SEPTAL STIMULATION INHIBITS SPINAL CORD DORSAL HORN NEURONAL ACTIVITY

by

CHRISTOPHER E. HAGAINS

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ABSTRACT

SEPTAL STIMULATION INHIBITS SPINAL CORD

DORSAL HORN NEURONAL ACTIVITY

Christopher E. Hagains, MS

The University of Texas at Arlington, 2008

Supervising Professor: Dr. Yuan Bo Peng

Deep Brain Stimulation is a useful technique for relieving chronic pain in patients that have exhausted their options. The septum has been a target for such treatment. The purpose of this study was to determine if electrical stimulation in the medial septum diagonal band of broca (MSDB) would reduce nociceptive neuronal activity in the spinal cord of rats. This interest was addressed using a Grass Stimulator to stimulate the MSDB on one side of the brain while recording mostly wide dynamic range neurons in the lumbar region of the spinal cord. Neuronal activity was initiated by graded mechanical stimulation of the hind paws (brush, pressure, and pinch). Responses to pressure were significantly reduced in both sides of the spinal cord by 1V, 5V, 10V, and 20V, 100Hz, and 0.1 ms duration MSDB stimulation. Responses to pinch

in the spinal cord were significantly reduced bilaterally by 1V, 5V, 10V, and 20V, 100Hz, and 0.1 ms duration. However, there was no change in responses to brush. Additionally, inhibition scores were calculated and used to examine the extent of inhibition for each parameter of electrical stimulation. These data suggested that 5V was adequate for achieving optimal inhibition. The ratio of stimulation-on and stimulation-off was also compared within each segment of mechanical stimulation. These results indicated that neuronal responses were being inhibited when the stimulation was on. In summary, it is concluded that unilateral stimulation of the MSDB produces bilateral inhibition of spinal cord dorsal horn neuronal responses to noxious mechanical stimuli.

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CHAPTER 1

INTRODUCTION

1.1 The Septum

The septum is a telencephalic structure of the forebrain. It is involved in multiple functions and has direct reciprocal connections to the diencephalon, mesencephalon, lower brain stem, spinal cord, and other limbic structures (Jakab & Leranth, 1995). The septum is involved in producing many behavioral outcomes. It influences learning and memory by way of connections to the hippocampus. Green and Arduini (1954) implicated the septum as a vital component of the hippocampal theta rhythm; they found that unilateral lesions to the septum or the pathways connecting the septum to the hippocampus abolished theta waves in the ipsilateral hippocampus. Morgane, Galler, and Mokler (2005) reviewed the influence of theta activation on long term potentiation (LTP) or neuroplasticity. Neuroplasticity is a necessary component of learning and memory, and Morgane et al. assert that theta activation is required for LTP based on previous research (Larson, Wong, & Lynch 1986; Balleine & Curthoys, 1991; Buzsaki, 2002; and Bronzino et al., 1996, 1996, 1997, 1999). If the septum is necessary for theta rhythm and theta rhythm is necessary for LTP, then the septum's function in learning and memory is deep-seated.

The septum plays a role in sexual behavior for both genders in rats. In males, septal innervation of the medial preoptic area may influence sexual behavior, since that

nucleus is critical to pelvic thrust (Hansen, Kohler, Goldstein, & Steinbusch, 1982). Also Dohanich and McEwen (1986) found that lesions of the horizontal limb in female rats led to unreceptive sexual behaviors.

Stimulation of the septum has been of interest for its capacity to be positively reinforcing in rats (Olds & Milner, 1954; Olds, 1958). In the famous Olds & Milner experiment, they found that rats will push a bar to receive electrical stimulation in the septum. This research led to ideas that the septum may be a pleasure-producing brain site.

In humans, Gol (1967) stimulated the septum in an attempt to treat patients suffering severe chronic pain. His efforts were fruitful but success was inconsistent. Gol reported that a side effect of septal stimulation was increased awareness or arousal. Schvarcz (1993) used septal stimulation to treat 19 patients with neurogenic pain over the course of 1-5 years. Each patient had initially reported severe pain. Although no patient reported total elimination of pain, septal stimulation effectively treated pain in 63%.

The septum is made up of several sections. Throughout the history of septum research, its borders and sections have been modified and difficult to define. Jakab and Leranth (1995) used a conglomerate of the most recent research and techniques to build a nomenclature. These sections include medial, lateral, and posterior septal divisions. These sections are further subdivided. The lateral septal division consists of dorsal, intermediate, and ventral components; the posterior section is made up of the bilateral septofimbrial nucleus and triangular septal nucleus; the medial septal division is divided

into the medial septal nucleus (MS) and the diagonal band of Broca (DB). The DB is divided once more into the vertical (VDB) and horizontal (HDB) limbs. Because of the interconnectedness and shared projections of the medial septal division as a whole, it is typically described in the literature as one nucleus referred to as the medial septum diagonal band complex (MSDB). Since this study involves stimulation of the HDB, this paper will focus on the contributions of the MSDB.

1.1.1 MSDB

The MSDB is located centrally in the septum along the midline of the brain ventral to the corpus collosum. Jakab & Leranth (1995) provide an overview of the organization of the MSDB. Afferent connections of the MSDB arrive from the hippocampus, diencephalon, brain stem, and spinal cord. The MSDB consists mostly of cholinergic and GABA-ergic projections. MSDB projections terminate in many limbic structures, cortical structures, thalamus, hypothalamus, and the periaqueductal gray (PAG) and raphe nuclei of the brain stem. There is also some evidence that the MSDB sends projections to the olfactory bulb (Divac, 1975). This paper will focus on projections that are antinociceptive.

1.1.1.1 Projections to the Diencephalon

One major projection of the MSDB terminates in the lateral hypothalamic area (LH) (Tomimoto, Kamo, Kameyama, McGeer, & Kimura, 1987). The LH is the strongest of the MSDB projections to the hypothalamus (Tomimoto et al., 1987). This projection is cholinergic in nature and appears to be excitatory, since microinjection of carbachol into the LH will activate descending inhibition of spinal cord neurons

(Holden & Naleway, 2001). The LH has been classified as a pain modulator because it meets a high standard for exerting control over pain (Dafny et al., 1996). Projections extending from the LH terminate in antinociceptive brain stem structures, such as periaqueductal gray (PAG), locus coeruleus, and raphe nuclei (Saper, Swanson, & Cowan, 1979; Swanson, 1976). Electrical stimulation in the LH of rats increases tolerance to aversive foot shock, which suggested that it increased the threshold for pain (Cox & Valenstein, 1965). In addition, stimulation of the LH attenuates tonic pain in rats (Lopez, Young, & Cox, 1991). This descending inhibition appears to be mostly attributable to a connection to the nucleus raphe magnus (NRM), since LH descending inhibition is attenuated by NRM lesions (Aimone et al., 1988) and acts on 5-HT_1 and 5-HT_3 receptors in the spinal cord (Holden, Farah, & Jeong, 2005). The LH also has a direct connection to the spinal cord that may play a role in sensory processing (Saper, Loewy, Swanson, & Cowan, 1976).

Another diencephalic structure that the MSDB projects to is the habenula (Swanson et al., 1979). The habenula efferent fiber fasciculus retroflexus also receives some septal projections (Murphy, DiCamillo, Haun, & Murray, 1996). Andres, von During, and Veh (1999) hypothesized that the habenula processes emotional information descending to the interpeduncular nucleus of the midbrain. Stimulation of the habenula produces antinociceptive effects in rats (Cohen & Melzack, 1985; Cohen & Melzack, 1986; Terenzi, Guimaraes, & Prado, 1990). It also has been shown to contribute to the analgesic properties of LH excitation (Fuchs & Cox, 1993).

1.1.1.2 Projections to the Brain Stem

The MSDB terminations in the midbrain include the PAG, dorsal raphe and medial raphe nuclei (Tomimoto et al., 1987). Projections at least to the dorsal raphe were shown to be bilateral (Swanson & Cowan, 1979). Basbaum and Fields (1984) review the antinociceptive qualities of these brain regions. The PAG projects to both the nucleus raphe magnus (NRM) and locus coeruleus (LC) and is well known for its antinociceptive qualities. The NRM descends to the spinal cord and inhibits nociceptive neuronal activity with serotonin (Millan, 2002). The LC has similar descending modulation of nociception using norepinephrine (Millan, 2002). This PAG-NRM-LC combination leads to the inhibition of dorsal horn spinal cord neurons.

1.1.2 Specific Aims

As mentioned above, the MSDB has direct projections to several nuclei involved in the inhibition of spinal nociception, and septum stimulation has shown some success in reducing pain in humans. The **goal** of this study is to clarify the underlying mechanisms of MSDB induced antinociception, by unilateral electrical stimulation in the MSDB while recording single unit spinal cord dorsal horn neuronal responses to mechanical stimuli. It was **hypothesized** that stimulation of the MSDB would induce inhibition of the spinal cord dorsal horn neurons, possibly through activation of the PAG descending inhibitory system. To test this hypothesis, the following specific aim was addressed.

Specific aim: To determine the effect of unilateral MSDB stimulation on responses of spinal cord dorsal horn neurons to peripheral graded mechanical stimuli. It

was expected that there would be inhibition of spinal dorsal horn neurons, possibly through direct and indirect projections from the MSDB to the PAG, NRM, and LC.

CHAPTER 2

METHODS

2.1 Methods

Seven adult male Sprague-Dawley rats were used for this project. All surgical procedures were approved by the University of Texas at Arlington Institutional Animal Care and Use Committee. The procedures were performed in accordance with the guidelines published by the Committee for Research and Ethical Issues of the International Association for the Study of Pain (Zimmermann, 1983).

2.1.1 Animal Preparation

Animals were anesthetized with an injection of sodium pentobarbital (50 mg/kg, i.p.). Once an animal was deemed unresponsive to tail-pinch, a laminectomy was performed to expose 3-4 cm of the spinal cord over the lumbosacral enlargement. A tracheotomy was performed in case artificial respiration was necessary. A jugular vein cannulation was performed to continuously administer sodium pentobarbital to the animals at a rate of 1.2 ml/hr. A craniotomy was performed to expose the brain for stereotaxic procedures. The head was then fixed in a stereotaxic frame. After surgeries the animals' spinal columns were fixed in a stereotaxic frame, the dura mater was removed, and mineral oil was poured over the spinal cord to preserve its moisture. Respiration was monitored, and body temperature was maintained at 37°C with a feedback controlled heating pad and rectal thermal sensor probe.

2.1.2 Histology

Following completion of all electrophysiological measurements, brains were extracted and stored in a 10% formalin solution. The brains were sliced into coronal sections 80µm thick and stained with thionin. Slides were viewed with a light microscope and photographed with a digital camera for confirmation of stimulation site (Figure 1).

2.1.3 Brain Stimulation

Brain stimulation was performed using a Grass Stimulator using a combination of the following parameters: 0.1 ms duration; 100 Hz; and 1V, 5V, 10V, and 20V. The stimulation was administered in train mode and was set for two second stimulation duration with two second intervals, respectively. A bipolar stimulating electrode was placed in the HDB (0.5 mm anterior to Bregma; 0.5 mm lateral to the left or right; 8.5 mm down).

2.1.4 Data Acquisition

Extracellular single unit recordings of spinal cord dorsal horn neurons were collected from both sides of spinal cord regions L4, L5, and L6 using a 10-12 MΩ tungsten microelectrode (FHS, Brunswick, ME). Cells were located by mechanical stimulation of the receptive field in the plantar region of the hind paw while navigating the electrode. Mechanical responses to brush, pressure, and pinch were recorded using SPIKE2 computer software program (CED, UK).

Wide Dynamic Range (WDR) spinal dorsal horn neurons, neurons associated with a response to innocuous and noxious mechanical stimuli (Willis and Coggeshall,

2004), were located for most measurements. Some high threshold spinal dorsal horn neurons were also used. Once the cell was identified, neuronal activity was recorded constantly to attain a baseline, experimental, and recovery measurement of brush, pressure, and pinch. The measurement was taken to assess the number of action potentials per second. The baseline and recovery measurements consisted of ten seconds of each mechanical stimulus with approximately twenty seconds between stimulations. The experimental conditions lasted about 12 seconds with 20 second intervals between mechanical stimulations and one minute between each electrical stimulation condition. Each experimental condition began with two seconds of mechanical stimulation alone followed by approximately two seconds of electrical stimulation. This occurred three times for a total of about twelve seconds. These segments were performed once per cell for each combination of parameters. Refer to Figures 2 and/or 3 for an illustration.

2.1.5 Data Analysis

2.1.5.1 Analysis 1

The data for this experiment consist of recordings of multiple cells from each rat. It was uncertain whether or not cell responses from an individual rat are more related to one another than cell responses across multiple rats; in other words, it was important to determine if 5 cells recorded from 1 rat is the same as recording 1 cell per rat for 5 rats. These two types of methods would result in experiments consisting of 10 rats with 50 cells or 50 rats with 50 cells, where the cells are the subject of interest. It was expected that cells' responses from one rat would not be more associated with each

other than they were to responses of cells in other rats. To test this hypothesis, an exploratory hierarchical cluster analysis (SPSS 15.0 for Windows statistical software) of neuronal responses to brush, pressure, and pinch from the control condition was used to determine if the cells should be categorized based on responses specific to a rat, groups of rats, or if they should be categorized based on responses unspecific to the rats. This type of analysis was useful for partitioning the data to reveal the number of groups that exist in this type of data set. After the data had been categorized, it allowed for determining if cells clustered based on rats or a group of rats. An analysis of only cell responses from the control condition was necessary for addressing this issue, since the interest of this analysis was in the relatedness of cells and not experimental manipulation.

2.1.5.2 Analysis 2

The average number of action potentials in two-second intervals corresponding to stimulation on/off was collected as follows: 2 s off, 2 s on, 2 s off, 2 s on, 2 s off, and 2 s on. Once all the numbers had been entered into the spreadsheet, an individual average was calculated for each cell in each experimental condition by using the following equation: $(2\text{ s off}_1 + 2\text{ s on}_1 + 2\text{ s off}_2 + 2\text{ s on}_2 + 2\text{ s off}_3 + 2\text{ s on}_3)/6$. This average was taken for each cell and then averaged one more time to get the experimental condition mean. These averages were compared to the control and recovery conditions (Figure 4). The data were used to run a 2 Side x (3 Mechanical x 6 Electrical) Mixed Factorial Design and post hoc Fisher LSD test (STATISTICA, StatSoft, OK). Comparisons between control and manipulated conditions were examined to determine

if the rate of action potentials were significantly reduced by electrical stimulation. The significance criterion was set at $p < 0.05$.

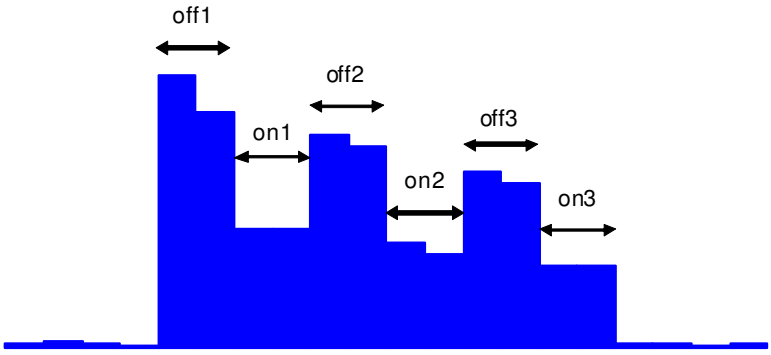


Figure 1. Representative diagram of a neuronal response to electrical stimulation presented as rate; sequence begins with stimulation off (off1) followed by stimulation (on1), etc.

It was expected that electrical stimulation in the HDB would produce inhibition of nociceptive spinal cord activity. This hypothesis was tested by considering the following: there would be an effect for Mechanical and Electrical stimulation; and there would be an interaction between Mechanical and Electrical stimulation. For the interaction, the following sub-hypotheses were considered in the post-hoc analysis for further support: control/pressure > pressure at 1V, 5V, 10V, and 20V; and control/pinch > pinch at 1V, 5V, 10V, and 20V.

2.1.5.3 Analysis 3

The amount of inhibition caused by each level of electrical stimulation was of interest. Inhibition scores were calculated for cell responses to 1V, 5V, 10V, and 20V using the formula: $2 \text{ s on}_1 + 2 \text{ s on}_2 + 2 \text{ s off}_3 + 2 \text{ s on}_3/2 \text{ s off}_1 + 2 \text{ s off}_2 + 2 \text{ s off}_3$. These scores were averaged for each condition, and a 2 Side x (3 Mechanical x 4

Electrical) Mixed Factorial Design and post hoc Fisher LSD test (STATISTICA, StatSoft, OK) was used to determine existing differences among the inhibition scores. It was expected that inhibition would be dependent upon the intensity of the electrical stimulus such that, assuming that a smaller score represents more inhibition, 1V < 5V < 10V < 20V.

2.1.5.4 Analysis 4

The final analysis was performed to examine overall differences between the times that the electrical stimulation was on and off. Scores for each cell were calculated for on with the formula $(on_1 + on_2 + on_3)/3$, and scores for off were calculated with $(off_1 + off_2 + off_3)/3$. These scores were analyzed using a 2 Sides x 3 Mechanical x 4 Electrical x (2 On/Off) Mixed Factorial Design and post hoc Fisher LSD test (STATISTICA, StatSoft, OK). It was expected that means for the times that electrical stimulation on for pressure and pinch would significantly less than when stimulation was off: pressure/1V, 5V, 10V, or 20V/on < pressure/1V, 5V, 10V, or 20V/off; pinch/1V, 5V, 10V, or 20V/on < pinch/1V, 5V, 10V, or 20V/off.

CHAPTER 3

RESULTS

3.1 Results

Forty-eight spinal dorsal horn neurons from ten rats were used in this study, 27 from the side of the spinal cord ipsilateral to brain stimulation and 21 from the contralateral side. Five additional rats were excluded from data analysis due to electrode placement outside of the targeted region. Rats' ages ranged between 60-200 days, and weights ranged from 265-520 g. Three independent observers examined the slides to confirm the location of the electrode tip for each brain. Electrode placement was observed on the right side of four brains; therefore, neurons are described in terms of their location relative to electrode placement (i.e. ipsilateral or contralateral to electrode). Electrode placement can be viewed for each rat in Figure 2.

3.1.1 Cell Clustering

Seventy-one cell responses to brush, pressure, and pinch were used in this analysis. There was no brain stimulation for this analysis; therefore, cell responses from 3 of the excluded animals were used here. A record of the number cells per rat and side can be seen in Table 1. An exploratory hierarchical cluster analysis using between groups average linkage and a measure of squared Euclidean Distance revealed two clusters of interest (Figure 3). The majority (67.6 %; n = 48) of the cells categorized into cluster 1, 18 (25.4 %) in cluster 2, and there were 5 outliers (7.0 %)

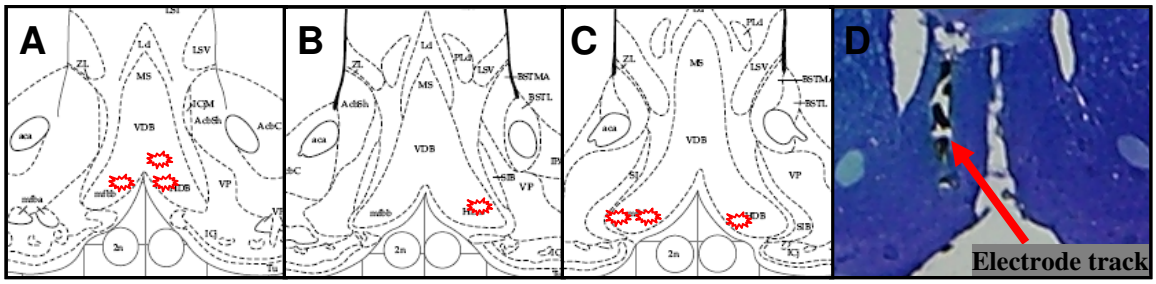


Figure 2. Histology: Images A, B, & C are coronal slices taken from Paxinos and Watson (1998) at A) 0.70 mm, B) 0.48 mm, & C) 0.20 mm anterior to Bregma and compared to brain slices from the experiment (image D); electrode tip placement for all brains is marked on the left images

(Table 1). The pattern of cell distribution for rats did not show a preference for any cluster, which supports the hypothesis that cells from one rat may be generalized to all rats. In other words, every rat but one had cell responses that clustered into at least the first and second cluster. Cells were also observed to not cluster according to the side that the recording was taken from, which makes sense considering that sensory input from both sides of the spinal cord should be similar.

Descriptive statistics for the two clusters were examined to determine the reason for such clusters (Table 2), and it was discovered that Cluster 1 was low responders, Cluster 2 was high responders, and Cluster 3 was outliers with the highest response rate. A two-tailed independent t-test was run to determine if the two main clusters were significantly different for brush, pressure, and pinch. Type I error for the three comparisons was controlled using a Bonferroni adjustment: $\alpha = 0.048$. A Levene's Test indicated that variance for the brush and pressure clusters was not equal, and the results for brush and pressure are reported assuming unequal variance. There was no difference between the two clusters for brush, $t\ (19.9) = -1.26$, $p > 0.048$. Cluster 2 was significantly higher for pressure, $t\ (23.6) = -8.3$, $p < 0.048$, and pinch, $t\ (64) = -12.6$, $p <$

0.048. These results indicate that cells from cluster 2 were responding at a significantly greater rate when being mechanically stimulated by pressure and pinch.

Table 1. Cluster Assignment

Rat	Cell	Side	Cluster	Rat	Cell	Side	Cluster	Rat	Cell	Side	Cluster
1	1	L	1	4	25	L	2	8	49	R	1
1	2	L	1	4	26	L	3	8	50	R	1
1	3	L	2	4	27	L	1	8	51	L	1
1	4	L	3	4	28	L	1	8	52	L	2
1	5	R	1	4	29	L	1	8	53	L	1
1	6	R	2	4	30	L	1	9	54	R	2
2	7	L	1	4	31	L	1	9	55	R	1
2	8	L	1	5	32	R	2	9	56	R	2
3	9	L	2	5	33	L	1	9	57	R	1
3	10	L	2	5	34	R	1	10	58	R	1
3	11	L	2	5	35	R	3	10	59	L	1
3	12	L	1	5	36	R	1	10	60	L	1
3	13	L	2	6	37	L	2	10	61	L	2
3	14	L	1	6	38	L	1	10	62	L	1
3	15	R	2	6	39	L	1	10	63	L	1
3	16	R	1	6	40	L	1	10	64	R	1
3	17	R	1	6	41	L	2	10	65	R	1
3	18	R	1	7	42	L	1	10	66	R	1
3	19	R	1	7	43	R	2	10	67	R	1
4	20	R	1	7	44	R	1	10	68	R	1
4	21	L	1	8	45	R	1	10	69	R	3
4	22	R	2	8	46	R	1	10	70	R	1
4	23	R	1	8	47	R	1	10	71	R	1
4	24	L	2	8	48	L	3				

Table 2. Descriptive Statistics for Clusters

	Cluster	N	Mean	SEM
Brush	low (1)	48	13.23	1.65
	high (2)	18	20.77	5.75
Pressure	low (1)	48	17.02	1.95
	high (2)	18	57.96	4.53
Pinch	low (1)	48	23.18	1.74
	high (2)	18	71.26	4.16

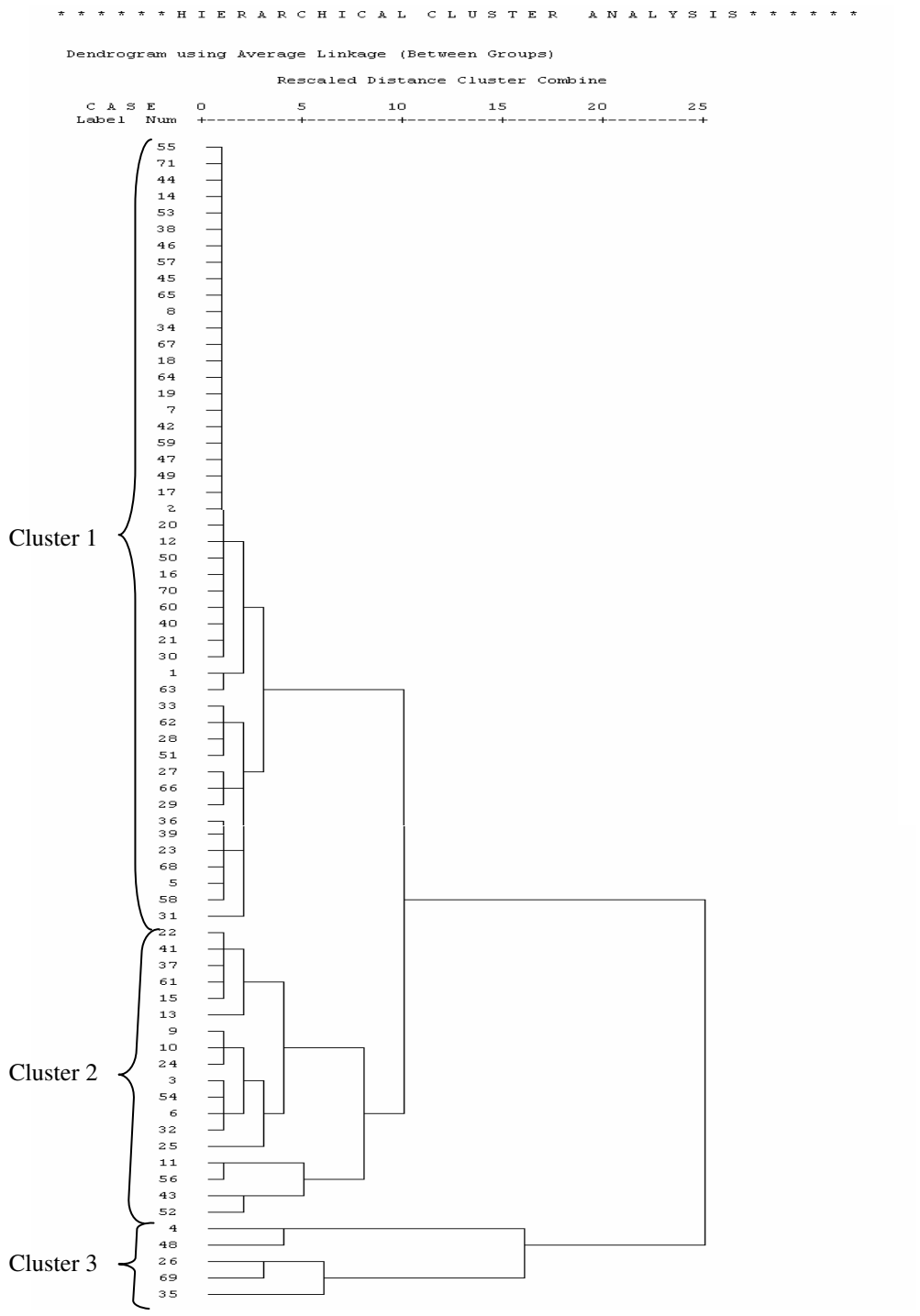


Figure 3. Cluster Analysis Dendogram: cell numbers are listed along the x-axis; Cluster 1 is low responders (n = 48), Cluster 2 is high responders (n = 18), and Cluster 3 is high responding outliers (n = 5)

3.1.2 Analysis of Cell Responses

Marginal means for the ipsilateral and contralateral sides of the spinal cord are reported as mean ± SEM in Table 3 and Figure 4. Analysis 2 was concerned with whether or not HDB stimulation would cause a reduction in nociceptive activity for projection neurons in the spinal cord. Statistical analysis produced effects for: Side, $F(1, 45) = 5.6$, $p < 0.05$; Electrical, $F(5, 225) = 8.9$, $p < 0.001$; Electrical x Side, $F(5, 225) = 3.2$, $p < 0.01$; Mechanical, $F(2, 90) = 27.8$, $p < 0.001$; Mechanical x Side, $F(2, 90) = 4.9$, $p < 0.01$; Electrical x Mechanical, $F(10, 450) = 4.8$, $p < 0.001$. A marginal effect was observed for Electrical x Mechanical x Side, $F(10, 450) = 1.7$, $p < 0.10$. These results suggested that there were discrepancies in the way that unilateral MSDB stimulation influenced neurons from the ipsilateral and contralateral side of the spinal cord; therefore, a post-hoc analysis was run using the Side x Electrical x Mechanical interaction to locate the more specific effects.

Table 3. Mean ± SEM Responses for Spinal Dorsal Horn Neurons

Ipsilateral Lumbar	**Control**	**1V**	**5V**	**10V**	**20V**	**Recovery**
Brush	14.00 ± 2.5	13.95 ± 2.1	14.45 ± 2.0	14.45 ± 2.0	14.00 ± 2.0	17.86 ± 1.9
Pressure	42.77 ± 6.4	36.75 ± 5.4	34.52 ± 5.6	33.36 ± 4.8	26.39 ± 3.8	52.38 ± 6.0
Pinch	49.95 ± 6.9	36.48 ± 5.0	37.95 ± 5.8	37.72 ± 5.3	41.20 ± 5.0	54.68 ± 6.0
Contralateral Lumbar						
Brush	14.38 ± 3.2	13.70 ± 2.8	14.55 ± 3.0	14.95 ± 3.0	14.83 ± 3.2	14.07 ± 2.7
Pressure	25.20 ± 6.4	21.84 ± 5.0	18.67 ± 4.3	18.80 ± 4.1	16.07 ± 3.9	23.13 ± 4.9
Pinch	35.02 ± 5.5	29.06 ± 6.5	30.31 ± 5.3	24.20 ± 4.1	22.88 ± 4.2	28.83 ± 5.3

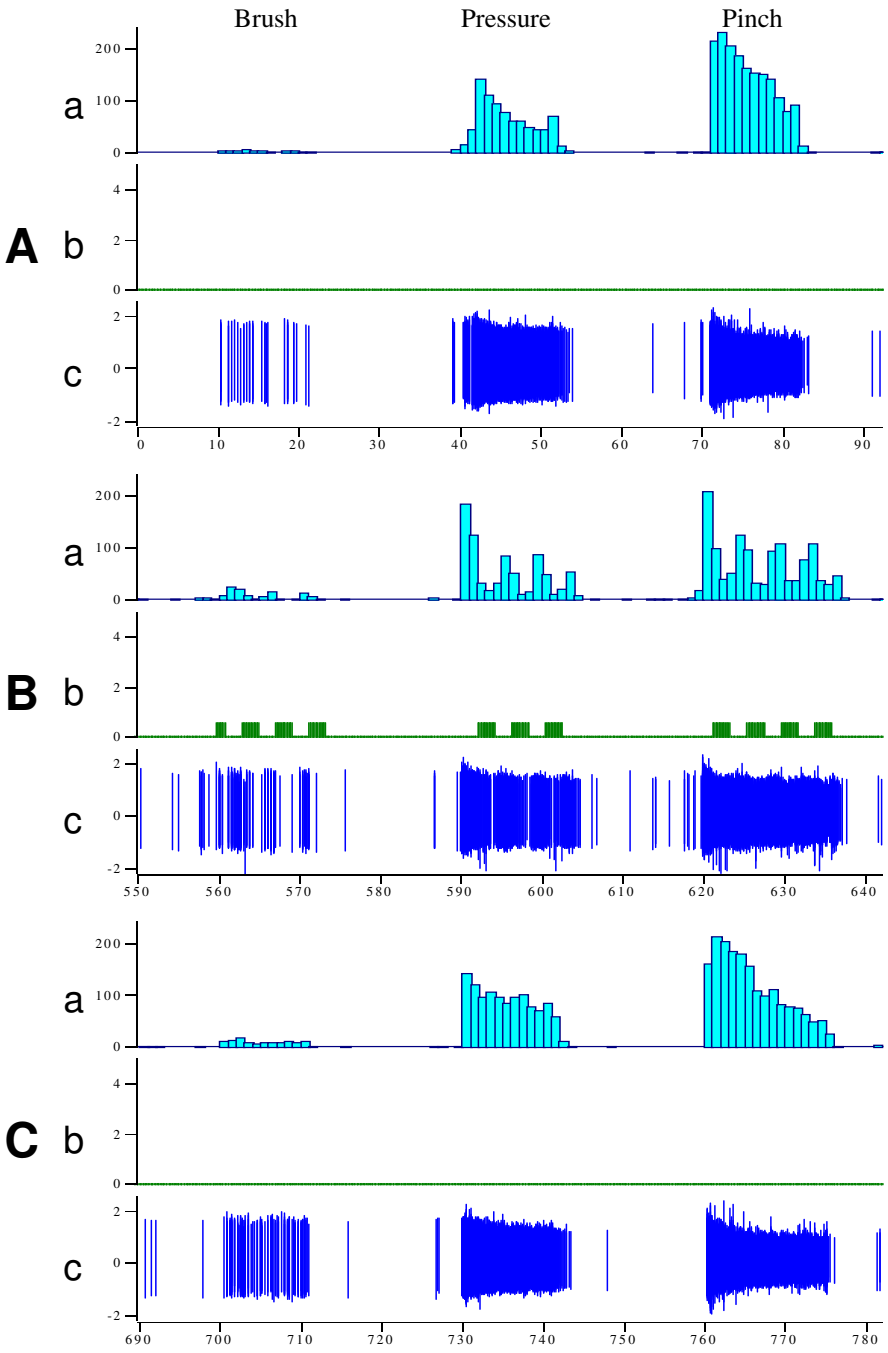


Figure 4. Electrophysiolgical Recording of a Dorsal Horn Neuron from the Ipsilateral Lumbar: A. Control B. 20V C. Recovery; a. rate AP/s* b. record of brain stimulation c. single APs; Each of the three pictures depict neuronal responses to brush, pressure, and pinch. The segments begin at 0 seconds (x axis) in A and end at 780 seconds in C. In B, inhibition is clearly demonstrated with the dips in the rate that correspond with the train stimulation. When brain stimulation is on, the rate is noticeably decreased. When it is off again, the rate increases. *AP: Action Potential

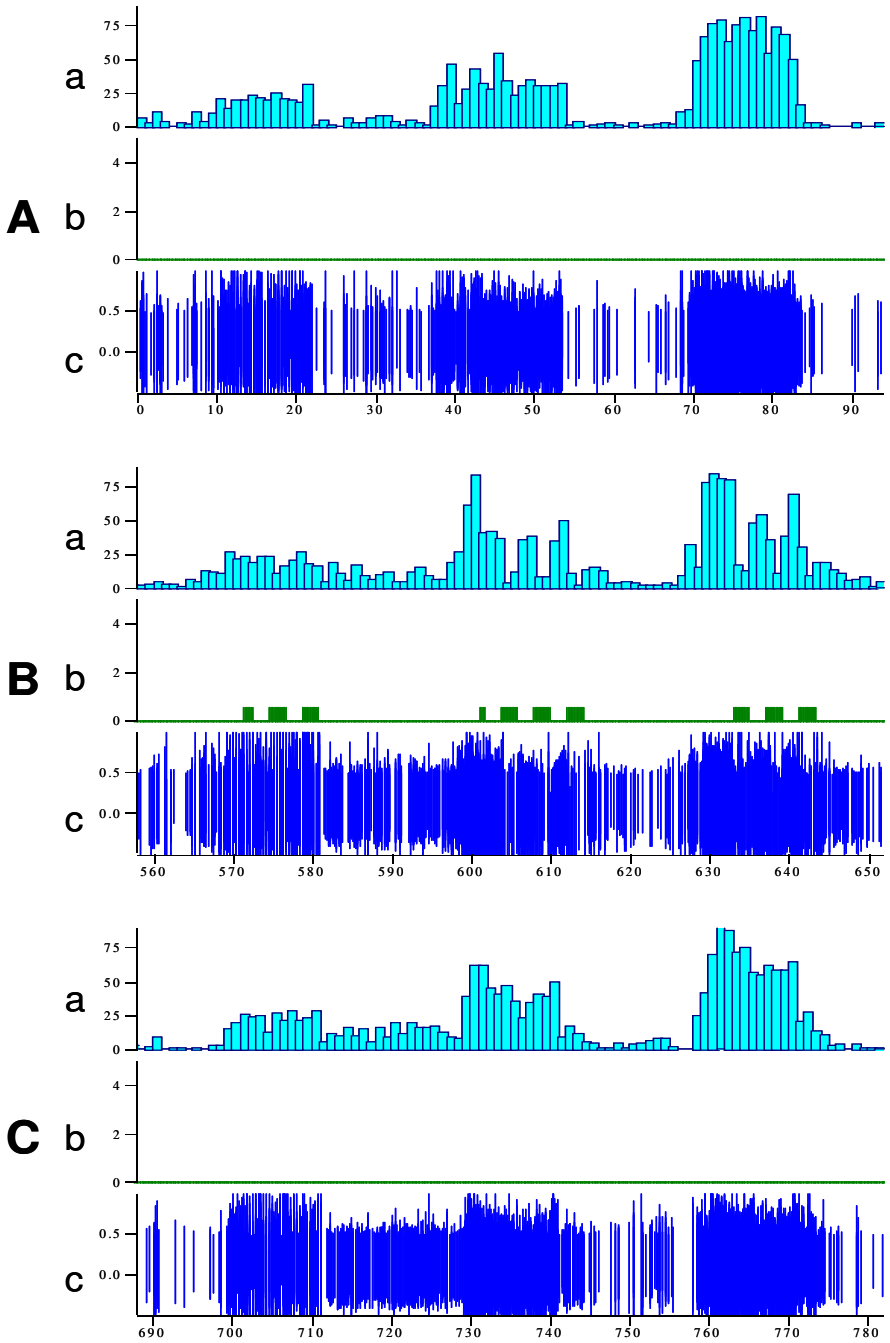


Figure 5. Electrophysiolgical Recording of a Dorsal Horn Neuron from the Contralateral Lumbar: A) Control B. 20V C) Recovery; a) rate AP/s* b) record of brain stimulation c) single APs; Each of the three pictures depict neuronal responses to brush, pressure, and pinch. The segments begin at 0 seconds (x axis) in A and end at 780 seconds in C. In B, inhibition is clearly demonstrated with the dips in the rate that correspond with the train stimulation. When brain stimulation is on, the rate is noticeably decreased. When it is off again, the rate increases. *AP: Action Potential

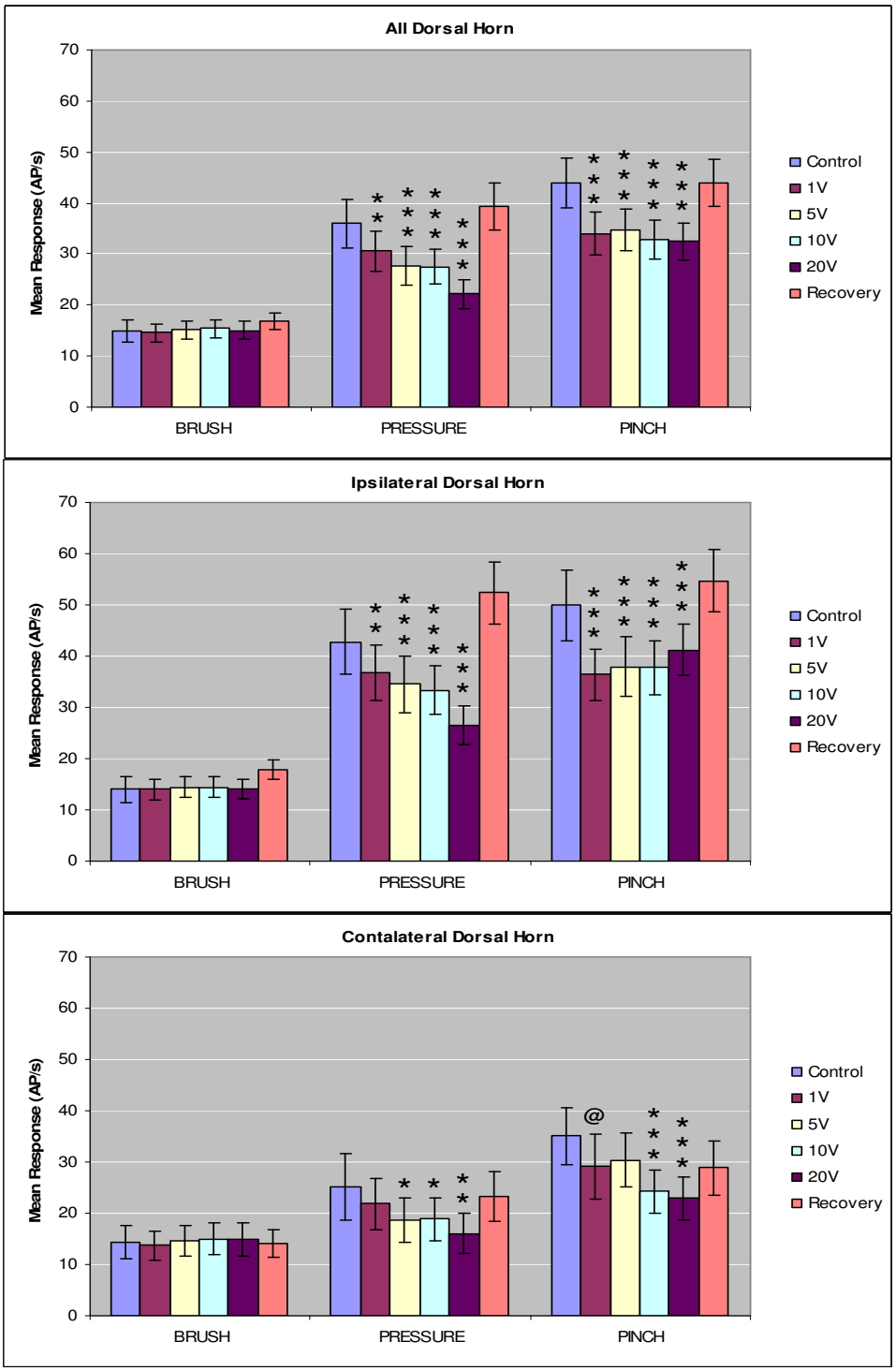


Figure 6. Mean Response of Dorsal Horn Neurons: A) All Spinal Dorsal Horn B) Ipsilateral Spinal Dorsal Horn C) Contralateral Spinal Dorsal Horn; comparisons were made between electrical stimulation conditions and respective controls

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$ @ $p < 0.10$

The Fisher LSD test was run to consider the sub-hypotheses. Results for each condition are presented in Figure 6, and raw data can be viewed in Figures 4 and 5. The first sub-hypothesis was that MSDB stimulation would reduce responses to pressure. Pressure was significantly inhibited on both sides for every condition except contralateral/1V/pressure: ipsilateral/control/pressure to ipsilateral/5V, 10V, and 20V/ pressure, $p < 0.001$; ipsilateral/control/pressure to ipsilateral/1V/pressure, $p < 0.01$; contralateral/control/pressure to contralateral/5V and 10V/ pressure, $p < 0.05$; contralateral/control/pressure to contralateral/20V/pressure, $p < 0.01$ (Figures 6). Additionally, the recovery condition on the ipsilateral side was significantly greater than the control, ipsilateral/recovery/pressure > ipsilateral/control/pressure, $p < 0.01$; and the contralateral recovery was not statistically different from the control. This suggests that cells in the ipsilateral spinal cord may have been sensitized. The second sub-hypothesis stated that pinch responses would be inhibited by MSDB stimulation, which was supported by the data in the following comparisons: ipsilateral/control/pinch to ipsilateral/1V, 5V, 10V, and 20V/pinch, $p < 0.001$; contralateral/control/pinch to contralateral/10V or 20V/pinch, $p < 0.001$ (Figures 6). Inhibition using 1V was marginally significant, contralateral/control/pinch to contralateral/1V/pinch, $p < 0.10$; but 5V did not significantly inhibit pinch on the contralateral side. Recovery responses to pinch were greater than controls on the ipsilateral side with marginal significance, $p < 0.10$; and recovery on the contralateral side was significantly less than control, $p < 0.05$. As for brush, there were no differences when comparing one brush condition to

any other. These data imply that unilateral MSDB stimulation will produce bilateral inhibition of nociceptive projection neurons, while leaving tactile neurons unaffected.

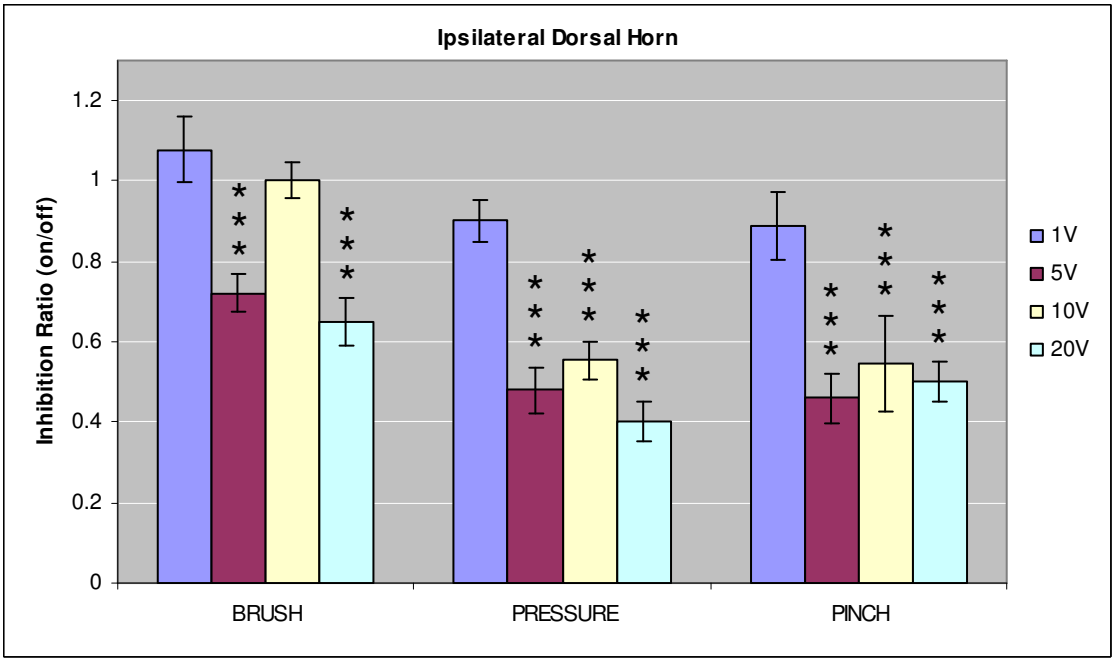
3.1.3 Inhibition Analysis

A ratio of on to off was calculated for each level of electrical stimulation to determine if one experimental condition was more inhibited than another. Mean inhibition scores are reported in Table 4. It was expected that inhibition would increase with the amount of voltage that was used, which is represented by decreases in inhibition values as voltage increased. This was partly supported as indicated below.

Table 4. Mean ± SEM Inhibition

Ipsilateral Lumbar	1V	5V	10V	20V
Brush	1.08 ± 0.08	0.72 ± 0.06	1.00 ± 0.12	0.65 ± 0.06
Pressure	0.90 ± 0.05	0.48 ± 0.06	0.55 ± 0.05	0.40 ± 0.05
Pinch	0.89 ± 0.08	0.46 ± 0.05	0.54 ± 0.04	0.50 ± 0.05
Contralateral Lumbar				
Brush	0.93 ± 0.05	0.77 ± 0.07	0.98 ± 0.05	0.84 ± 0.07
Pressure	0.70 ± 0.06	0.59 ± 0.08	0.75 ± 0.05	0.49 ± 0.07
Pinch	0.78 ± 0.05	0.61 ± 0.07	0.64 ± 0.04	0.53 ± 0.06

A 2 Side x (4 Electrical x 3 Mechanical) ANOVA produced significant effects for the following: Electrical, $F(3, 135) = 26.6$, $p < 0.001$; Electrical x Side, $F(3, 135) = 4.0$, $p < 0.01$; Mechanical, $F(2, 90) = 52.7$, $p < 0.001$; and Electrical x Mechanical, $F(6, 270) = 2.4$, $p < 0.05$. Effects were not observed for: Side, $F(1, 45) = 0.6$, $p > 0.05$; Mechanical x Side, $F(2, 90) = 0.01$, $p > 0.05$; Electrical x Mechanical x Side, $F(6, 270) = 1.3$, $p = 0.26$. Although the three-way interaction was not significant, it was used for the post hoc analysis so that the results would match the comparisons made previously.



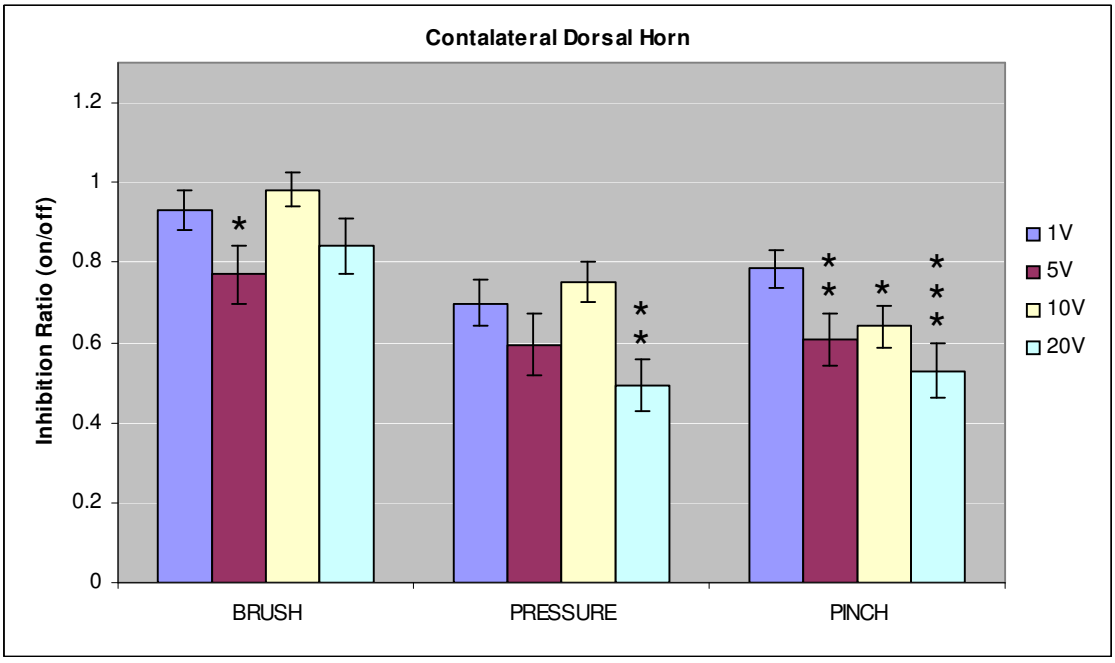


Figure 7. Mean Inhibition: mean scores for brush, pressure, and pinch at 1V, 5V, 10V, and 20V on both sides of the spinal cord; statistical comparisons were made between the 1V condition and 5V, 10V, and 20V

* $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

The Fisher LSD test produced significant differences (Figure 7) for brush on the ipsilateral side when comparing brush/1V to brush/5V or brush/20V, $p < 0.001$, and brush/10V was inhibited less than brush/5V and brush/20V, $p < 0.001$. On the contralateral side, brush/1V had less inhibition than brush/5V, $p < 0.05$; brush/10V was less inhibited than brush/5V, $p < 0.001$, and brush/20V, $p < 0.05$. All other comparisons for brush were non-significant. Inhibition for pressure was significantly greater for every condition on the ipsilateral side when compared to pressure/1V, $p < 0.001$, and pressure/20V was inhibited significantly more than pressure/10V, $p < 0.01$; but on the contralateral side, the only significant differences were between pressure/1V and pressure/20V, $p < 0.01$, and pressure/10V and pressure/20V, $p < 0.001$. All other pressure comparisons were non-significant. Pinch was inhibited significantly more on the ipsilateral side by 5V, 10V, and 20V than 1V, $p < 0.001$, and 5V produced more inhibition than 10V, $p < 0.05$. On the contralateral side, 1V had less inhibition than 5V, $p < 0.01$; 10V, $p < 0.05$; and 20V, $p < 0.001$. Inhibition for pinch/20V was slightly more than pinch/10V, $p < 0.10$. Comparisons between pressure and pinch or across sides were all non-significant. These results partially support the hypothesis. All electrical stimuli on the ipsilateral side and one from the contralateral side increase inhibition with increases in electrical output; on the contrary, inhibition using 5V was sometimes greater than 10V, and inhibition using 20V was never greater than 5V.

3.1.4 On/Off Analysis

The last analysis was performed to look at differences that existed between the times that electrical stimulation was *on* and *off*. Pressure and pinch were expected to

have lower means when electrical stimulation was on versus when it was off. Means and standard error can be viewed in Table 5.

A 2 Side x 4 Electrical x 3 Mechanical x (2 On/Off) ANOVA was run to test the differences among these means. Significant effects included: Mechanical, $F(2, 551) = 32.9$, $p < 0.001$; Side, $F(1, 551) = 24.6$, $p < 0.001$; Mechanical x Side, $F(2, 551) = 5.6$, $p < 0.01$; On/Off, $F(1, 551) = 314.9$, $p < 0.001$; On/Off x Mechanical, $F(2, 551) = 44.4$, $p< 0.001$; On/Off x Electrical, $F(3, 551) = 14.5$, $p < 0.001$; On/Off x Side, $F(1, 551) = 38.4$, $p < 0.001$; On/Off x Mechanical x Electrical, $F(6, 551) = 2.3$, $p < 0.05$; On/Off x Mechanical x Side, $F(2, 551) = 6.6$, $p < 0.01$; On/Off x Electrical x Side, $F(3, 551) = 2.9$, $p < 0.05$. All other effects were non-significant: Electrical, $F(3, 551) = 0.61$, $p > 0.05$; Mechanical x Electrical, $F(6, 551) = 0.90$, $p > 0.05$; Electrical x Side, $F(3, 551) = 0.06$, $p > 0.05$; Mechanical x Electrical x Side, $F(6, 551) = 0.31$, $p > 0.05$; On/Off x

Table 5. Mean ± SEM Neuronal Responses when Electrical Stimulation was On & Off

Ipsilateral Lumbar	**1V**		**5V**		**10V**		**20V**	
	on	**off**	**on**	**off**	**on**	**off**	**on**	**off**
Brush	14.5 ± 2.0	15.9 ± 2.3	12.7 ± 2.0	17.4 ± 2.4	13.6 ± 1.9	16.7 ± 2.4	12.7 ± 2.0	16.6 ± 2.2
Pressure	34.7 ± 5.6	40.2 ± 5.7	24.3 ± 5.2	45.0 ± 6.6	24.4 ± 3.9	44.0 ± 6.3	15.9 ± 3.1	37.9 ± 5.4
Pinch	34.5 ± 5.1	41.2 ± 5.8	25.3 ± 5.0	51.3 ± 7.3	28.8 ± 4.7	50.0 ± 7.0	26.8 ± 4.7	53.2 ± 6.5
Contralateral Lumbar								
Brush	13.2 ± 2.7	14.2 ± 3.0	13.0 ± 2.9	16.1 ± 3.1	14.6 ± 3.0	15.3 ± 3.1	13.1 ± 2.9	16.6 ± 3.5
Pressure	19.4 ± 4.7	24.3 ± 5.4	14.9 ± 4.1	22.4 ± 4.9	15.9 ± 3.6	21.7 ± 4.7	10.7 ± 2.7	21.5 ± 5.4
Pinch	27.1 ± 6.7	31.0 ± 6.4	23.7 ± 4.9	36.9 ± 6.1	19.4 ± 3.7	29.0 ± 4.7	16.0 ± 3.4	29.8 ± 5.2

Mechanical x Electrical x Side, $F(6, 551) = 0.61$, $p > 0.05$. Once again, to stay consistent with previous analyses, the On/Off x Mechanical x Electrical x Side interaction was used to run the post hocs (Figure 8).

The Fisher LSD test produced one marginally significant on/off difference for brush at 5V on the ipsilateral side, $p < 0.10$. All pressure comparisons for on/off were significant or marginally significant: pressure/1V/ipsilateral/on < pressure/1V/ ipsilateral/off, $p < 0.05$; pressure/5V, 10V or 20V/ipsilateral/on < pressure/5V, 10V, or 20V/ipsilateral/off, $p < 0.001$; pressure/1V or 10V/contralateral/on < pressure/1V or 10V/contralateral/off, $p < 0.10$; pressure/5V/contralateral/on < pressure/contralateral/ off, $p < 0.01$; pressure/20V/contralateral/on < pressure/20V/contralateral/off, $p < 0.001$. All but one on/off comparison for pinch was significant: pinch/1V/ipsilateral/on < pinch/1V/ipsilateral/off, $p < 0.01$; pinch/5V, 10V, or 20V/ipsilateral/on < pinch/5V, 10V, or 20V/ ipsilateral/off, $p < 0.001$; pinch/5V or 20V/contralateral/on < pinch/5V or 20V/off, $p < 0.001$; pinch/10V/contralateral/on < pinch/10V/contralateral/off, $p < 0.01$.

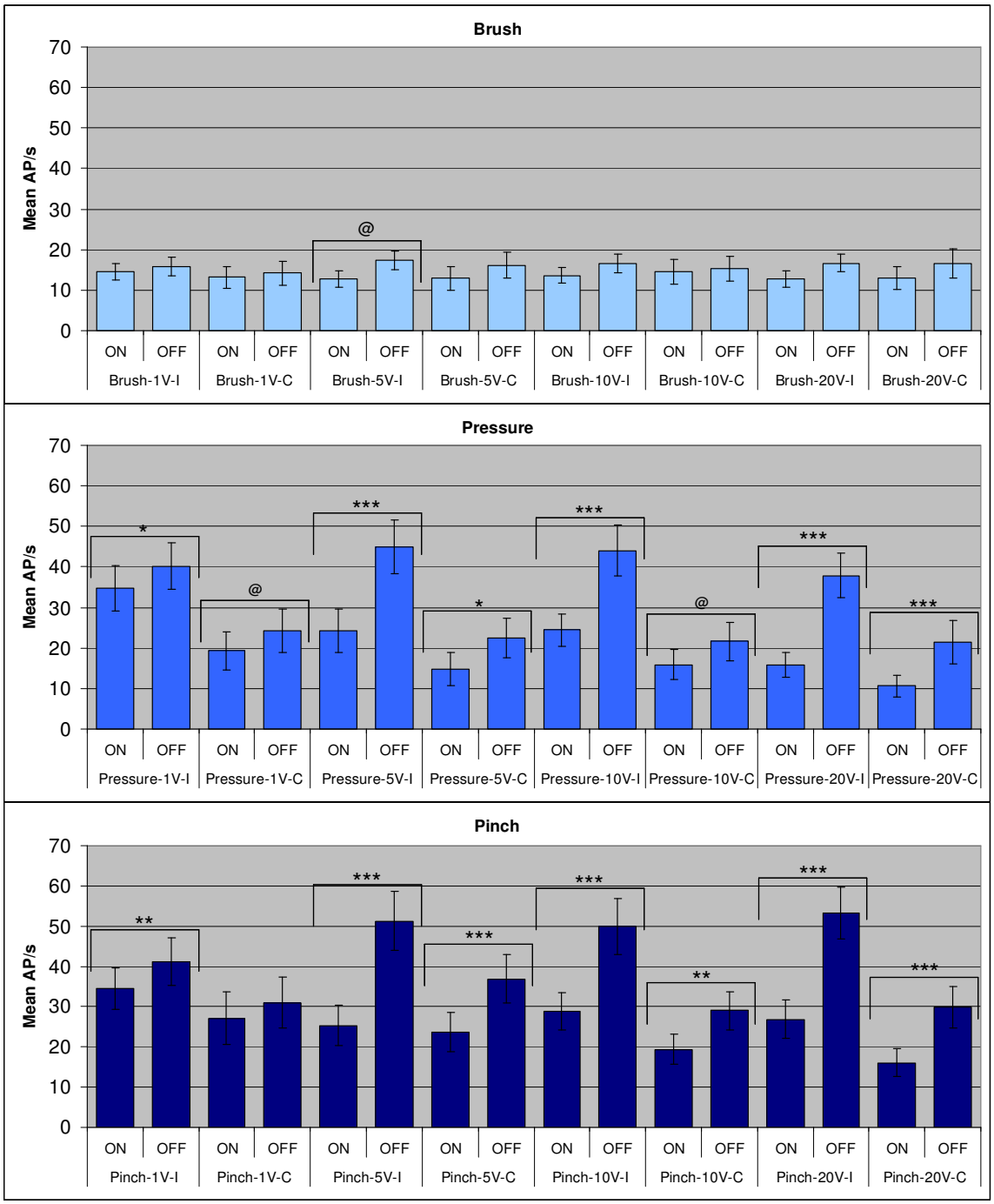


Figure 8. On/Off Comparisons: Mean ± SEM neuronal responses to when electrical stimulation was *on* and *off*; comparisons were made for each condition between *on* and *off* @ $p < 0.10$ * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$

CHAPTER 4

DISCUSSION

4.1 Discussion

4.1.1 Cell Clustering

The purpose of this analysis was to determine if multiple cells from individual rats would be usable for generalizing cell responses to all rats. It was expected that cell clustering would not be specific to rats. The cluster analysis produced two distinct clusters. Cells from these clusters were not specific to any one rat. These results suggest that an experiment which collects recordings for seventy cells from one rat is equivalent to an experiment that collects single-cell recordings for seventy rats; therefore, the hypothesis set forth was supported.

The two clusters found from these analyses indicated that neurons were high rate responders and low rate responders. There are two major possibilities for the reason the cell responses created these two clusters. One is that these cells are inherently responding the way that was observed here. Another more plausible explanation is that cells are being sensitized by continuous and sometimes noxious stimulation. Although special care is taken to ensure that the integrity of a rat's paw remains intact, the fact remains that the paw of interest must be stimulated in order to search for a cell. This procedure takes place before any recording, and time spent searching can range from finding a cell immediately to spending hours in exploration. For that reason, receptors

in the skin (Perl et al., 1976) to receptors on cells in the spinal cord (Woolf, 1983; Baranauskas & Nistri, 1997) can be sensitized, resulting in increased nerve activity and pain. A minority of cells in the spinal cord have a mechanism called "action potential windup" that results when cells are under constant stimulation (Baranauskas & Nestri, 1997). This finding is consistent with what was observed from the current study, which was a smaller group of cells that have higher response rates. It does not negate using multiple cell recordings from one rat, since searching for the first cell is likely to produce sensitization. Additionally, inclusion of sensitized cells into an experiment working on descending inhibition of spinal cord neurons and pain will only compliment the findings. If a sensitized neuron, which is common to pain, is inhibited, this further supports any results that are found for inhibiting pain. Therefore, neither possibility interferes with the conclusions made for this study.

These data are in full support of using a minimum number of animals to do research on dorsal horn neurons. It demonstrates that the cells can be the subject of interest without considering the rat that the cells are recorded from. One possible confound here is that there is not a cluster analysis of data that uses very few cells per rat to see if it would match the current results. Since data of this nature has already been collected in our lab, the next task will be to compare the current data to a data set of many rats to ensure the present findings.

4.1.2 Analysis of Cell Responses

The post hoc analysis revealed that most every level of electrical stimulation significantly inhibits pressure and pinch bilaterally when compared to respective

controls. In the contralateral spinal cord, 1V reduced the firing rate for pressure and pinch, and only pinch was marginally significant inhibition. Five volts also decreased pinch responses in the contralateral spinal cord but not significantly. All other responses to pressure and pinch were significantly inhibited by the remaining electrical stimuli. Additionally, the pressure/recovery condition on the ipsilateral side of the spinal cord was significantly greater than its control, and the pinch/recovery was greater than its control with marginal significance. This may have been a result of sensitization of the dorsal horn neurons or their presynaptic counterparts. Contrary to that, the contralateral pinch responses during recovery were significantly less than the control. Other results of interest were a lack of effect for any brush condition compared to controls. Overall these results suggest that neural activity in both sides of the spinal cord related pain is being inhibited by unilateral electrical stimulation, while leaving tactile sensory signals intact.

4.1.3 Inhibition Analysis

The inhibition analysis was run to explore the extent of inhibition and determine if increased electrical stimulation would cause greater inhibition. Mean inhibition for pressure was greater using 5V, 10V, 20V ipsilateral to stimulation and 20V contralateral to stimulation. Pinch was inhibited more for every condition when compared to 1V, partially suggesting that inhibition increases with electrical intensity; however, all other electrical stimuli were equally effective barring the few occasions where 10V was not as inhibitory as 5V or 20V. This suggests that 5V is better than 1V and as effective as 20V. Determining the least amount of electricity needed to get the best effect is

important in the septum, since this nucleus is prone to inducing seizure with overstimulation (Tress & Herberg, 1972; Duchowny & Burchfiel, 1981). There were no differences in the amount of inhibition any one parameter of electrical stimulation caused for pressure and pinch. Inhibition comparisons across sides were also non-significant. These results also demonstrated some mild inhibition for brush in both sides of the spinal cord. Pressure and pinch, however, were always significantly more inhibited than brush. This still implies that the majority of inhibition of neuronal responses is occurring for noxious stimuli, leaving tactile sensation intact.

4.1.4 On/Off Analysis

This analysis was performed to consider neuronal responses when electrical stimulation was on versus when it was off. One mild inhibition was observed in this analysis for brush; all others were non-significant suggesting that tactile sensory input is mostly unaffected in the spinal cord by MSDB stimulation. Pressure was marginally or significantly inhibited in both sides of the spinal cord using any parameter of electrical stimulation; and pinch was significantly inhibited by all but one parameter, which was 1V used on the contralateral side. Taken together, these results indicate that MSDB stimulation inhibits dorsal horn neuronal responses to noxious mechanical stimulation.

4.1.5 Possible Mechanisms

More physiological properties would need to be determined to confirm the mechanisms for this type of descending inhibition. The proposed mechanism was that the MSDB has connections to brain sites known to decrease dorsal horn activity, such as the LH, PAG, and raphe nuclei; and inhibition in the spinal cord probably comes

from these connections (Figure 9). As mentioned above, the LH receives a strong cholinergic projection from the MSDB. Spinal cord neuronal responses to noxious skin heating were inhibited by LH stimulation in both cats (Carstens, Fraunhoffer, & Suberg, 1983) and rats (Carstens, 1986). Not much detail was provided for data on LH stimulation in the rat (Carstens, 1986), but inhibition from LH stimulation in the cat required more current to generate inhibition in the ipsilateral spinal cord than in the contralateral (Carstens, Fraunhoffer, & Suberg, 1983). This was similar to the results of the present experiment. The projection of the MSDB to the LH is at least ipsilateral in nature, but a bilateral projection is not confirmed or denied by the literature (Swanson & Cowan, 1979; Tomimoto et al., 1987). Also Holden and Naleway (2001) microinjected the cholinergic agonist carbachol into the LH and found that it had an antinociceptive effect for tail-flick and paw-withdrawal latency responses to heat. In addition they found that intrathecal injection of naltrexone or the α_2-adrenoceptor antagonist yohimbine attenuated the antinociceptive effect, suggesting that LH stimulation involves opioid receptors and adrenoceptors in the spinal cord (Holden & Naleway, 2001). Holden, Poppel, & Thomas (2002) later found that the pontine tegmentum A_7 (subcoeruleus) region may be the source of the noradrenergic influence in the spinal cord that comes from LH stimulation, since blocking A_7 activity attenuates LH stimulation analgesia. The PAG and NRM are key sites in LH descending inhibition as was demonstrated by blocking nerve conduction and lesioning these sites and weakening the effect of LH descending inhibition (Aimone, Bauer, & Gebhart, 1988). This suggests that serotonin is also part of the LH descending transmitters that

are important to inhibiting dorsal horn neurons, as was demonstrated by Holden, Naleway, & Jeong (2005).

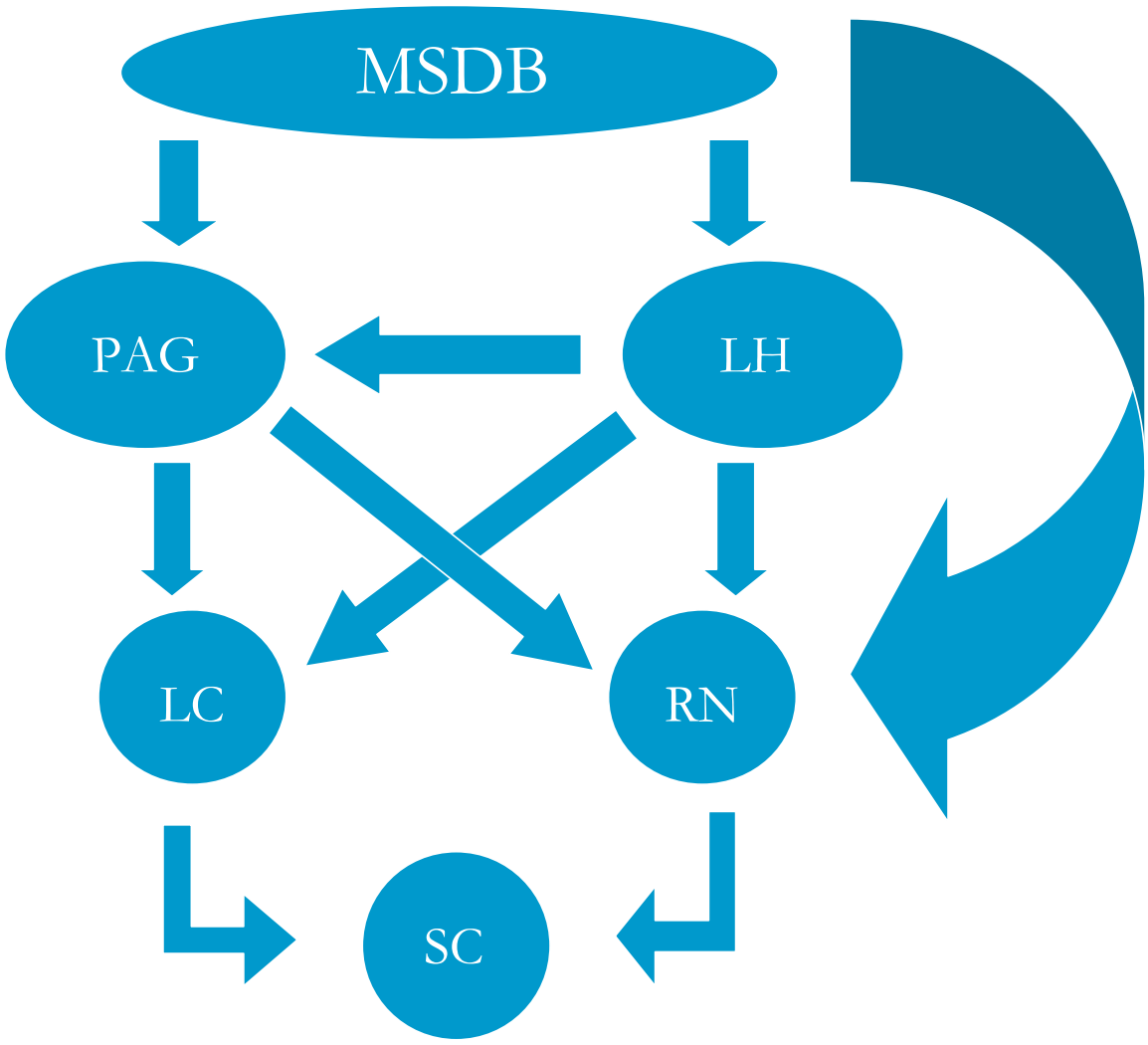


Figure 9. Proposed Mechanism: This figure illustrates a chain of projections to the spinal cord that begin in the MSDB. These projections are the proposed mechanism responsible for inhibition of spinal cord dorsal horn neurons.

4.1.6 Conclusions and Future Directions

The purpose of this study was to determine if electrical stimulation in the MSDB would have antinociceptive affects in the spinal cord. The results provide ample evidence to support that activating the MSDB leads to inhibition of nociceptive spinal neuronal responses to mechanical stimulation. These data indicate that MSDB stimulation will inhibit spinal dorsal horn neurons and therefore pain. The mechanism that leads to inhibition is unknown. Future studies could involve lesions or blocks along the proposed pathway as a way to attenuate the inhibition. Such data will help to better understand the interaction between the forebrain and hindbrain in descending modulation.

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BIOGRAPHICAL INFORMATION

Christopher Hagains received his Bachelor's of Science with a major in psychology from Texas Wesleyan University in December 2003. He began graduate work at The University of Texas at Arlington in January 2005 working under the supervision of Dr. Yuan Bo Peng. Since that time, he has participated in studies of descending mechanisms of pain modulation, pain related to multiple sclerosis, neuropathic pain, and neurogenic inflammation. Chris plans to finish his academic career under Dr. Peng upon completion of a PhD. After doing so, he will continue pursuing a career of research that will involve understanding physiological mechanisms related to pain.