into the spinal cord. The picture on the right is an overhead view of the setup. The head is located in the lower portion of the picture and the tail is in the upper portion. From the left, the stimulating electrode that was placed into the left cerebellar cortex can be seen. From the top, the stimulating electrode placed in the right can be seen. And the cannula is beneath the equipment at the bottom. In the upper portion of the picture are the exposed spinal cord and recording electrode (very thin).

activity of the interposed nucleus in the same way that the Purkinje cells inhibit the IPN. And the final group received bicucilline to block $GABA_A$ receptors in the IPN and therefore block the GABA being released from Purkinje cells.

2.3 Cannula

The guide cannula was constructed by sawing off the tip of a 23 gauge needle leaving a length of 5.5 mm. The injection cannula was a hypodermic stainless steel tube with a 0.0120 inch diameter (AM-Systems, Inc., Carlsborg, WA). It was made by running a bare stainless steel wire (AM-Systems, Inc., Carlsborg, WA) with a diameter of 0.002 inches through the 30 gauge needle. This prevented the needle from crimping on the side that was cut. The needle was then cut to a length long enough to bend slightly (without crimping) to be able to provide a stopping place for the cannula once it was placed inside the guide. To bend the cannula, it was placed in the guide to where it extended out 0.5 mm. Then the cannula was bent slightly where it entered the guide. Finally a tube was attached to the cannula. This tube would later be attached to a Hamilton syringe. A dummy cannula 5.5 mm long was constructed in the same fashion as the injection cannula; the dummy was used when inserting the guide to prevent the guide from clogging. The dummy also had a long end that was used to clean the guide.

Before each experiment, a 1 ml syringe with a 25 gauge needle was used to fill the line and cannula with a drug. This ensured that there were no occlusions at the beginning of each experiment. Next the cannula was dangled in a downward fashion, and a drop of drug was pushed through to the tip of the cannula. With the drop present, the syringe was replaced with a Hamilton syringe. To be sure that there was not backflow of drug from the tip of the needle, the tip was dried and the Hamilton syringe was used to push out another drop (0.3-0.5 µl). Upon injection, a micro infusion pump was used to inject 0.5 µl of a drug at a constant rate in 90 seconds.

Following the completion of each experiment, each cannula was retrieved to be cleaned. After the guide cannula was cleaned, the dummy cannula was used to clear any

remaining water inside the guide. For the injection cannula, ACSF was used to flush the line and the cannula followed by air to flush the ACSF.

2.4 Data Acquisition

Spinal cord dorsal horn neurons were recorded electrophysiologically in either side of the L5 spinal cord region using a 10-12 MΩ tungsten microelectrode (FHS, Brunswick, ME). Cells were located by applying mechanical stimulation to the hind paw while navigating the electrode through the dorsal horn. Mechanical responses to brush, pressure, and pinch were recorded using SPIKE2 computer software (CED, UK). Wide Dynamic Range (WDR) spinal dorsal horn neurons were located and used for each recording. Once a WDR cell was identified, a single-unit extracellular recording was collected. WDR activity was recorded constantly in the following manner (Figure 2): brush, pressure, pinch; left cerebellar cortex electrical stimulation during brush, pressure, pinch; brush, pressure, pinch; right cerebellar cortex electrical stimulation during brush, pressure, pinch; brush, pressure, pinch; wait 60 seconds before injecting drug for 90 seconds; wait 5 minutes; brush, pressure, pinch; left cerebellar cortex stimulation during brush, pressure, pinch; brush, pressure, pinch; right cerebellar cortex stimulation during brush, pressure, pinch; brush, pressure, pinch. To avoid any order effects that might occur from the drug being more concentrated in the IPN at the time of the first electrical stimulation following injection, the order of electrical stimulation was switched in half of the rats. In other words, half received right cerebellar cortex electrical stimulation before left cerebellar cortex electrical stimulation before and after a drug was injected into the IPN.

The recordings were used to assess the number of action potentials per second. For the mechanical stimuli, each lasted 10 seconds with 20 second inter-stimulus intervals and one minute between each brush, pressure, pinch condition so that there were 60 seconds from the end of a pinch to the beginning of the next brush. Data was stored offline to edit recordings and

ensure proper cell classification. All data were then organized into a spreadsheet to be used for statistical analyses.

2.5 Histology

Following data collection, brains were extracted from each rat to confirm cannula placement. The brains were stored in 10% formalin until histology (at least 24 hours). Brains were removed from formalin and placed in a sucrose solution for dehydration about 24 hours before being sliced. They were then sliced into coronal sections 80 µm thick and mounted on slides. The slices dried on slides for about one day and were then stained with thionin. Following histology, the slides were reviewed by the experimenter and two independent raters to identify cannula placements.

Due to complications in the aforementioned histology technique, a different approach was used to try to improve the quality of the slides. The following histology methods ended up being used on the majority of the animals in this study.

Immediately following electrophysiological recordings, all animals remained deeply anesthetized and were perfused transcardially with 200 ml of 0.01 M PBS (approximately 7 minutes) followed by 400 ml of 4% paraformaldehyde made in 0.01 M PBS (approximately 20 minutes). Brains were removed, placed in 4% paraformaldehyde and stored at 4ºC overnight. The next day, brains were placed in 20% glycerol for a minimum of 24 hours. Coronal brain sections (40 µm) were taken using a freezing microtome and stored in .01% PBS-azide at 4℃. Sections from the cerebellum were selected from each brain and stained with cresyl violet.

2.6 Drugs

A total of four experimental drugs were used in this study. All drugs were microinjected into the left IPN. ACSF was injected in the first group as a control. Lidocaine hydrochloride was used in the second group block all nerve activity in the IPN (.5 µl; 2%; Sigma-Aldrich, St. Louis, MO). The third group received gamma-Aminobutyric acid (GABA; .5 µl; 1 M), and the fourth group received bicuculline (.5 µl; 50 µM). GABA (Sigma-Aldrich, St. Louis, MO) was dissolved

in ACSF to make a 1 M solution. A 50 µM solution of bicuculline was made by first dissolving 0.002 g of bicuculline (Sigma-Aldrich, St. Louis, MO) in 11 ml of dimethyl sulfoxide (DMSO; Sigma-Aldrich, St. Louis, MO). The first solution was used as a stock solution from which 0.1 ml was drawn to be mixed with 9.9 ml of ACSF. The final/experimental solution then consisted of 10 ml of bicuculline with a concentration of 50 µM.

2.7 Data Analysis

2.7.1 General Analysis

The mean number of action potentials for each ten second interval of mechanical stimulation (with and without electrical stimulation) was calculated for all cells and transferred to a spreadsheet. The calculation was performed with Spike2 software by placing a cursor at the beginning and end of a mechanical stimulation event. Spike2 calculated the mean across those 10 seconds and that score was transferred to a spreadsheet. Therefore, a total of 30 scores were calculated for each cell. Those scores were arranged by group (which drug was received). Then the mean ± SEM was calculated for each condition of each group. Those data were then statistically analyzed using a 4 Drugs (ACSF, Lidocaine, GABA, and Bicucilline) x 2 Treatments (pre- and post-drug) x 5 Electrical (Control 1, Left Stimulation, Control 2, Right Stimulation, Control 3) x Mechanical (brush, pressure, and pinch) Mixed ANOVA followed by Fischer LSD post hoc analysis (STATISTICA, StatSoft, OK). A significance criterion of $p < 0.05$ was used in all comparisons; and if a comparison was made but not significantly different, it was reported NS (not significant).

Comparisons were made within groups across time (30 time points or conditions) to analyze the dorsal horn neuronal response changes evoked by mechanical stimulation with or without electrical stimulation. It was expected that electrical stimulation of either side of the cerebellar cortex would inhibit nociceptive (pressure/pinch) dorsal horn responses. The expected inhibition produced by cerebellar cortex stimulation was then manipulated by microinjecting a drug into the IPN. This would be evident in comparing pre- and post-treatment

conditions. ACSF was not expected to produce a change in neuronal responding from pre- to post-treatment. In the post-treatment condition, bicucilline in the left IPN was expected to attenuate the antinociception produced by electrical stimulation of the left cerebellar cortex, but it was not expected to attenuate the antinociception produced by right cerebellar cortex stimulation. This is because bicuculline would bind to $GABA_A$ receptors in the left IPN, which should block the effect of the GABA being released from Purkinje cells activated during electrical stimulation of the left cerebellar cortex. On the other hand, GABA was expected to intensify the antinociception produced by left cerebellar cortex stimulation but not right. Lidocaine was expected to block the inhibitory effect produced by electrical stimulation in the left cerebellar cortex but not the right. All groups were expected to have similar inhibitory effects on nociceptive responses during left and right cerebellar cortex stimulation before any drugs were injected.

2.7.2 Inhibition Analysis

An additional analysis was run to test the between groups differences because the control conditions for pressure and pinch differed too much. To acquire an inhibition score, Control 1 was used to get a ratio for each raw score: Condition X/Control 1. Each condition was divided by its respective Control 1 for each group. This provided a way to observe the amount of change that took place for each condition; therefore, all Control 1 conditions had a ratio of 1, or no change, since Control 1 was both numerator and denominator. The closer a score was to zero the more inhibition there was; whereas scores above one indicated facilitative activity. Once the inhibition scores were calculated, the means and SEMs were calculated for those scores. To analyze statistical differences, a 3 Mechanical (Brush, Pressure, Pinch) x 2 Treatment (Pre- and Post-Treatment) x 5 Electrical (Control 1, Left Stimulation, Control 2, Right Stimulation, Control 3) x Drug (ACSF, Lidocaine, GABA, Bicuculline) Mixed ANOVA was run followed by Fisher LSD post hoc analysis (STATISTICA, StatSoft, OK).

2.7.3 Counterbalancing

To eliminate any order effects that might occur from one side of the brain being stimulated closer in time than the other side of the brain, the order of stimulation was reversed in half of the rats. In other words, Right Stimulation was delivered before Left Stimulation half of the time. After all the data were collected, the data that contained Right Stimulation first was modified to balance any order effect. Right Stimulation conditions switched places with Left Stimulation conditions, and Control 2 conditions were switched with Control 3 conditions. This was performed in all groups in both Pre- and Post-Treatment conditions.

CHAPTER 3

RESULTS

A total of 28 cells were recorded from 23 rats for this experiment. Fifteen rats had to be excluded due to improper placement of the cannula, because the histology did not yield clear enough results for a definite confirmation, or because the cell was lost before data collection could be completed. Rats' ages ranged from 178-244 days, and weights ranged from 400-582 g.

To be included in the study, it was determined that the tip of the cannula had to be either in the IPN or no farther than 1 mm above it. One electrophysiology study observed the direct effects of similar drugs on spinal neurons after the drugs diffused at that deep (Peng, Lin, & Willis, 1996). All other placements were too far anterior (n = 8) or could not be confirmed (n = 4). Cannula placement was determined by the experimenter and two independent observers. After personal judgments, the three met to compare notes and make a final decision for each brain. Twenty-three brains had accurate placement and can be viewed in Figure 5.

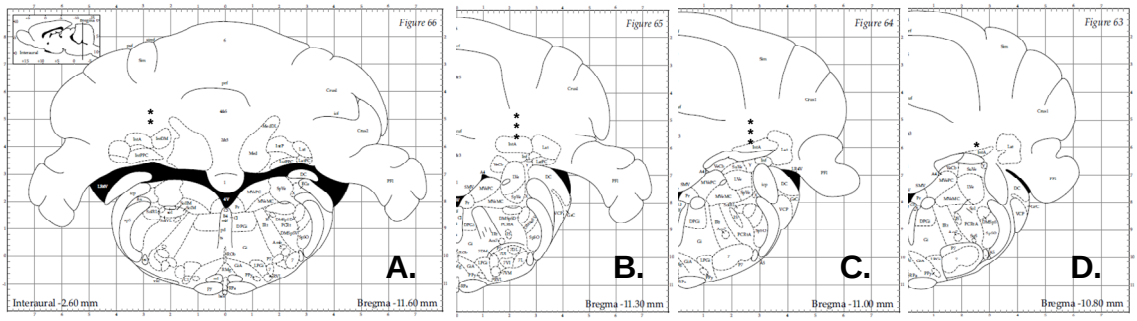

Figure 5. Cannula Placement. Coronal sections from the Rat Atlas (Paxinos & Watson, 1998). Stars are positioned where the cannula tips were observed. All cannulae were in the left side, but B.-D. are shown on the right because there is no IntA represented on the map for B. and D. The following indicates how many cannulae were positioned at each star and the numbers indicate the distance above the interposed nucleus. A. 1mm – 1; 0.5mm – 1; B. 1mm – 1; 0.5mm – 3; 0mm – 8; C. 1mm – 1; 0.5mm – 3; 0mm – 4; D. 0mm – 1; IntA – anterior interposed nucleus

3.1 Analysis of Cell Responses

This analysis was performed to consider the overall cellular responses for each condition. It was useful for determining the pattern of responses within each group. However, it was not useful for comparing between groups, since the cells across groups had different control rates. Between groups comparisons were performed in an additional analysis (Section 3.2).

A total of 28 cells were recorded from 23 rats: ACSF (n = 9; 7 rats); Lidocaine (n = 4; 3 rats; Appendix A); GABA (n = 8; 7 rats); Bicuculline (n = 7; 6 rats; Appendix B). Means ± SEM were calculated for each cell's response to brush, pressure, and pinch at *Control 1*, *Left Stimulation*, *Control 2*, *Right Stimulation*, *Control 3*, *Control 1 Drug*, *Left Stimulation Drug*, *Control 2 Drug*, *Right Stimulation Drug*, and *Control 3 Drug* (Table 1). Each condition included brush, pressure, and pinch mechanical stimuli and was manipulated according to the condition. Control indicates that no electrical stimulation was used, Left Stimulation indicates that the left cerebellar cortex was stimulated, Right Stimulation indicates that the right cerebellar cortex was stimulated, and Drug indicates that the condition follows the microinjection of a drug into the

Table 1. Mean ± SEM Responses to Brush (Br), Pressure (PR), and Pinch (Pi)

Drug	Tx	C1			LS			C2			RS			C3		
		Br	Pr	Pi	Br	Pr	Pi	Br	Pr	Pi	Br	Pr	Pi	Br	Pr	Pi
ACSF	Pre	15± 4	55±17	74±18	14±3	55±15	58±14	19±4	68±21	70±17	17±4	66±19	65±15	17±4	64±19	66±17
	Post	14±3	67±17	71±18	14±3	55±16	52±14	15±3	67±18	61±17	15±3	54±14	52±11	17±4	68±17	61±13
GABA	Pre	15±3	43±12	48±8	13±3	30±9	46±8	17±4	33±8	43±8	16±3	33±8	45±9	14±3	31±5	45±9
	Post	15±3	31±7	37±7	15±4	25±7	35±9	12±4	18±6	35±11	15±3	28±6	38±9	14±3	24±7	41±11

** C – Control; LS – Left Stimulation; RS – Right Stimulation; Tx - Treatment

IPN. Due to the small sample size and unusual response patterns, results for the Lidocaine and Bucuculline groups are reported in Appendices A and B.

To determine statistical differences, a 4 Drug (ACSF, Lidocaine, GABA, Bicuculline) x 5 Electrical (Control 1, Left Stimulation, Control 2, Right Stimulation, Control 3) x 3 Mechanical (Brush, Pressure, Pinch) x 2 Treatment (Pre- and Post-Drug) Mixed ANOVA was run followed by Fisher LSD post hocs (STATISTICA, StatSoft, OK). This analysis produced the following

effects: Drug, $F(3, 22) = 1.0$, NS; Treatment, $F(1, 22) = 4.2$, $p = 0.051$; Treatment x Drug, $F(3, 22) = 2.3$, NS; Electrical, $F(4, 88) = 4.8$, $p < 0.01$; Electrical x Drug, $F(12, 88) = 1.2$, NS; Mechanical, $F(2, 44) = 23.2$, $p < 0.001$; Mechanical x Drug, $F(6, 44) = 1.4$, NS; Treatment x Electrical, $F(4, 88) = 4.0$, $p < 0.01$; Treatment x Electrical x Drug, $F(12, 88) = 2.3$, $p < 0.05$; Treatment x Mechanical, $F(2, 44) = 5.5$, $p < 0.01$; Treatment x Mechanical x Drug, $F(6, 44) = 1.7$, NS; Electrical x Mechanical, $F(8, 176) = 2.8$, $p < 0.01$; Electrical x Mechanical x Drug, $F(24, 176) = 1.8$, $p < 0.05$; Treatment x Electrical x Mechanical, $F(8, 176) = 3.2$, $p < 0.01$; Treatment x Electrical x Mechanical x Drug, $F(24, 176) = 2.2$, $p < 0.01$. These results suggest that there are differences among many conditions that can only be determined by a post hoc analysis. The Treatment x Electrical x Mechanical x Drug interaction was used to run the Fisher LSD test. Only relevant comparisons within groups were considered (Figures 6 & 7).

For each of the following analyses, brain stimulation conditions were compared to the control condition immediately prior to the brain stimulation condition. For example, Right Stimulation was compared to Control 2 rather than Control 1. This was done to try to rule out any order effects that might have occurred from repeated antinociceptive electrical stimulation.

3.1.1 ACSF Analysis

The ACSF group had a sample of n = 9 (Right Stimulation first = 7; Left Stimulation first = 2). The first hypothesis to be tested was that both left and right cerebellar cortex stimulation would inhibit noxious spinal dorsal horn cellular responses. This hypothesis was not necessarily supported for pressure, since pressure was not inhibited by brain stimulation: ACSF/Pressure/Control 1/Pre-Treatment – ACSF/Pressure/Left Stimulation/Pre-Treatment, NS; ACSF/Pressure/Control 2/ Pre-Treatment – ACSF/Pressure/Right Stimulation/ Pre-Treatment, NS (Figure 6). It was, on the other hand, supported for pinch with Left Stimulation inhibition but not Right Stimulation: ACSF/Pinch/Control 1/Pre-Treatment – ACSF/Pinch/Left Stimulation/Pre-Treatment, $p < 0.05$; ACSF/Pinch/Control 2/Pre-Treatment – ACSF/Pinch/Right Stimulation/Pre-Treatment (Figure 6). Although Right Stimulation was not significantly less than its control

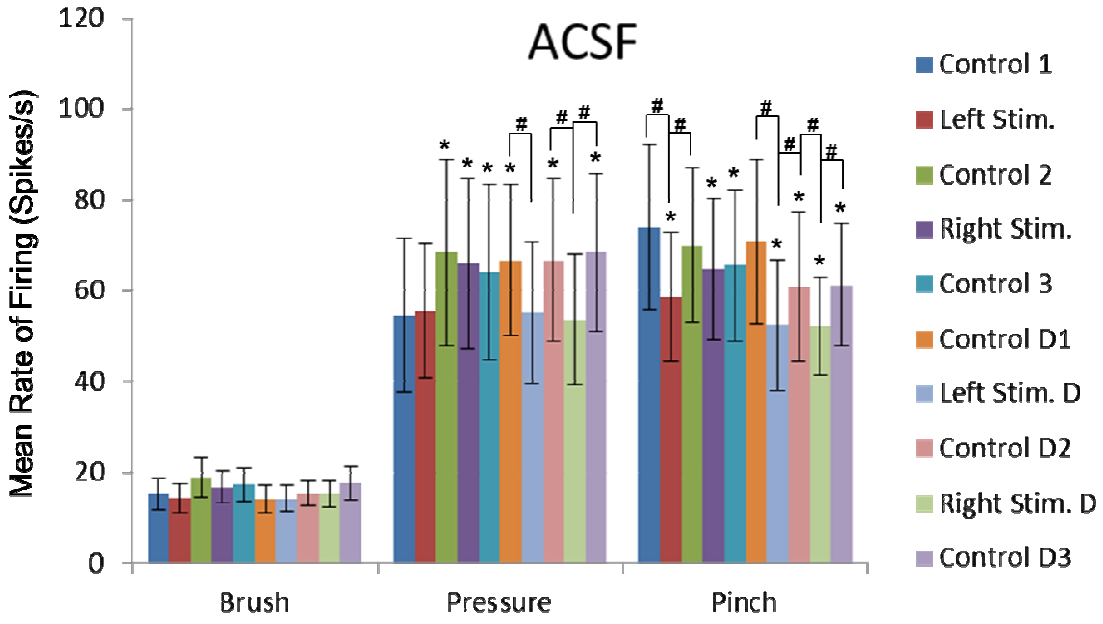

Figure 6. Mean ± SEM responses of dorsal horn neurons from the ACSF group. Comparisons for this graph were made with respective Control 1 conditions. Significant differences are represented by: * $p < 0.05$ compared to Control 1; # $p < 0.05$; + $p < 0.10$.

(Control 2), it was significantly less than the first control: ACSF/Pinch/Control 1/Pre-Treatment–ACSF/Pinch/Right Stimulation/Pre-Treatment, $p < 0.05$ (Figure 6). Furthermore, the hypothesis was partially supported considering cell responses following ACSF injection. Both pressure and pinch were inhibited by both Left Stimulation and Right Stimulation: ACSF/Pressure/Control 1/Post-Treatment – ACSF/Pressure/Left Stimulation/Post-Treatment; ACSF/Pressure/Control 2/Post-Treatment – ACSF/Pressure/Right Stimulation/Post-Treatment; ACSF/Pinch/Control 1/Post-Treatment – ACSF/Pinch/Left Stimulation/Post-Treatment; ACSF/Pinch/Control 2/Post-Treatment – ACSF/Pinch/Right Stimulation/Post-Treatment (Figure 6).

Ideally there would be no statistical differences for pressure or pinch from one control to the next, but this was not exactly the case. For pressure, each control condition had significantly greater response rates than the initial control: ACSF/Pressure/Control 1/Pre-Treatment – ACSF/Pressure/Control 2/Pre-Treatment, $p < 0.05$; ACSF/Pressure/Control 1/Pre-Treatment – ACSF/Pressure/Control 3/Pre-Treatment, $p < 0.05$; ACSF/Pressure/Control 1/Pre-Treatment – ACSF/Pressure/Control 1/Post-Treatment, $p < 0.05$; ACSF/Pressure/Control 1/Pre-

Treatment – ACSF/Pressure/Control 2/Post-Treatment, $p < 0.05$; ACSF/Pressure/Control 1/Pre-Treatment – ACSF/Pressure/Control 3/Post-Treatment, $p < 0.05$ (Figure 6). This may suggest that pressure responses were producing a wind-up phenomenon where the cells' firing rates increase from sensitization caused by repeated mechanical stimulation. However, it would also be expected to occur in the pinch condition as well, which was not the case. The greatest mean response to pinch occurred during the initial control. The three controls that had significantly lower responses than the first control were Control 3/Pre-Treatment, Control 2/Post-Treatment, and Control 3/Post-Treatment: ACSF/Pinch/Control 1/Pre-Treatment – ACSF/Pinch/Control 3/Pre-Treatment, $p < 0.05$; ACSF/Pinch/Control 1/Pre-Treatment – ACSF/Pinch/Control 2/Post-Treatment, $p < 0.05$; ACSF/Pinch/Control 1/Pre-Treatment – ACSF/Pinch/Control 3/Post-Treatment, $p < 0.05$ (Figure 6). These results conflict with a wind-up theory since response rates are decreasing in later control conditions. The results actually compliment an inhibitory trend from repeated antinociceptive electrical stimulation, which was evident from inhibition when each brain stimulation condition was compared to their respective controls.

There were no inhibitory effects or differences between any of the brush conditions. This suggests that tactile sensation is not noticeably affected by cerebellar cortex stimulation.

3.1.2 GABA Analysis

The GABA group had a sample size of n = 8 (Right Stimulation first = 5; Left Stimulation first = 3). GABA was expected to facilitate inhibition Post-Treatment. There is some evidence to support that hypothesis, but it too failed to provide clear proof. To start, stimulation in the left cerebellar cortex caused inhibition of responses to pressure, but then the other controls never recovered to the initial firing rate in the Pre-Treatment condition: GABA/Pressure/Control 1/Pre-Treatment – GABA/Pressure/Left Stimulation/Pre-Treatment, $p < 0.05$; GABA/Pressure/Control 1/Pre-Treatment – GABA/Pressure/Control 2/Pre-Treatment, $p < 0.05$; GABA/Pressure/Control 1/Pre-Treatment – GABA/Pressure/Control 3/Pre-Treatment, $p < 0.05$ (Figure 7). Electrical stimulation on either side of the cerebellar cortex did not inhibit any pinch responses Pre- or

Post-Treatment. Pressure and pinch responses considered together make it difficult to draw strong conclusions.

GABA alone without electrical stimulation should be sufficient to inhibit noxious mechanical stimulation. The most obvious evidence for GABA-evoked inhibition would be if Control 1/Post-Treatment was significantly lower than Control 3/Pre-Treatment. This was the case for pinch but not pressure: GABA/Pinch/Control 3/Pre-Treatment – GABA/Pinch/Control 1/Post-Treatment, $p < 0.05$ (Figure 7). Pinch was also significantly lower for the Post-Treatment condition at Left Stimulation, Control 2 (marginal), and Right Stimulation: GABA/Pinch/Control 3/Pre-Treatment – GABA/Pinch/Left Stimulation/Post-Treatment, $p < 0.05$; GABA/Pinch/Control 3/Pre-Treatment – GABA/Pinch/Control 2/Post-Treatment, $p < 0.10$; GABA/Pinch/Control 3/Pre-Treatment – GABA/Pinch/Right Stimulation/Post-Treatment, $p < 0.05$ (Figure 7). Additionally all pinch responses in the Post-Treatment condition were significantly less than the initial pinch response: GABA/Pinch/Control 1/Pre-Treatment – all GABA/Pinch/Post-Treatment conditions,

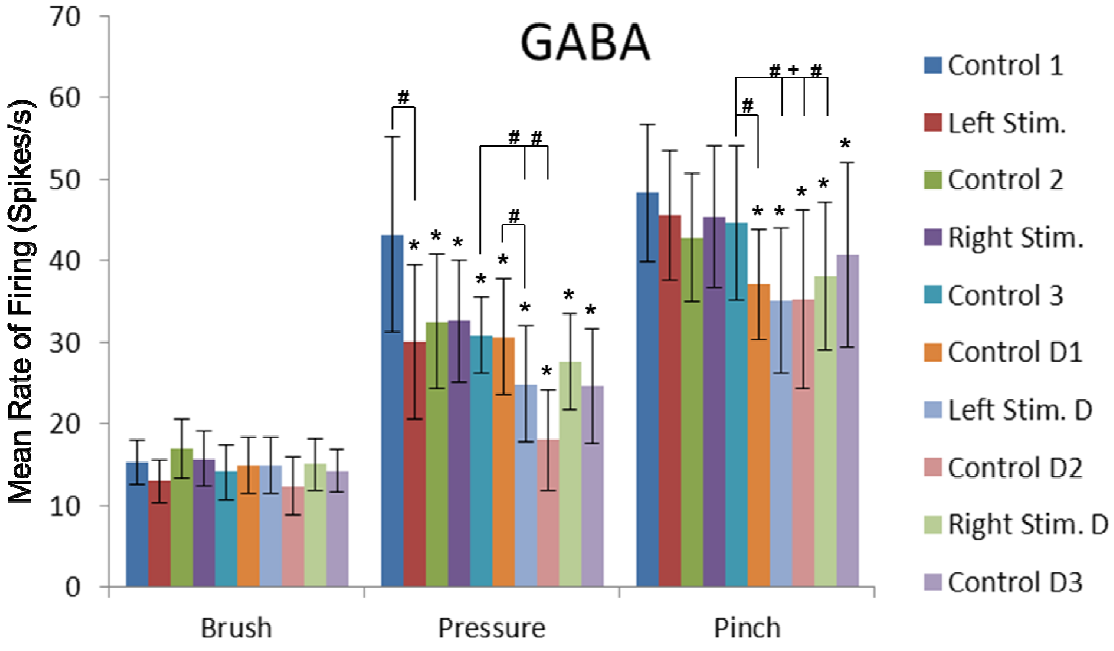

Figure 7. Mean ± SEM responses of dorsal horn neurons from the GABA group. Comparisons for this graph were made with respective Control 1 conditions. Significant differences are represented by: * $p < 0.05$ compared to Control 1; # $p < 0.05$; + $p < 0.10$.

$p < 0.05$ (Figure 7). One last component that suggests GABA alone was able to inhibit nociceptive spinal activity was that pressure was inhibited at Left Stimulation and Control 2: GABA/Pressure/Control 3/Pre-Treatment – GABA/Pressure/Left Stimulation/Post-Treatment, $p < 0.05$; GABA/Pressure/Control 3/Pre-Treatment – GABA/Pressure/Control 2/Post-Treatment, $p < 0.05$ (Figure 7). All together, these results suggest that GABA in the IPN likely played a facilitative role in producing descending inhibition.

There were no significant changes in brush.

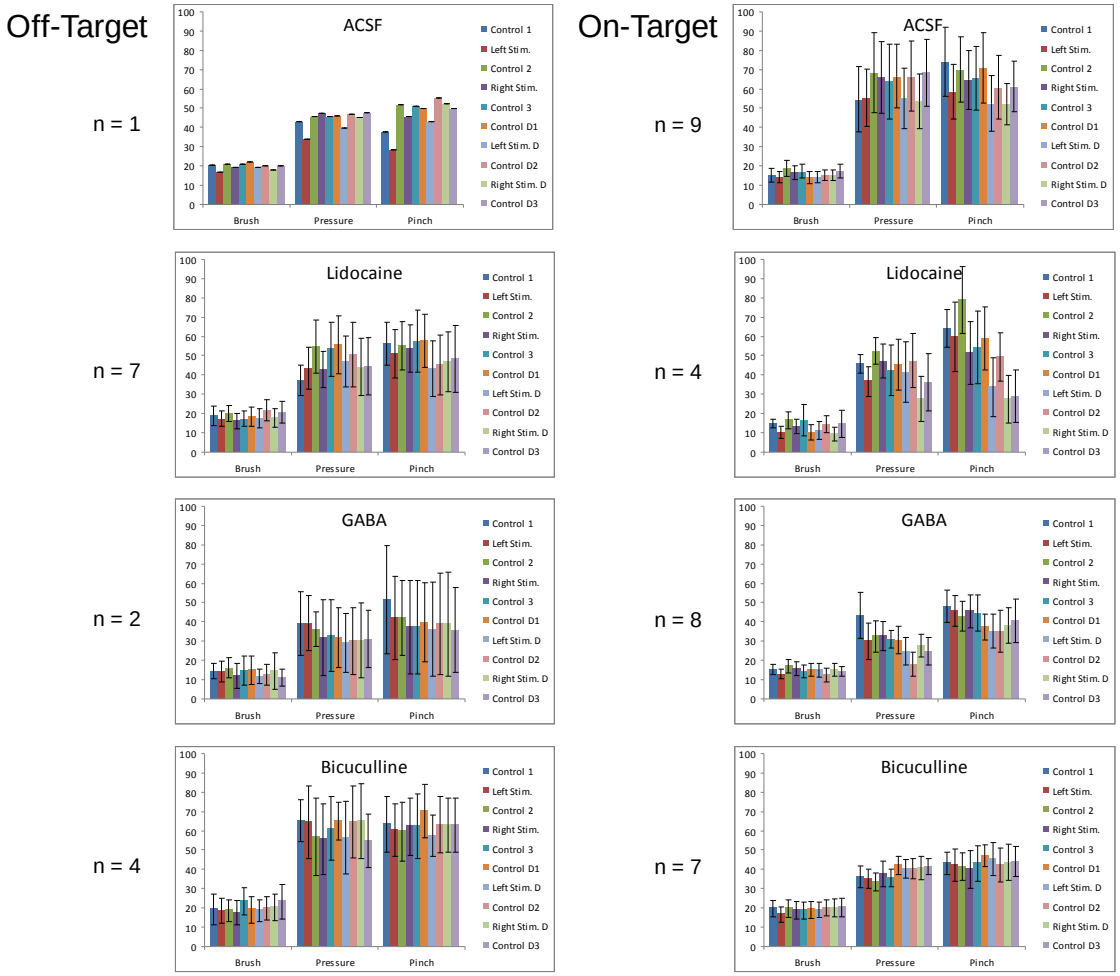

Figure 8. Mean ± SEM responses of dorsal horn neurons for all groups including the excluded data. Y-axes are mean firing rates (Spikes/s).

3.2 Inhibition Analysis

This analysis was necessary to compare between groups. All raw scores were converted into inhibition scores. Means ± SEM were calculated to determine the amount of inhibition on brush, pressure, and pinch at *Control 1*, *Left Stimulation*, *Control 2*, *Right Stimulation*, *Control 3*, *Control 1 Drug*, *Left Stimulation Drug*, *Control 2 Drug*, *Right Stimulation Drug*, and *Control 3 Drug* (Tables 2-7). A 3 Mechanical (Brush, Pressure, Pinch) x 2 Treatment (Pre- and Post-Treatment) x 5 Electrical (Control 1, Left Stimulation, Control 2, Right Stimulation, Control 3) x Drug (ACSF, Lidocaine, GABA, Bicuculline) Mixed ANOVA produced the following results: Drug, $F(3, 22) = 0.7$, NS; Mechanical, $F(2, 44) = 1.5$, NS; Mechanical x Drug, $F(6, 44) = 0.7$, NS; Treatment, $F(1, 22) = 0.1$, NS; Treatment x Drug, $F(3, 22) = 2.0$, NS; Electrical, $F(4, 88) = 1.4$, NS; Electrical x Drug, $F(12, 88) = 0.9$, NS; Mechanical x Treatment, $F(2, 44) = 1.0$, NS; Mechanical x Treatment x Drug, $F(6, 44) = 0.5$, NS; Mechanical x Electrical, $F(8, 176) = 1.0$ NS; Mechanical x Electrical x Drug, $F(24, 176) = 1.1$, NS; Treatment x Electrical, $F(4, 88) = 1.1$, NS; Treatment x Electrical x Drug, $F(12, 88) = 1.1$, NS; Mechanical x Treatment x Electrical, $F(8, 176) = 0.9$, NS; Mechanical x Treatment x Electrical x Drug, $F(24, 176) = 1.4$, NS. Although there were no effects, the Mechanical x Treatment x Electrical x Drug interaction was used to run a Fisher LSD post hoc test to make planned comparisons.

No differences were observed between groups for the brush conditions (Figure 9). This is further support that brush was unaffected by any drugs or electrical stimulation. It also illustrates that brush was very consistent across time and groups.

Pressure inhibition was significantly lower across several conditions between groups when compared to ACSF (Figure 10); however, the results are not reported here because the ACSF group had skewed inhibition scores due to a low Control 1 response. There were no other differences observed for pressure.

Table 2. ACSF Brush Inhibition Means ± SEM

Tx	C1	LS	C2	RS	C3
Pre	1.0± 0	1.0± 0.1	1.3± 0.1	1.2± 0.1	1.3± 0.2
Post	1.0± 0.1	1.1± 0.2	1.2± 0.2	1.2± 0.2	1.4± 0.3

Table 3. ACSF Press Inhibition Means ± SEM

Tx	C1	LS	C2	RS	C3
Pre	1.0± 0	2.5± 1.2	2.0± 0.9	2.7± 1.2	1.5± 0.4
Post	2.1± 0.8	1.5± 0.5	1.8± 0.6	1.5± 0.5	2.2± 0.9

Table 4. ACSF Pinch Inhibition Means ± SEM

Tx	C1	LS	C2	RS	C3
Pre	1.0± 0	0.8± 0.1	0.9± 0.1	0.8± 0.1	0.8± 0.1
Post	0.9± 0.1	0.6± 0.1	0.7± 0.1	0.7± 0.1	0.8± 0.1

Table 5. GABA Brush Inhibition Means ±

Tx	C1	LS	C2	RS	C3
Pre	1.0± 0	0.9± 0.1	1.1± 0.2	1.0± 0.1	0.9± 0.1
Post	1.0± 0.1	1.0± 0.2	0.8± 0.1	1.0± 0.1	0.9± 0.1

Table 6. GABA Press Inhibition Means ±

Tx	C1	LS	C2	RS	C3
Pre	1.0± 0	0.7± 0.1	1.0± 0.2	0.9± 0.1	1.4± 0.5
Post	1.1± 0.4	0.8± 0.2	1.0± 0.6	1.2± 0.4	1.4± 0.6

Table 7. GABA Pinch Inhibition Means ±

Tx	C1	LS	C2	RS	C3
Pre	1.0± 0	0.9± 0	0.9± 0.1	1.0± 0.1	0.9± 0.1
Post	0.9± 0.1	0.7± 0.1	0.9± 0.2	0.8± 0.1	1.0± 0.3

Pinch inhibition comparisons did not yield any meaningful data either. There were

no significant differences between ACSF and GABA conditions (Figure 11).

Within group effects were also compared across brush, pressure, and pinch to give an alternative perspective to the overall cell responses; however, no relevant differences were observed.

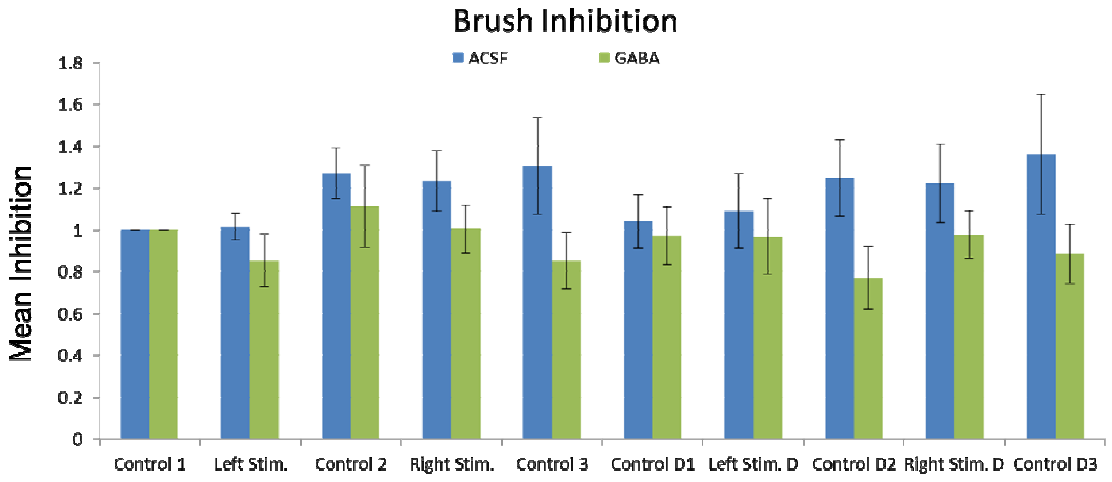

Figure 9. Mean ± SEM inhibition for brush. Comparisons for this graph were made between all 20 conditions. There were no differences.

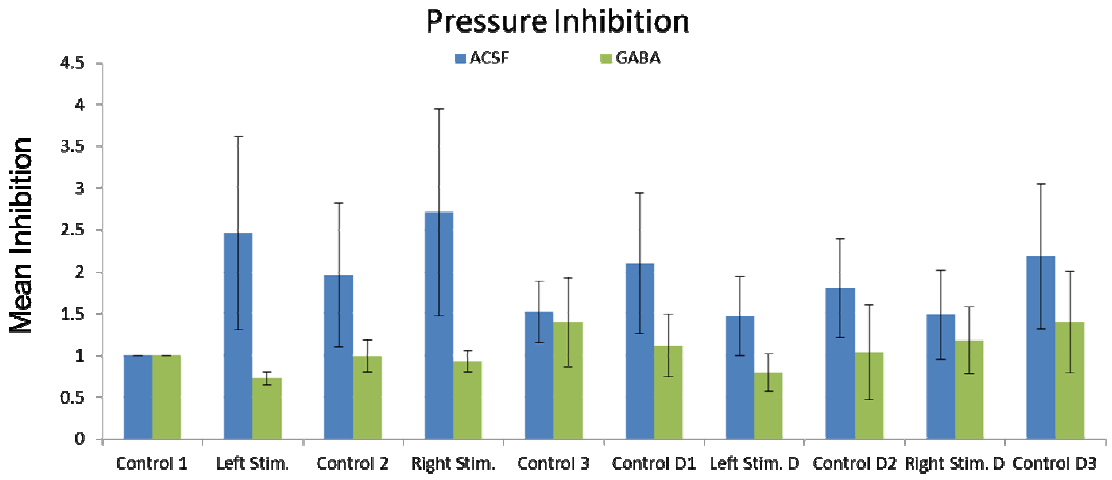

Figure 10. Mean ± SEM inhibition for pressure. Comparisons for this graph were made within and between groups. There were no differences.

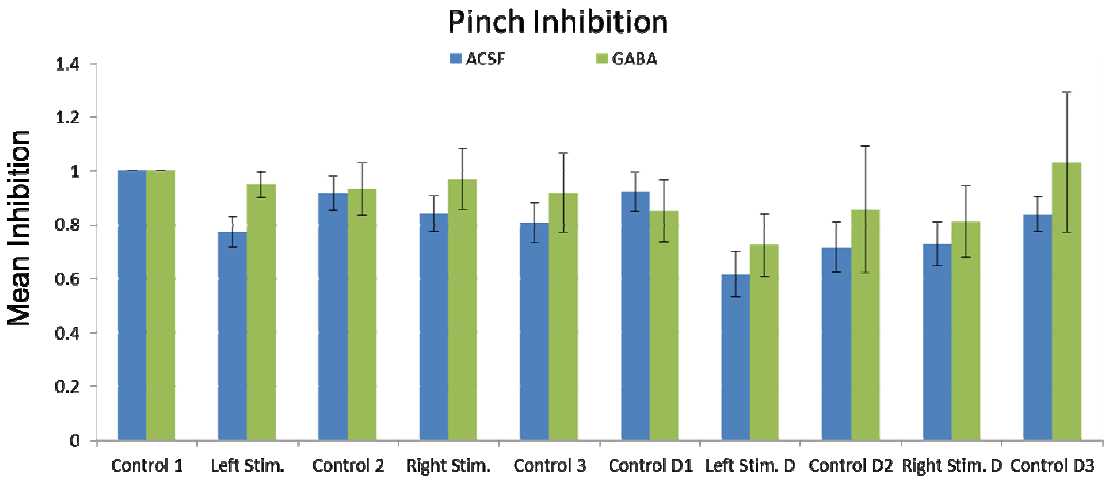

Figure 11. Mean ± SEM inhibition for pinch. Comparisons for this graph were made within and between groups. There were no differences.

CHAPTER 4

DISCUSSION

The purpose of the experiment was to explore some of the qualities of the cerebellum as it relates to pain. More specifically, the goal was to uncover physiological mechanisms that contribute to descending inhibition produced by electrical stimulation of the intermediate hemisphere of the cerebellar cortex. A previous study found that electrical stimulation in the intermediate hemisphere of the cerebellar cortex is capable of inhibiting nociceptive responses to pressure and pinch in spinal dorsal horn neurons (Hagains et al., 2011). The current study utilized similar techniques and incorporated pharmacological manipulations to try to identify some of cerebellar physiology responsible for descending inhibition.

4.1 ACSF Findings

ACSF was used as a control drug to compare with the drugs of interest. It was expected that this group would have responses similar to those observed previously (Hagains et al., 2011), but the results did not all turn out as expected. The main hypothesis was that electrical stimulation in both right and left cerebellar cortices would produce inhibition of nociceptive responses in spinal dorsal horn neurons. Pressure responses were not inhibited by electrical stimulation of either side of the cerebellar cortex. One issue regarding pressure is that it started off with a low control. This may have influenced the way that it was affected by cerebellar cortex stimulation early on because pressure responses after ACSF injection were significantly inhibited by brain stimulation (Figure 6). This was a little problematic when considering pressure responses in other drug groups. If inhibition is sporadic in one group, how can it be reliable in other groups? In fact, other groups also failed to replicate the inhibition trends observed previously. It does still imply that pressure can be inhibited by cerebellar stimulation; the timing just needs to be more predictable.

Neuronal responses to pinch responded better to electrical stimulation in the cerebellar cortex. In the ACSF group, pinch responses were inhibited by Left Stimulation in both Pre- and Post-Treatment conditions and by Right Stimulation in the Post-Treatment condition. And although Right Stimulation was not significant compared to its control, it was significantly less than the initial control. These results were very close to what was hypothesized, and it suggests that stimulating either side of the cerebellar cortex will activate descending inhibition.

One issue for both pressure and pinch is a lack of consistency across Pre- and Post-treatment conditions. A lack of consistency makes for a poor between groups control, but it is certainly premature to suggest that injecting ACSF into the IPN is affecting descending inhibition. Another issue was the lack of consistency with control conditions. Other studies have consistently observed almost no difference between brush, pressure, pinch measurements taken before and after electrical stimulation, which is the same as the control conditions in the current experiment (Senapati et al., 2005a; Senapati et al., 2005b; Senapati et al., 2005c; Hagains et al., 2011a; Hagains et al., 2011b). And one last issue was concerning the facilitative activity in pressure. This may have been an issue that would have been washed out with a larger sample size. The ACSF group had 9 total cells from 7 rats. A higher cell count may provide a more normal response.

4.2 GABA Findings

GABA in the IPN was expected to facilitate descending inhibition. The best evidence to support this hypothesis was that Left Stimulation/Post-Treatment and Control 2/Post-Treatment had significantly lower pressure responses than Control 3/Pre-Treatment. Additionally, there was not any return to baseline rates for the control conditions pre-treatment following Left Stimulation; but when the left cerebellar cortex was stimulated post-treatment it produced further significant inhibition (Figure 7). And although there was no inhibition for any Pre-Treatment conditions, pinch had significantly lower firing rates post-treatment at Control 1, Left Stimulation, Control 2 (marginal), and Right Stimulation when compared to Control 3/Pre-

Treatment. These findings are all in support of the hypothesis and suggest that GABA was having an antinociceptive effect by inhibiting the IPN. That would mean most likely that the antinociceptive effect from electrical stimulation in the intermediate hemisphere of the cerebellar cortex is producing its effect by inhibiting the IPN.

4.3 Overall Findings, Implications, & Confounds

Considering the results as a whole, there is a small amount of evidence to support the hypotheses. The results imply that the IPN may play a nociceptive role that is inhibited by activation of the intermediate hemisphere of the cerebellar cortex. This was best supported by 1) antinociceptive effects produced by Left and Right Stimulation in the ACSF group; 2) GABA in the IPN was able to facilitate descending inhibition by inhibiting the output of the IPN during noxious stimulation. Another important finding was that there were no changes in brush. This is consistent with previous descending inhibition studies (Senapati et al., 2005a; Senapati et al., 2005b; Senapati et al., 2005c; Hagains et al., 2011a; Hagains et al., 2011b) and suggests that there is minimal, if any, influence on tactile sensation at the spinal level.

While the results of this experiment suggest that IPN is nociceptive, it is still unclear how the IPN produces its nociceptive influence. The IPN consists mostly of glutamatergic neurons (Nieuwenhuys, Voogd, & Huijzen, 2008). One site of interest that the IPN projects to is the PAG (Teune et al., 2000). The nature of the connection of the PAG is unknown. It is possible that the IPN sends excitatory signals to the PAG that activate inhibitory interneurons, thereby inhibiting the PAG and descending facilitation. This would account for the nociceptive nature of the IPN. Alternatively, the IPN also sends out minor inhibitory afferents (Nieuwenhuys, Voogd, & Huijzen, 2008). These afferents could synapse with the PAG and inhibit descending inhibition that way. Understanding the nature of the projection from the IPN to other sites would be useful for understanding how it is affecting the descending inhibitory system.

While this experiment does not define the cerebellum's role in pain, it does bring us a step closer to understanding how it modulates pain. Discovering that the IPN is a nociceptive nucleus is a novel finding. It explains why stimulation of the anterior cerebellar cortex is so effective at inhibiting nociception. It may also explain why the cerebellum is so active during pain. Whenever an organism gets injured, it needs to protect its wound to promote healing; the cerebellum may have a protective mechanism that facilitates nociception in order to support more expedient healing. In a similar situation, an injured organism that is being threatened may need to escape further injury. The cerebellar cortex may play a larger role here to help provide antinociception for escape/movement.

There are some confounds that need to be taken into consideration. Sample sizes in all groups could have been larger, but this was especially true for Lidocaine. Having a sample size of n = 4 greatly reduces the power to observe an effect. Other effects too though may have manifested with more power from larger samples, especially the Pre-Treatment electrical stimulation effects. Also because some rats had to be excluded from the study, the counterbalancing for ACSF was not very balanced and GABA was a little unbalanced as well. Ideally there would be enough cells for both conditions so that if one had a more than the other, it would be less influenced by a normal distribution of responses from both conditions.

4.4 Conclusion & Future Directions

This experiment was performed to better understand cerebellum-produced descending inhibition. Understanding descending inhibition and antinociception will empower us with more techniques to use to treat pain. It was partially determined here that stimulation of the intermediate hemisphere of the cerebellar cortex produces descending inhibition most likely by inhibiting the output of the interposed nucleus. Therefore, it was also determined that the interposed nucleus is a nociceptive brain site. To build on this, it would be useful to look at spinal dorsal horn responses to electrical stimulation in the interposed nucleus. It would also be

beneficial to further explore the mechanism responsible for producing descending inhibition via inhibition of the interposed nucleus.

APPENDIX A

LIDOCAINE GROUP

The Lidocaine group had a sample size of n =4 (Right Stimulation first = 2; Left Stimulation first = 2). Lidocaine was expected to have a blocking or attenuating effect upon the antinociceptive effects from brain stimulation on the left side but not the right, since the drug was injected into the left IPN. To test such a hypothesis, there would first need to be inhibition produced by the brain stimulation in either side of the cerebellar cortex. For pressure, neither Left nor Right Stimulation produced any inhibition for Pre-Treatment conditions: Lidocaine/Pressure/Control 1/Pre-Treatment – Lidocaine/Pressure/Left Stimulation/Pre-Treatment, NS; Lidocaine/Pressure/Control 2/Pre-Treatment – Lidocaine/Pressure/Right Stimulation/Pre-Treatment, NS (Figure A). Interestingly though, Left Stimulation again did not inhibit pressure in the Post-Treatment condition but Right Stimulation did: Lidocaine/Pressure/Control 1/Post-Treatment – Lidocaine/Pressure/Left Stimulation/Post-Treatment, NS; Lidocaine/Pressure/Control 2/Post-Treatment – Lidocaine/Pressure/Right Stimulation/Post-Treatment, $p < 0.05$ (Figure A). Additionally, Right Stimulation was

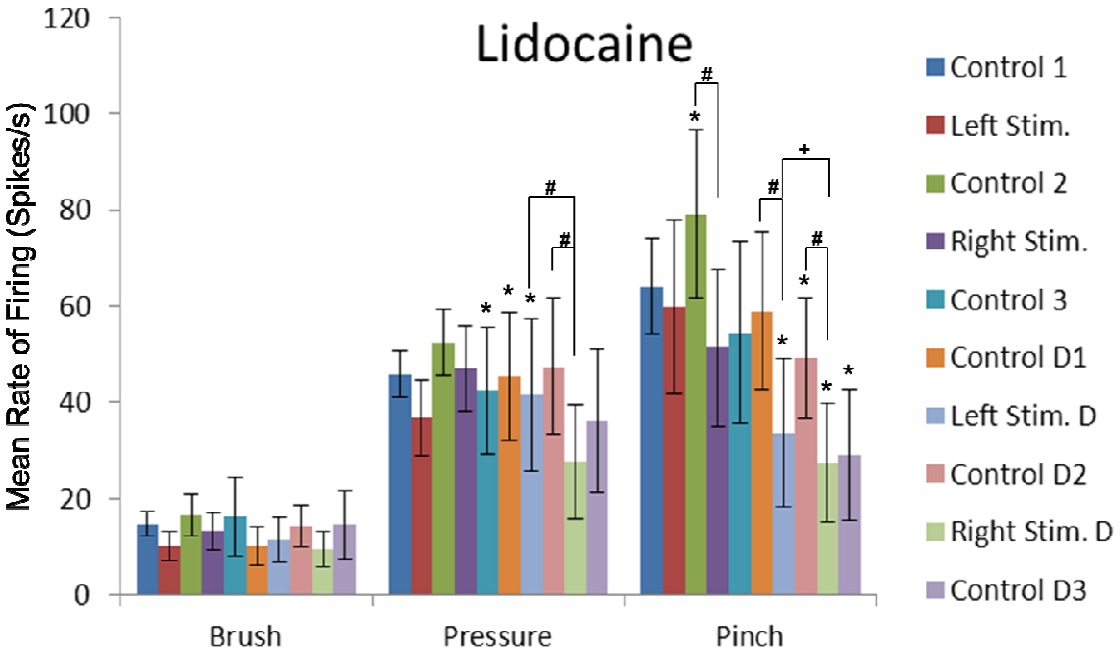

Figure A. Mean ± SEM responses of dorsal horn neurons from the Lidocained group. Comparisons for this graph were made with respective Control 1 conditions. Significant differences are represented by * $p < 0.05$.

significantly lower than Left Stimulation: Lidocaine/Pressure/Left Stimulation/Post-Treatment – Lidocaine/Pressure/Right Stimulation/Post-Treatment. In support of the hypothesis, these results may suggest that the lidocaine was successfully blocking the inhibitory effect of cerebellar cortex stimulation, but the results would be better supported with Pre-Treatment inhibition from both Left and Right Stimulation.

Pinch had a slightly different pattern of responding and did not provide strong support for the hypothesis either. In the Pre-Treatment condition, Right Stimulation inhibited pinch but Left Stimulation did not: Lidocaine/Pinch/Control 1/Pre-Treatment – Lidocaine/Pinch/Left Stimulation/Pre-Treatment, NS; Lidocaine/Pinch/Control 2/Pre-Treatment – Lidocaine/Pinch/Right Stimulation/Pre-Treatment, $p < 0.05$. In the Post-Treatment condition, both Left and Right Stimulation inhibited pinch: Lidocaine/Pinch/Control 1/Post-Treatment – Lidocaine/Pinch/Left Stimulation/Post-Treatment, $p < 0.05$; Lidocaine/Pinch/Control 2/Post-Treatment – Lidocaine/Pinch/Right Stimulation/Post-Treatment, $p < 0.05$. And finally, pinch was inhibited somewhat more (marginally significant) during Right Stimulation than during Left Stimulation, which offers a little support to the hypothesis: Lidocaine/Pinch/Left Stimulation/Post-Treatment – Lidocaine/Pinch/Right Stimulation/Post-Treatment, $p < 0.10$. These results suggest that there may be some support for the hypothesis, but there is not enough evidence to give a definitive statement.

Brush did not have any statistical differences again leaving tactile activity unaffected. Other comparisons of interest though were between the controls. The controls were not stable throughout the experiment for pressure or pinch. Pressure responses were different from the initial control at Control 3/Pre-Treatment and Control 1/Post-Treatment: Lidocaine/Pressure/Control 1/Pre-Treatment – Lidocaine/Pressure/Control 3/Pre-Treatment, $p < 0.05$; Lidocaine/Pressure/Control 1/Pre-Treatment – Lidocaine/Pressure/Control 1/Post-Treatment, $p < 0.05$ (Figure A). Pinch became significantly less active across time except for Control 2 in the Pre-Treatment: Lidocaine/Pinch/Control 1/Pre-Treatment –

Lidocaine/Pinch/Control 2/Pre-Treatment, $p < 0.05$; Lidocaine/Pinch/Control 1/Pre-Treatment – Lidocaine/Pinch/Control 2/Post-Treatment, $p < 0.05$; Lidocaine/Pinch/Control 3/Post-Treatment – Lidocaine/Pinch/Control 2/Pre-Treatment, $p < 0.05$. Pressure responses did not provide any clear indication the mechanism. The results for the responses to pinch suggest that the lidocaine was facilitating inhibition, especially given how drastic the differences were Pre- and Post-Treatment.

Lidocaine had some potentially interesting results. It was expected to block the effects of antinociception from cerebellar cortex electrical stimulation on the left side but not the right. Although pressure responses were not inhibited for the Pre-Treatment condition, Right Stimulation did inhibit pressure responses in the Post-Treatment condition in contrast to Left Stimulation failing to produce inhibition. This evidence would have been rather convincing if the Pre-Treatment condition had inhibition during both brain stimulations; as is though, this weakly suggests that lidocaine was blocking the antinociceptive effects of Left Stimulation. This effect may have been more apparent with a larger sample size.

Pinch responses also provided some support for the hypothesis. In the Pre-Treatment condition, Pinch was inhibited by Right Stimulation but not Left Stimulation. In the Post-Treatment, Pinch was inhibited by both Right and Left Stimulation. This differed from Pressure; however, Right Stimulation was marginally lower than Left Stimulation meaning that Right Stimulation was being inhibited slightly more. While this is in support of the hypothesis, it would have been more convincing had Left Stimulation inhibited pinch responses in the Pre-Treatment condition. Instead, similar patterns emerged from Pre- to Post-Treatment with Right Stimulation also having a more inhibitory effect in the Pre-Treatment.

Lidocaine also seemed to produce an inhibitory trend following injection. In spite of similar control means in the Pre-Treatment condition, Control 3 dropped quite bit in the Post-Treatment condition. Right Stimulation too had a much lower response post-treatment. This may indicate that lidocaine inhibits both the nociceptive and antinociceptive effects of noxious

mechanical stimulation. This would result from lidocaine blocking nociception in the IPN; if this were the case, pressure and/or pinch would be inhibited by Left Stimulation/Pre-Treatment and then be unaffected during Left Stimulation Post-Treatment. This was not able to be tested though because Pressure and Pinch were not inhibited by Left Stimulation/Pre-Treatment. If lidocaine was inhibiting the IPN from nociceptive processing, it could also potentially lead to a new baseline following lidocaine injection. This idea was partially supported by the inhibitory trend for both Pressure and Pinch. It would also help here to have a good control (ACSF) here to determine if Lidocaine was truly facilitating any antinociception.

APPENDIX B

BICUCULLINE GROUP

Bicuculline had a sample size of n = 7 (Right Stimulation first = 3; Left Stimulation first = 4). Bicuculline was expected to block the inhibition produced by electrical stimulation in the left cerebellar cortex while leaving the inhibition produced by stimulating the right cerebellar cortex intact. This hypothesis was able to be tested because there were no changes observed for all of the Bicuculline condition.

The bicuculline group did not have any significant effects. Without any Pre-Treatment effects, it was impossible to test any hypotheses concerning bicuculline. It may be worth noting though that Bicuculline/Post-Treatment consistently had higher means than those in the Pre-Treatment. Had Post-Treatment means been significantly higher than Pre-Treatment, there would have been some support for bicuculline blocking any GABA activity in the IPN, which would create a sensitizing effect. This in turn would suggest that the IPN has a nociceptive role and that activation of the intermediate cerebellar cortex is capable of inhibiting that nociceptive activity. It would also fit well with the inhibitory effects observed during GABA/Post-Treatment.

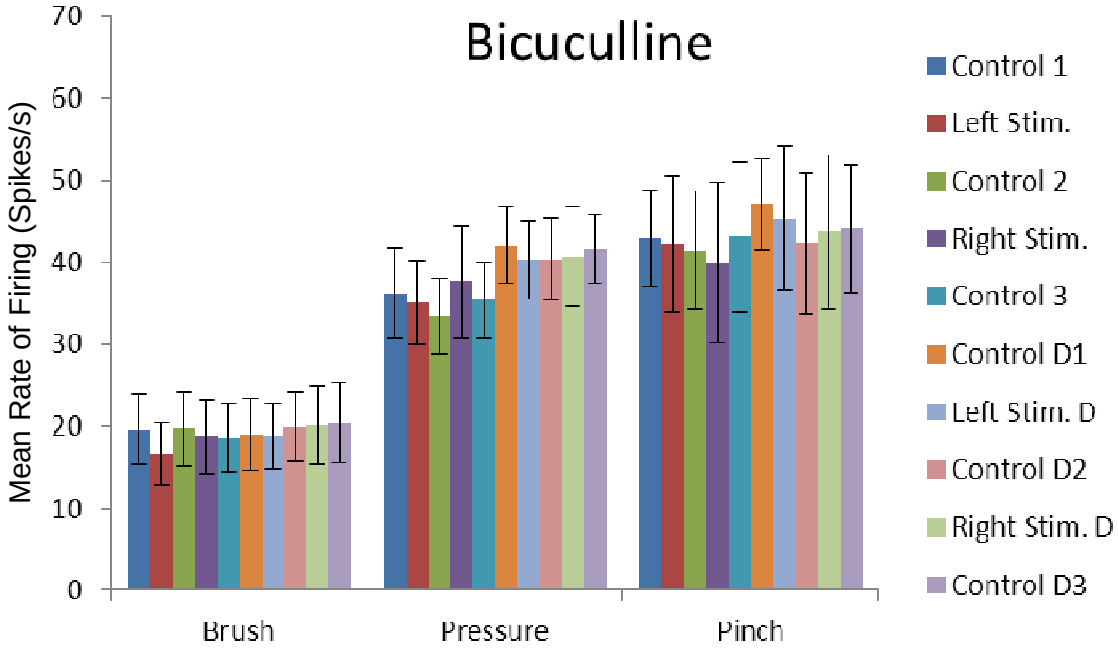

Figure B. Mean ± SEM responses of dorsal horn neurons from the Bicuculline group. Comparisons for this graph were made with respective Control 1 conditions. Significant differences are represented by * $p < 0.05$.

REFERENCES

Basbaum A.I. & Fields H.L. (1979). The origin of descending pathways in the dorsolateral funiculus of the spinal cord of the cat and rat: further studies on the anatomy of pain modulation. *J Comp Neurol* 187: 513-531.

Basbaum A.I. & Fields H.L. (1984). Endogenous pain control systems: brainstem spinal pathways and endorphin circuitry. *Annu Rev Neurosci* 7: 309-338.

Bengtsson S.L., Ehrsson H.H., Forssberg H. & Ullen F. (2004). Dissociating brain regions controlling the temporal and ordinal structure of learned movement sequences. *Eur J Neurosci* 19: 2591-2602.

Bolk L. (1906). *Das Cerebellum der Saugetiere F. Bohn*. Fischer: Jena.

Buisseret-Delmas C., Yatim N., Buisseret P. & Angaut P. (1993). The X zone and CX subzone of the cerebellum in the rat. *Neurosci Res* 16: 195-207.

Casey K.L., Minoshima S., Berger K.L., Koeppe R.A., Morrow T.J. & Frey K.A. (1994). Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. *J Neurophysiol* 71: 802-807.

Casey K.L., Minoshima S., Morrow T.J. & Koeppe R.A. (1996). Comparison of human cerebral activation pattern during cutaneous warmth, heat pain, and deep cold pain. *J Neurophysiol* 76: 571-581.

Chambers W.W. & Sprague J.M. (1955). Functional localization in the cerebellum. I. Organization in longitudinal cortico-nuclear zones and their contribution to the control of posture, both extrapyramidal and pyramidal. *J Comp Neurol* 103: 105-129.

Chambers W.W. & Sprague J.M. (1975). Functional localization in the cerebellum. II. Somatotopic organization in cortex and nuclei. *AMA Arch Neurol Psychiatry* 74: 653-680.

Chiang C.Y., Hu J.W. & Sessle B.J. (1994). Parabrachial area and nucleus raphe magnus-induced modulation of nociceptive and nonnociceptive trigeminal subnucleus caudalis neurons activated by cutaneous or deep inputs. *J Neurophysiol* 71: 2430-2445.

Chiang C.Y., Sessle B.J. & Hu J.W. (1995). Parabrachial area and nucleus raphe magnus-induced modulation of electrically evoked trigeminal subnucleus caudalis neuronal responses to cutaneous or deep A-fiber and C-fiber inputs in rats. *Pain* 62: 61-68.

Condés-Lara M., Martínez-Lorenzana G., Rodríguez-Jiménez J. & Rojas-Piloni G. (2008). Paraventricular hypothalamic nucleus stimulation modulates nociceptive responses in dorsal horn wide dynamic range neurons. *Neurosci Lett* 444: 199-202.

Condés-Lara M., Rojas-Piloni G., Martínez-Lorenzana G., López-Hidalgo M. & Rodríguez-Jiménez J. (2009). Hypothalamospinal oxytocinergic antinociception is mediated by GABAergic and opiate neurons that reduce A-delta and C fiber primary afferent excitation of spinal cord cells. *Brain Res* 1247: 38-49.

Cox V.C. & Valenstein E.S. (1965). Attenuation of aversive properties of peripheral shock by hypothalamic stimulation. *Science* 149: 323-325.

Dey P.K. & Ray A.K. (1982). Anterior cerebellum as a site for morphine analgesia and post-stimulation analgesia. *Indian J Physiol Pharmacol* 26: 3-12.

Doyon J. & Benali H. (2005). Reorganization and plasticity in the adult brain during learning of motor skills. *Curr Opin Neurobiol* 15: 161-167.

Doyon J., Song A.W., Karni A., Lalonde F., Adams M.M. & Ungerleider L.G. (2002). Experience-dependent changes in cerebellar contributions to motor sequence learning. *Proc Natl Acad Sci USA* 99: 1017-1022.

Duncan G.H., Kupers R.C., Marchand S., Villemure J.G., Gybels J.M. & Bushnell M.C. (1998). Stimulation of human thalamus for pain relief: possible modulatory circuits revealed by positron emission tomography. *J Neurophysiol* 80: 3326-3330.

Ekerot C.F., Garwicz M. & Schouenborg J. (1991a). The postsynaptic dorsal column pathway mediates cutaneous nociceptive information to cerebellar climbing fibres in the cat. *J Physiol (Lond)* 441: 275-284.

Ekerot C.F., Garwicz M. & Schouenborg J. (1991b). Topography and nociceptive receptive fields of climbing fibres projecting to the cerebellar anterior lobe in the cat. *J Physiol (Lond)* 441: 257-274.

Ekerot C.F., Gustavsson P., Oscarsson O. & Schouenborg J. (1987a). Climbing fibres projecting to cat cerebellar anterior lobe activated by cutaneous A and C fibres. *J Physiol (Lond)* 386: 529-538.

Ekerot C.F., Oscarsson O. & Schouenborg J. (1987b)Stimulation of cat cutaneous nociceptive C fibres causing tonic and synchronous activity in climbing fibres. *J Physiol (Lond)* 386: 539-546.

Fields H.L. & Basbaum A.I. (1984). Endogenous Pain Control Mechanisms. In: *Textbook of Pain*, edited by Wall P.D. & Melzack R.M. New York: Churchill Livingstone, p. 142-152.

Fields H.L., Basbaum A.I., Clanton C.H. & Anderson S.D. (1977). Nucleus raphe magnus inhibition of spinal cord dorsal horn neurons. *Brain Res* 126: 441-453.

Fields H.L., Wagner G.M. & Anderson S.D. (1975). Some properties of spinal neurons projecting to the medial brain-stem reticular formation. *Exp Neurol* 47: 118-134.

Fiez J.A. 1996). Cerebellar contributions to cognition. *Neuron* 16: 13-15.

Follett K.A. & Gebhart G.F. 1992). Modulation of cortical evoked potentials by stimulation of nucleus raphe magnus in rats. *J Neurophysiol* 67: 820-828.

Ghez C. & Thach W.T. (2000). The cerebellum. In: *Principles of Neural Science*, edited by Kandel E.R., Schwartz J.H. & Jessell T.M. New York: McGraw-Hill, p. 832-852.

Glickstein M. & Doron K. (2008). Cerebellum: connections and functions. *Cerebellum* 7: 589-594.

Gybels J. (2001). Thalamic stimulation in neuropathic pain: 27 years later. *Acta Neurol Belg* 101: 65-71.

Hagains C.E., He J.W., Chiao J.C., & Peng Y.B. (2011a). Septal stimulation inhibits spinal cord dorsal horn neuronal activity. *Brain Res* 1382: 189-197.

Hagains C.E., Senapati A.K., Huntington P.J., He J.W. & Peng Y.B. (2011b). Inhibition of spinal cord dorsal horn neuronal activity by electrical stimulation of the cerebellar cortex. *J Neurophysiol* 106: 2515-2522.

Hagbarth K.E. & Kerr D.I.B. (1954). Central influences on spinal afferent conduction. *J Neurophysiol* 17: 295-307.

Hartmann-von Monakow K., Akert K. & Kunzle H. (1981). Projection of precentral, premotor and prefrontal cortex to the basilar pontine grey and to nucleus reticularis tegmenti pontis in the monkey (Macaca fascicularis). *Schweiz Arch Neurol Neurochir Psychiatr* 129: 189-208.

Hofbauer R.K., Fiset P., Plourde G., Backman S.B. & Bushnell M.C. (2004). Dose-dependent effects of propofol on the central processing of thermal pain. *Anesthesiology* 100: 386-394.

Holden J.E., Farah E.N. & Jeong Y. (2005). Stimulation of the lateral hypothalamus produces antinociception mediated by 5-HT1A, 5-HT1B and 5-HT3 receptors in the rat spinal cord dorsal horn. *Neurosci* 135: 1255-1268.

Holden J.E. & Pizzi J.A. 2008). Lateral hypothalamic-induced antinociception may be mediated by a substance P connection with the rostral ventromedial medulla. *Brain Res* 1214: 40-49.

Holden J.E., Pizzi J.A. & Jeong Y. (2009). An NK1 receptor antagonist microinjected into the periaqueductal gray blocks lateral hypothalamic-induced antinociception in rats. *Neurosci Lett* 453: 115-119.

Holmes G. (1939). The cerebellum of man. *Brain* 62: 1-30.

Hoover J.E. & Strick P.L. (1999). The organization of cerebellar and basal ganglia outputs to primary motor cortex as revealed by retrograde transneuronal transport of herpes simplex virus type 1. *J Neurosci* 19: 1446-1463.

Hosobuchi Y., Adams J.E. & Rutkin B. (1975). Chronic Thalamic and Internal Capsule Stimulation for the Control of Central Pain. *Surg Neurol* 4: 91-92.

Huisman A.M., Kuypers H.G. & Verburgh C.A. (1981). Quantitative differences in collateralization of the descending spinal pathways from red nucleus and other brain stem cell groups in rat as demonstrated with the multiple fluorescent retrograde tracer technique. *Brain Res* 209: 271-286.

Iadarola M.J., Berman K.F., Zeffiro T.A., Byas-Smith M.G., Gracely R.H., Max M.B. & Bennet (1998). Neural activation during acute capsaicin-evoked pain and allodynia assessed with PET. *Brain* 121: 931-947.

Ingvar M. & Hsieh J.C. (1999). The image of pain. In: *Textbook of Pain*, edited by Wall P.D. & Melzack R.M. New York: Churchill Livingstone, p. 215-233.

Ito M. (1984). *The Cerebellum and Neural Control*. New York: Raven Press.

Ivry R.B. and Keele S.W. (1989). Timing functions of the cerebellum. *J Cognitive Neurosci* 1: 136-152.

Jörntell H., Bengtsson F., Schonewille M. & De Zeeuw C.I. (2010). Cerebellar molecular layer interneurons – computational properties and roles in learning. *Trends in Neuroscience* 33: 524-532.

Kelly R.M. & Strick P.L. (2003). Cerebellar loops with motor cortex and prefrontal cortex of a nonhuman primate. *J Neurosci* 23: 8432-8444.

Kuypers H.G. & Lawrence D.G. (1967). Cortical projections to the red nucleus and the brain stem in the Rhesus monkey. *Brain Res* 4: 151-188.

Larsell O. (1952). The morphogenesis and adult pattern of the lobules and tissues of the cerebellum of the white rat. *J Comp Neurol* 97: 281-356.

Larsell O (1970). *The comparative anatomy and histology of the cerebellum from monotremes through apes*, edited by Janson J. Minnesota: University of Minnesota Press.

Liu M., Liu X. & Liu B. (1991). The analgesic effect of red nucleus and strengthening effect thereof to the acupuncture analgesia. *Zhen Ci Yan Jiu* 16: 48-53.

Lopez R., Young S.L. & Cox V.C. (1991). Analgesia for formalin-induced pain by lateral hypothalamic stimulation. *Brain Res* 563: 1-6.

Marchand S., Kupers R.C., Bushnell M.C. & Duncan G.H. (2003). Analgesic and placebo effects of thalamic stimulation. *Pain* 105: 481-488.

Mason S.T. & Iversen S.D. (1977). An investigation of the role of cortical and cerebellar noradrenaline in associative motor learning in the rat. *Brain Res* 134: 513-527.

Meng I.D., Hu J.W. & Bereiter D.A. (2000). Parabrachial area and nucleus raphe magnus inhibition of corneal units in rostral and caudal portions of trigeminal subnucleus caudalis in the rat. *Pain* 87: 241-251.

Mihailoff G.A. (1983). Intra- and interhemispheric collateral branching in the rat pontocerebellar system, a fluorescence double-label study. *Neuroscience* 10: 141-160.

Moulton E.A., Schmahmann J.D., Becerra L. & Borsook D. (2010). The cerebellum and pain: passive integrator or active participator? *Brain Res Rev* 65: 14-27.

Nieuwenhuys R., Voogd J. & van Huijzen C. (2008). *The Human Central Nervous System*. New York: Springer.

Owen S.L., Green A.L., Nandi D.D., Bittar R.G., Wang S. & Aziz T.Z. (2007). Deep brain stimulation for neuropathic pain. *Acta Neurochir Suppl* 97: 111-116.

Owen S.L., Green A.L., Stein J.F. & Aziz T.Z. 2006). Deep brain stimulation for the alleviation of post-stroke neuropathic pain. *Pain* 120: 202-206.

Palay S.L. & Chan-Palay V. (1974). *Cerebellar Cortex: Cytology and Organization*. New York: Springer.

Peng Y.B., Lin Q., & Willis W.D. (1996). Effects of GABA and glycine receptor antagonists on the activity and PAG-induced inhibition of rat dorsal horn neurons. *Brain Res* 736: 189-201.

Petersen S.E., Fox P.T., Posner M.I., Mintun M. & Raichle M.E. (1988). Positron emission tomographic studies of the cortical anatomy of single-word processing. *Nature* 331: 585-589.

Rees H. & Roberts M.H. (1993). The anterior pretectal nucleus: a proposed role in sensory processing. *Pain* 53: 121-135.

Rees H., Terenzi M.G. & Roberts M.H. (1995). Anterior pretectal nucleus facilitation of superficial dorsal horn neurones and modulation of deafferentation pain in the rat. *J Physiol (Lond)* 489: 159-169.

Reis D.J., Doba N & Nathan M.A. (1973). Predatory attack, grooming, and consummatory behaviors evoked by electrical stimulation of cat cerebellar nuclei. *Science* 182: 845-847.

Reynolds D.V. (1969). Surgery in the rat during electrical analgesia induced by focal brain stimulation. *Science* 164: 444-445.

Ruigrok T.J. & Cella F. (1995). Precerebellar nuclei and red nucleus. In: *The Rat Nervous System*, edited by Paxinos G. New York: Academic Press, p. 277-308.

Rutherford J.G., Anderson W.A. & Gwyn D.G. (1984). A reevaluation of midbrain and diencephalic projections to the inferior olive in rat with particular reference to the rubro-olivary pathway. *J Comp Neurol* 229: 285-300.

Saab C.Y., Kawasaki M., Al-Chaer E.D. & Willis W.D. (2001). Cerebellar cortical stimulation increases spinal visceral nociceptive responses. *J Neurophysiol* 85: 2359-2363.

Saab C.Y. & Willis W.D. (2001). Nociceptive visceral stimulation modulates the activity of cerebellar Purkinje cells. *Exp Brain Res* 140: 122-126.

Saab C.Y. & Willis W.D. (2003). The cerebellum: organization, functions and its role in nociception. *Brain Res Brain Res Rev* 42: 85-95.

Scheibel M.E. & Scheibel A.B. (1954). Observations on the intracortical relations of the climbing fibers of the cerebellum; a Golgi study. *J Comp Neurol* 101: 733-763.

Schmahmann J.D. & Sherman J.C. (1998). The cerebellar cognitive affective syndrome. *Brain* 121: 561-579.

Senapati A.K., Huntington P.J., & Peng Y.B. (2005a). Spinal dorsal horn neuron response to mechanical stimuli is decreased by electrical stimulation of primary motor cortex. *Brain Res* 1036: 173-179.

Senapati A.K., Huntington P.J., LaGraize S.C., Wilson H.D., Fuchs P.N., & Peng Y.B. (2005b). Electrical stimulation of the primary somatosensory cortex inhibits spinal dorsal horn neuron activity. *Brain Res* 1057: 134-140.

Senapati A.K., LaGraize S.C., Huntington P.J., Wilson H.D., Fuchs P.N., & Peng Y.B. (2005c). Electrical stimulation of the anterior cingulate cortex reduces responses of rat dorsal horn neurons to mechanical stimuli. *J Neurophysiol* 94: 845-851.

Shinoda Y., Sugihara I., Wu H.S. & Sugiuchi Y. (2000). The entire trajectory of single climbing and mossy fibers in the cerebellar nuclei and cortex. *Prog Brain Res* 124: 173-186.

Siegel P. & Wepsic J.G. (1974). Alteration of nociception by stimulation of cerebellar structures in the monkey. *Physiol Behav* 13: 189-194.

Strick P.L., Dum R.P. & Fiez J.A. (2009). Cerebellum and nonmotor function. *Annu Rev Neurosci* 32: 413-434.

Svensson P., Minoshima S., Beydoun A., Morrow T.J. & Casey K.L. (1997). Cerebral processing of acute skin and muscle pain in humans. *J Neurophysiol* 78: 450-460.

Terenzi M.G., Rees H. & Roberts M.H. (1992). The pontine parabrachial region mediates some of the descending inhibitory effects of stimulating the anterior pretectal nucleus. *Brain Res* 594: 205-214.

Teune T.M., van der Burg J., van der Moer J., Voogd J. & Ruigrok T.J. (2000). Topography of cerebellar nuclear projections to the brain stem in the rat. *Prog Brain Res* 124: 141-172.

Timmann D., Brandauer B., Hermsdorfer J., Ilg W., Konczak J. Gerwig M., Gizewski E.R. & Schoch B. (2008). Lesion-symptom mapping of the human cerebellum. *Cerebellum* 7: 602-606.

Villarreal C.F., Kina V.A. & Prado W.A. (2004). Antinociception induced by stimulating the anterior pretectal nucleus in two models of pain in rats. *Clin Exp Pharmacol Physiol* 31: 608-613.

Voogd J. (1995). Cerebellum. In: *The Rat Nervous System*, edited by Paxinos G. New York: Academic Press, p. 309-350.

Voogd J. (2003). The human cerebellum. *Journal of Chemical Neuroanatomy* 26: 243-252.

Voogd J. & Glickstein M. (1998). The anatomy of the cerebellum. *Trends Neurosci* 21: 370-375.

Wilson D.G., Rees H. & Roberts M.H. (1991). The antinociceptive effects of anterior pretectal stimulation in tests using thermal, mechanical and chemical noxious stimuli. *Pain* 44: 195-200.

Wu J. & Chen P.X. (1990). Cerebellar evoked potential elicited by stimulation of C-fiber in saphenous nerve of cat. *Brain Res* 522: 144-146.

Yang S. & Follett K.A. (2003). Electrical stimulation of thalamic Nucleus Submedius inhibits responses of spinal dorsal horn neurons to colorectal distension in the rat. *Brain Res Bull* 59: 413-420.

Zimmermann M. (1983). Ethical guidelines for investigations of experimental pain in conscious animals. *Pain* 16: 109-110.

BIOGRAPHICAL INFORMATION

Chris earned a B.S. in Psychology from Texas Wesleyan University. There he developed an interest in biological psychology. He did his graduate work at the University of Texas at Arlington. In his time there, he worked in Dr. Peng's lab studying physiological mechanisms of pain. He earned an M.S. in Experimental Psychology mentored under Dr. Peng and is now completing his Ph.D. He authored and co-authored six peer-reviewed scholarly papers while at UTA and earned the Verne Cox Outstanding Graduate Research Award. He plans to use his degree to continue studying physiological mechanisms in pain.