THE ROLE OF CINGULATE CORTEX IN SPATIAL LEARNING AND PAIN PROCESSING

by

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ABSTRACT

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Research implicates the Anterior Cingulate Cortex (ACC) mediates the affective component of pain processing but not spatial learning. The Posterior Cingulate Cortex (PCC) mediates spatial learning but not pain processes. This study investigated the roles of each cortex in spatial learning and pain processing. Lesions were made using standard stereotaxic procedures on 96 animals. Inflammatory condition was induced via carageenan. Animals were randomly assigned between both MPWT and PEAP followed by Morris Swim Test or vice versa in order to prevent an order effect. As hypothesized, ACC lesioned animals resulted in no spatial learning decrement, but showed no avoidance behavior in PEAP within the inflammatory condition. PCC

lesioned animals resulted in no spatial learning decrement and showed no significant avoidance behavior in PEAP that goes against the hypotheses.

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CHAPTER 1

INTRODUCTION

Papez (1937) proposed a brain circuit that modulates emotional experience (include a figure of the Papez circuit). This circuit includes the thalamus, hippocampus and cingulate cortex. Mechanisms mediating pain processing in the cingulate cortex are not well defined. Evidence for cingulate cortex modulation of pain processing indicates multiple components; an affective component, a motor component as well as a learning component (Devinsky, Morrell, and Vogt, 1995).

Many studies show that the ACC mediates the affective component or emotional aspect of pain processing. Lesioning (Johansen et al., 2001; LaBuda & Fuchs, 2002; LaGraize et al., 2004) or stimulating (Fuchs et al. 1996) the ACC results with a decrease of emotional expression after nociceptive stimulation. The ACC also mediates the motor response. ACC damage may also disturb motor response in cognitive tasks associated with avoidance/escape behavior (Vogt, 2005). Current research investigates whether various findings after damage to the ACC with nociceptive stimulation could be a result of this motor response deficit especially involving reward based tasks (Bush, G. et al., 2002). The learning component includes the acquisition of the appropriate and repeated avoidance behavior within a task. Research supports the notion that the ACC mediates the affective component of pain processing and motor response; however,

research does not convincingly support the implication that the ACC mediates learning (Devinsky et al., 1995; Vogt et al., 1993).

Some researchers question whether the ACC moderates spatial learning and memory. Whishaw and colleagues (2001) conducted a study that resulted in spatial learning deficits after ACC damage. However, the ablated lesion disrupted the interconnections between the ACC, posterior cingulate cortex (PCC), hippocampal formation and the anterior thalamic nuclei. Though all of these regions are interconnected, some of those areas contain denser connections to other areas. For instance, the PCC densely connects reciprocally to the CA1, CA2 and CA3 regions of the hippocampus; where as, the ACC densely connects to the anterior thalamic nuclei (Isaacson, 1982; O'Keefe, 1983). Most evidence supports other structures and not the ACC to be major contributors to spatial learning and memory.

The hippocampus and the hippocampal formation moderate spatial learning and memory. Sutherland and Hoesing (1993) reason that since proper acquisition of spatial representation requires intact function of the hippocampal formation which is indirectly linked with the ACC, it is reasonable to conclude that the ACC is not involved in the spatial learning process simply due to its lack of necessity. In other words, ACC damage should not result in behaviorally expressed learning deficit. Therefore, the interconnections between the ACC and the hippocampal formation are insignificant when it comes to learning acquisition.

In fact, the differences between the functions mediated by either the ACC or PCC seem more mutually exclusive than previously implicated. The PCC varies in

cytoarchitecture, function, and connections as compared to the ACC (for review see Devinsky et al., 1995; Sutherland et al., 199;, Vogt et al., 1995; Vogt, 2005). A change in afferent and efferent connections within the cingulate cortex begins and continues caudally from the splenial of the corpus callosum (Isaacson, 1982). As a result, the PCC is synonymous with the retrosplenial cortex in the rat. The PCC has reciprocal connections to the hippocampal formation and consequently contributes to learning and memory but more specifically spatial learning and memory (Olton, 1983). Using Sutherland's (1993) logic, destruction of the PCC and not the ACC should result in spatial learning deficit due to connections to the hippocampal formation lost.

Acquisition of spatial learning is expressed via avoidance behavior. As previously mentioned, Devinsky and colleagues (1995) implicated the ACC mediates the learned avoidance response. Avoidance responses found as a result of either ACC or PCC damage should be inversely proportionate (Gabriel, 1993). More specifically, damage to the ACC initially resulted with no impairment of avoidance behavior acquisition but over time avoidance behavior expression declines. Inversely, PCC damage initially resulted with impaired avoidance expression followed by an increase in the avoidance behavior. However, research suggests the opposite.

Studies have investigated the avoidance response of either PCC or ACC lesion. Findings suggested that ACC damage resulted with a loss of affect resulting in decreased avoidance behavior in pain processing. ACC damage led to a lack of escape/avoidance behavior in place escape/avoidance paradigm (LaBuda & Fuchs, 2000). A similar effect was found for conditioned place avoidance paradigm (Johansen,

Fields & Manning, 2001) where damage to the ACC decreased the avoidance behavior of an adversely associated room. Research also implicates that the PCC moderates acquired avoidance response in spatial learning.

PCC lesion studies show a variety of avoidance response deficits in spatial learning tasks. Lesions to retrosplenial area B not A resulted in longer duration to escape the given aversiveness of the Morris Swim Test (Thomas van Groen et al., 2004). Retrosplenial damage resulted with a decreased task completion in the radial arm maze (Vann & Aggleton, 2005).

1.1 Experimental Purpose

The purpose of this study was to investigate the role in which the ACC and the PCC play in spatial learning as well as avoidance behavior. ACC or PCC electrolytic lesions were performed on rats in order to analyze their avoidance behavior resulting from the lesion. The Morris swim test (Morris, 1984) and place escape/avoidance paradigm (PEAP) (LaBuda & Fuchs, 2000) after induction of inflammatory condition was used in order to analyze the avoidance behavior within each lesion treatment. Mechanical hyperalgesia after induction of inflammatory condition was assessed using the up/down method (Dixon, 1980).

1.2 Hypotheses

It was hypothesized that animals receiving ACC lesions would show no decrement in duration of escape behavior for the Morris Swim test but would decrease escape/avoidance behavior in the PEAP. Furthermore, animals receiving PCC lesions would show decrement in duration of escape behavior in the Morris swim test but

would not decrease escape/avoidance behavior for the PEAP. MPWT would result with the inflammatory induced animals would develop allodynia across groups as compared to control groups. Analysis of swim velocity for the Morris Swim test investigated possible motor impairment resulting from the lesions. It was hypothesized that there would be no significant differences between groups across time. Analysis of the swim path distance for the Morris Swim test investigated the consistency of the pool entry randomization. It was hypothesized that no significant differences with-in groups would be found.

CHAPTER 2

METHOD

2.1 Subjects

Ninety five male Sprague-Dawley rats (University of Texas at Arlington vivarium) weighing approximately 250-450 g at the time of surgery were housed in groups of three or four and maintained on a 12:12 light/dark cycle with access to food and water ad lib throughout the duration of the study. One animal was lost due to development of a broken forepaw. Two animals were excluded from analysis due to errors in the experimental process. The animals were maintained and cared for in accordance to the guidelines outlined by the International Association for the Study of Pain (Zimmerman, 1983). The experimental protocol was approved by the Institutional Animal Care and Use Committee at the University of Texas at Arlington.

2.2 Surgical Methods

All animals were administered a subcutaneous injection of acepromazine (0.65 mg/kg). After five minutes, the animals were deeply anesthetized by an intramuscular injection of ketamine (50 mg/kg) and xylazine (2.61 mg/kg) then positioned in a stereotaxic frame with blunt-tipped ear bars. Both the ACC and PCC lesions were performed by applying 15 seconds of 1.5 mA constant current. The single insertion of the electrode at the 20º angle created a bilateral ACC or PCC lesion due to the cingulate cortex being a midline structure. The experimenter performed sham ACC or PCC

lesions in the same manner as the bilateral ACC and PCC lesions with the exception that no current was passed through the electrode.

2.2.1 ACC Lesions

Stereotaxic surgery was performed using traditional methods. Electrolytic lesions of the anterior cingulate cortex were performed seven days prior to the start of behavioral tests (Sham ACC lesion/saline, n = 12, Sham ACC lesion/carrageenan, n = 13, ACC lesion/saline, n = 5, ACC lesion/carrageenan, n = 6, Incomplete ACC lesion/saline, n = 7, Incomplete ACC lesion/carrageenan, n = 7). A midline incision was then made along the scalp. For the ACC lesion, a burr hole was drilled 0.9 mm lateral and 1.70 mm anterior to bregma and an insulated stainless steel electrode with no insulation on the tip was lowered 3.4 mm from the skull at a 20° angle.

2.2.2 PCC Lesions

Electrolytic lesion of the posterior cingulate cortex was also performed seven days prior to the start of behavioral tests (Sham PCC lesion/saline, n = 10, Sham PCC lesion/carrageenan, n = 12, PCC lesion/saline, n = 4, PCC lesion/carrageenan, n = 1, Incomplete PCC lesion/saline, n = 7, Incomplete PCC lesion/carrageenan, n = 11). A midline incision was made along the scalp. Drilling a burr hole 1.0 mm lateral and 2.0 mm made the PCC lesion posterior to bregma and using the same electrode as the ACC lesion descending 3.1 mm from the skull at a 20° angle.

2.3 Induction of Inflammatory Condition

The experimental inflammatory condition was induced via 0.05 cc subcutaneous injection into the left hindpaw of 1% Carrageenan (2mg/200µl, Sigma). The

experimental control condition was induced via 0.05 cc of normal saline and injected in the same manner as the experimental inflammatory condition.

2.4 Behavioral Testing

2.4.1 Mechanical Paw Withdrawal Threshold

MPWT behavioral testing was performed three and a half to four hours after injection of saline or carrageenan and seven days following ACC or PCC lesions. Each animal was placed within a Plexiglas chamber (20 x 10.5 x 40.5 cm) and allowed to habituate for 15 minutes. The chamber was positioned on top of a mesh screen so that mechanical stimuli could be administered to the plantar surface of both hindpaws. Mechanical threshold measurements for each hindpaw were obtained using the up/down method (Dixon, 1980) with eight von Frey monofilaments (3.91, 5.91, 9.97, 19.81, 38.82, 78.14, 141.99 and 239.04 mN). Each trial began with a von Frey force of 9.97 mN delivered to the right hindpaw for approximately 1 s. Immediately following, the left paw was stimulated in the same manner. If there were no withdrawal response, the next higher force was delivered. If there were a response, the next lower force was delivered. This procedure was performed until no response is made at the highest force level (216.58 mN) or until five stimuli are administered following the initial response. The MPWT for each paw was calculated using the following formula: [Xth]log = [vFr]log +ky where [vFr] is the force of the last von Frey used, k = 0.2492 which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. If an animal did not respond to the highest von Frey monofilament (239.04 mN), then y = 1.00 and the MPWT response for

that paw was calculated to be 424 mN. MPWT testing was performed three times and the withdrawal values were averaged over the three trials to determine the mean MPWT for the right and left paw of each animal.

2.4.2 Place/Escape Avoidance Paradigm

Place escape/avoidance testing (LaBuda & Fuchs, 2000) was performed immediately following MPWT testing. Animals were placed within a 60 x 30 x 30 cm Plexiglas chamber positioned on top of a mesh screen. Half of the chamber is painted white (i.e. the light side) and the other half of the chamber is painted black (i.e. the dark side). During behavioral testing, animals were allowed unrestricted movement throughout the test chamber for the duration of a 30 minute test period. Testing began immediately upon the animal's placement within the chamber with suprathreshold mechanical stimulation (476mN von Frey monofilament) applied to the plantar surface of the hindpaws at 15 second intervals. The mechanical stimulus was applied to the left hindpaw (carrageenan/saline injected) when the animal was within the preferred dark side of the test chamber and the right paw (control paw) when the animal was within the non-preferred light side of the test chamber. Both the experimental inflammatory condition and the experimental control condition were mechanically stimulated in an identical manner. The experimenter's record of the animal's location depends upon the chamber side where both hindpaws are located. In the case where one paw is in each side of the chamber, the stimulation was given to the same paw previously stimulated (as if the animal had not crossed to the other side of the chamber). Based on the location of the animal at each 15 second interval, an overall mean percentage of time spent in

each side of the chamber was calculated and the percentage of time spent in the light side of the chamber was analyzed.

2.4.3 Morris Swim Test

Spatial navigation was tested using a Morris swim test (MST) in an open field water maze (Morris, 1984). The arena consists of a large circular hard plastic tub (approximately 165 cm in diameter and 100 cm high). The water was maintained at room temperature (23 ± 1ºC). The arena was positioned in the center of the room beneath a camera. The camera connected to a computer that used Ethovision 3.1 to collect data for analysis. The water was rendered opaque using a non-toxic tempered white paint. The pool was divided into five different quadrants including a perimeter, center, and four main body areas. An escape platform was placed in one main quadrant and remained stationary for five days of acquisition. On the sixth day, the platform was moved and maintained in an opposite main quadrant for three more days of testing. Random assignment of direction (north, south, east, or west) was conducted using the Latin square technique in order to minimize any order effects. The animal was placed into the pool at the assigned direction facing the edge of the pool. Testing was conducted for a duration of two minutes or until the animal had successfully remained upon the platform for a duration of ten seconds. If at two minutes the animal had not found the platform or had not successfully remained upon it for the required time, the experimenter then placed the animal upon the platform until the time requirement was met. Eight trials were conducted per day with at least one hour break at the half way point. After all daily trials, a probe test was conducted. The animal was placed within

the tub at the assigned random position and allowed to swim freely for two minutes without the platform in place. The duration taken to find the platform, swim velocity and total distance traveled was recorded and analyzed.

2.5 Experimental Procedure

Behavioral testing was performed blind with respect to ACC or PCC lesion conditions (sham versus lesion). Experimenters were blind to the behavioral results while conducting the histological analysis. Histological analysis was performed blind to inflammatory condition (carrageenan versus saline) and behavioral outcome. Following a seven day recovery period, random assignment to behavioral tests was conducted for each animal. Each animal was given the three behavioral tests in order to minimize error. The tests were counterbalanced to avoid order effects. Mechanical paw withdrawal threshold (MPWT) and place escape/avoidance paradigm (PEAP) or Morris swim test (MST) was then conducted depending upon random assignment.

2.6 Data Analysis

MPWT quantifies the withdrawal threshold after application of various forced von Frey hairs. PEAP quantifies the affective/emotional aspect of pain processing by recording the amount of time spent within the light or undesired side of the chamber. Morris Swim test measures duration to find platform, swim distance and swim velocity within the pool which permits analysis of spatial acquisition impairment, motor impairment and position effects. Each DV was examined using a fully factorialized mixed design ANOVA with 3 between-subjects factors and 1 within-subjects factor. The experimenter implemented a 2 (Treatment: ACC, PCC) x 3 (Lesion: bilateral,

incomplete, sham) x 2 (Injection type: carrageenan, saline) design for the between subjects factors (see Table 1 for design details). Time was manipulated within-subjects, with a different number of levels for each DV: 8 levels (Day 1-8) for Morris; 2 levels (pretest, posttest) for MPWT; and 6 levels (5, 10, 15, 20, 25, 30 min) for PEAP. The levels of Time were nested within the Test Type. Each test (Morris, MPWT and PEAP) had a different dependent variable.

Morris Swim Test measured duration to find platform, mean swim velocity and mean distance traveled with time nested within each. MPWT measured thresholds prior to (pretest) and after (posttest) inflammatory induction. The mean difference (posttest – pretest) was used for analysis collapsing time across variables. PEAP measured mean percentage of time spent within the light side of the chamber collapsed across time. For simplification, the overall analysis was broken down into five separate four factor analysis of variance (ANOVA) to evaluate the between subject conditions across Time of one Test type.

A four factor mixed ANOVA was used to analyze the mean MPWT difference scores for each of the ACC and PCC groups across trials. A four factor mixed ANOVA was used to analyze the percentage of time spent within the light side of the PEAP for each of the ACC and PCC groups across trials. A four factor mixed ANOVA was used to analyze the duration to find the platform as well as the swim velocity and total distance traveled within the Morris Swim Test (refer to Table 1 for group details).

2.7 Histology

Following all behavioral testing, animals were sacrificed using carbon dioxide gas and decapitated. Brains were extracted then stored in 10% formaldehyde for at least 24 hours to ensure fixation of tissue. The tissues were then drained of formaldehyde and allowed to soak in 30% sucrose solution in order to protect the tissue during the slicing process. Coronal sections were sliced 80 μm thick using a cryostat, mounted and stained with thionin. Tissues were examined under magnification to determine the degree of tissue damage according to the atlas of Paxinos and Watson (1998).

CHAPTER 3

RESULTS

3.1 Inflammatory Condition and MPWT

MPWT measured thresholds pretest and posttest for induction of inflammatory induction. The mean difference score (posttest – pretest) of the right paw was analyzed in order to rule significant differences within the right paw across time. The ANOVA revealed no main effect for time ($F_{1, 82} = 0.385$, $p > .05$). No interaction affects were found between conditions across time. The ANOVA revealed no significant main effects for treatment ($F_{1, 82} = 0.152$, $p > .05$), condition ($F_{1, 82} = 0.571$, $p > .05$), or injection type ($F_{1, 82} = 0.494$, $p > .05$). No interaction effects were found between subjects across groups. The consistency of the right paw withdrawal threshold permits the right paw to serve as a control in comparison to the inflammatory condition induced in the left paw.

The mean difference scores (posttest – pretest) across time are illustrated in Figure 1. As hypothesized, the ANOVA revealed a significant interaction effect for Time X Injection ($F_{1, 82} = 4.529$, $p < .05$). The ANOVA revealed no significant interaction effect for Time X Condition ($F_{1, 82} = 0.568$, $p > .05$) or Time X Treatment ($F_{1, 82} = 0.114$, $p > .05$). The ANOVA revealed a significant main effect for Injection ($F_{1, 82} = 12.645$, $p < .05$). The ANOVA revealed no significant main effect for Condition

($F_{2, 82} = 0.389$, $p > .05$) or Treatment ($F_{1, 82} = 0.353$, $p > .05$). No significant interaction effects were found between groups.

3.2 Electrolytic Lesions and PEAP

The time spent within the light side of the PEAP and number of crosses between dark and light sides was recorded in order to measure the affective component of pain processing. Analyses were conducted of the mean percentage of time spent across time between treatment, condition and injection type. The mean percentage of time spent within the light side of the chamber across time for each group is shown in Figure 2. The ANOVA revealed no significant interaction effect for Time crossed with Treatment ($F_{5, 420} = 1.508$, $p > .05$), Condition ($F_{5, 420} = 0.754$, $p > .05$) or Injection ($F_{5, 420} = 1.899$, $p > .05$). The ANOVA revealed a significant Treatment X Injection ($F_{1, 84} = 6.890$, $p < .05$) interaction effect as hypothesized. However, the ANOVA revealed no significant interaction effect for Treatment X Condition ($F_{2, 84} = 2.131$, $p > .05$) or Injection X Condition ($F_{2, 84} = 2.231$, $p > .05$). The ANOVA revealed no significant main effect for Treatment ($F_{1, 84} = 1.116$, $p > .05$), Condition ($F_{2, 84} = 0.2.362$, $p > .05$) or Injection ($F_{1, 84} = 1.056$, $p > .05$).

3.3 Electrolytic Lesions and Morris Swim Test

Escape to the platform duration (sec), distance (cm) of swim path and swim velocity (cm/sec) were recorded in the Morris Swim Test.

3.3.1 Escape Duration

Sums of the eight daily trials were average across subjects within groups. The mean daily escape durations for treatment type and lesion type are shown in Figure 3.

The ANOVA revealed no significant interaction effect for Time crossed with Treatment ($F_{7, 623} = 0.855$, $p > .05$), Condition ($F_{14, 623} = 0.961$, $p > .05$) or Treatment X Condition ($F_{14, 623} = 1.278$, $p > .05$) unlike hypothesized. The ANOVA revealed a significant main effect for Time ($F_{7, 623} = 117.45$, $p < .05$) as expected. The ANOVA revealed no significant interaction effect for Treatment X Condition ($F_{2, 89} = 1.204$, $p > .05$). The ANOVA also revealed no significant main effects for Treatment ($F_{1, 89} = 0.046$, $p > .05$) or Condition ($F_{2, 89} = 0.863$, $p > .05$).

3.3.2 Distance

Mean distance of the swim path was calculated across daily trials, time and between groups in order to analyze the possibility of position effect. The ANOVA revealed no significant interaction affects for Time crossed with Treatment ($F_{7, 623} = 1.275$, $p > .05$), Condition ($F_{14, 623} = 1.122$, $p > .05$) or Treatment X Condition ($F_{14, 623} = 1.202$, $p > .05$) as hypothesized. The ANOVA revealed a significant main effect for Time ($F_{7, 623} = 116.37$, $p < .05$) as expected. The ANOVA revealed no significant interaction effect for Treatment X Condition ($F_{2, 89} = 0.166$, $p > .05$). The ANOVA also revealed no significant main effects for Treatment ($F_{1, 89} = 0.354$, $p > .05$) or Condition ($F_{2, 89} = 0.505$, $p > .05$).

3.3.3 Velocity

Mean swim velocity was calculated across daily trials, time and between groups in order to analyze the possibility of motor impairment. The ANOVA revealed no significant interaction affects for Time crossed with Treatment ($F_{7, 623} = 1.735$, $p > .05$) or Treatment X Condition ($F_{14, 623} = 0.795$, $p > .05$) as hypothesized. However, the

ANOVA also revealed a Time X Condition ($F_{14, 623} = 1.758$, $p < .05$) interaction affect. The ANOVA revealed a significant main effect for Time ($F_{7, 623} = 44.45$, $p < .05$) as expected. The ANOVA revealed no significant interaction effect for Treatment X Condition ($F_{2, 89} = 0.603$, $p > .05$). The ANOVA also revealed no significant main effects for Treatment ($F_{1, 89} = 1.903$, $p > .05$) or Condition ($F_{2, 89} = 1.030$, $p > .05$).

3.4 Histology

Histological analysis was performed blind to behavioral analysis. Histological findings confirmed PCC or ACC lesions to encompass greater than 75% bilateral damage (to see percent of animals with tissue damage to figure 4). Unilateral lesions encompassed less than 75% bilateral damage. One animal was excluded from analysis due to corpus callosum damage.

CHAPTER 4

DISCUSSION

The purpose of this experiment was to investigate the role of the ACC or PCC in escape/avoidance behavior associated with pain processing and spatial learning. It was hypothesized that animals receiving ACC lesions would show no decrement in escape behavior in the Morris Swim test but would decrease escape/avoidance behavior in the PEAP. These hypotheses were supported. Duration to escape the adversiveness of the Morris Swim test for ACC lesioned animals compared to ACC sham and PCC sham showed no significant differences between groups. ACC lesioned group also showed differences in PEAP scores as compared to the control groups. The ACC moderates the affective component of pain processing similar to Papez's original implication.

It was also hypothesized that animals receiving PCC lesions would show decrement in escape behavior in the Morris swim test but would not decrease escape/avoidance behavior in the PEAP. However, neither of these hypotheses was supported. The duration of escape behavior for the PCC lesioned group as compared to the control groups revealed no differences. The PCC lesioned group also revealed no differences in PEAP scores as compared to control group.

Some methodological problems may have contributed to the lack of hypothesized effect within this study. The cingulate cortex contributes to motor response of escape/avoidance behavior in pain processing as previously discussed. The

hippocampus also moderates motor response (Isaacson, 1982). Rats changed from a spatial learning strategy in a problem solving task to a motor turning strategy after damage to the retrosplenial cortex (Vann & Aggleton, 2005). Since the reciprocal connections between the PCC and the hippocampus are dense, damage to the motor behavior in this study may have been affected forcing the animal to use an unknown strategy to solve the escape task.

Lesion quality also affects the spatial learning deficit. The PCC lesion size affects the significance of the deficit. Vann and Aggleton (2004) found a significant difference in learning deficit dependant upon the inclusiveness of the retrosplenial lesion. Another determining factor affecting the deficit may be recovery time. In traumatic brain damaged areas of the rat brain, retrosplenial damage repairs itself within 48 hours of injury (Bayly et al., 2006). Though this damage quality is not comparable to the damage quality of a lesion, this result suggests neuroregeneration within this brain region occurs rapidly.

Analysis of MPWT revealed differences between inflammatory conditions. Subjects injected with carageenan showed significantly decreased threshold scores as compared to saline injected groups. The subjects injected with the inflammatory agent developed the allodynia necessary in order to test for escape/avoidance behavior in PEAP.

Analyses of the distance traveled and mean velocity also revealed no differences between groups. The starting location was randomized using Latin square techniques. Therefore, distance traveled between groups per day was analyzed in order to ensure

that there were no starting location effects due to variations in starting position to platform distance. The analysis revealed no differences between groups per day for distance traveled suggesting there was no starting location effect as anticipated.

The mean velocity of subjects was analyzed to determine any possible motor impairment caused by the lesions. The lack of differences suggests there was no motor impairment caused by lesions.

APPENDIX A

TABLE

Table 1. A total of 95 experimental animals were used for the analysis. The overall design of the 6 factor mixed design is detailed below. Treatment, Condition, and Injection were analyzed as the between subject factors. The Test Type was the repeated subject factor with Time being nested within each Test Type and subjects being nested within each condition.

				Type of Test														
				Morris								MPWT		PEAP				
Treatment	Condition	Injection	Subjects	Day1	Day2	Day3	Day4	Day5	Day6	Day7	Day8	Pre-test	Post-test	5 min	10 min	15 min	20 min	25 min	30 min
ACC	Lesion	CARR	N = 6	Each of the following: Duration to find platform; Swim Velocity; Total Distance Traveled								Mean Difference scores		% Time spent in the Light side of the chamber					
		Saline	N = 5																
	Incomplete Lesion	CARR	N = 7																
		Saline	N = 7																
	Sham	CARR	N = 13																
		Saline	N = 12																
PCC	Lesion	CARR	N = 4																
		Saline	N = 1																
	Incomplete Lesion	CARR	N = 11																
		Saline	N = 7																
	Sham	CARR	N = 12																
		Saline	N = 10																

APPENDIX B

FIGURES

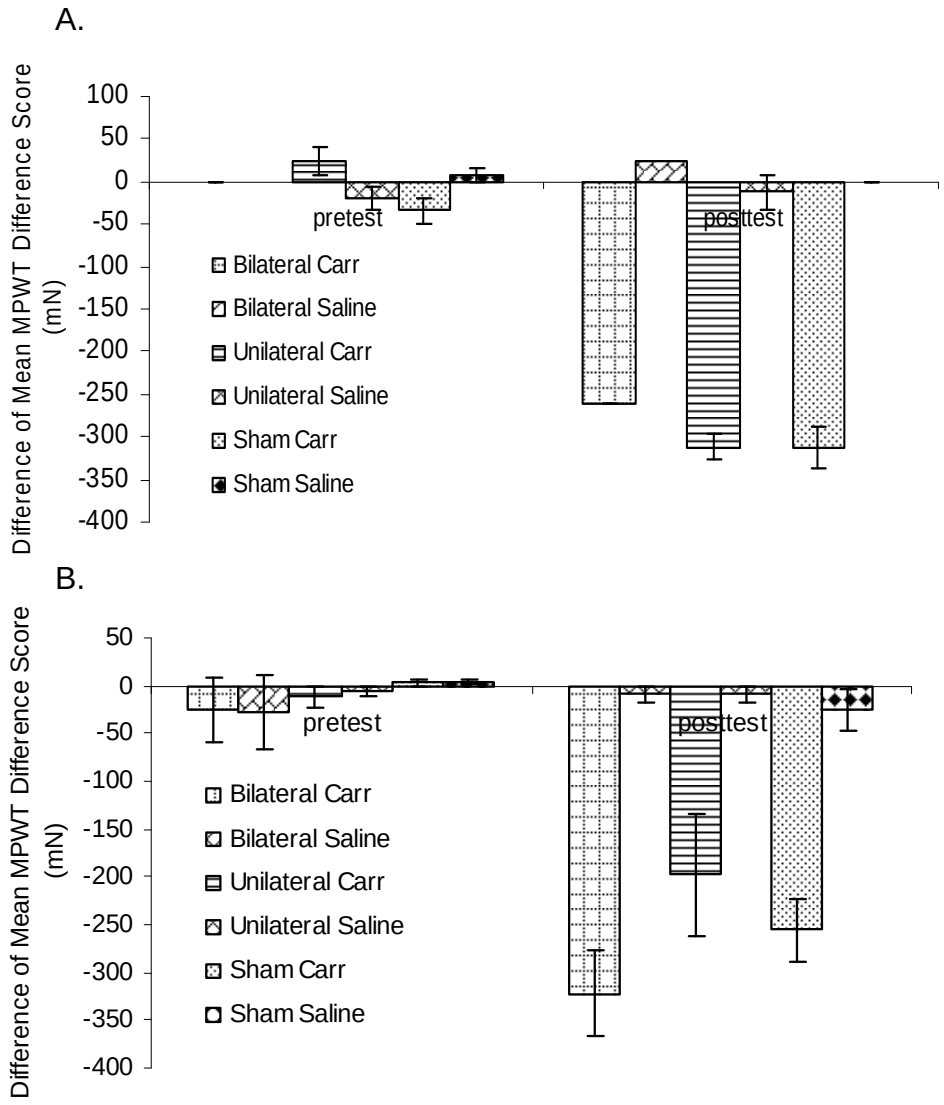
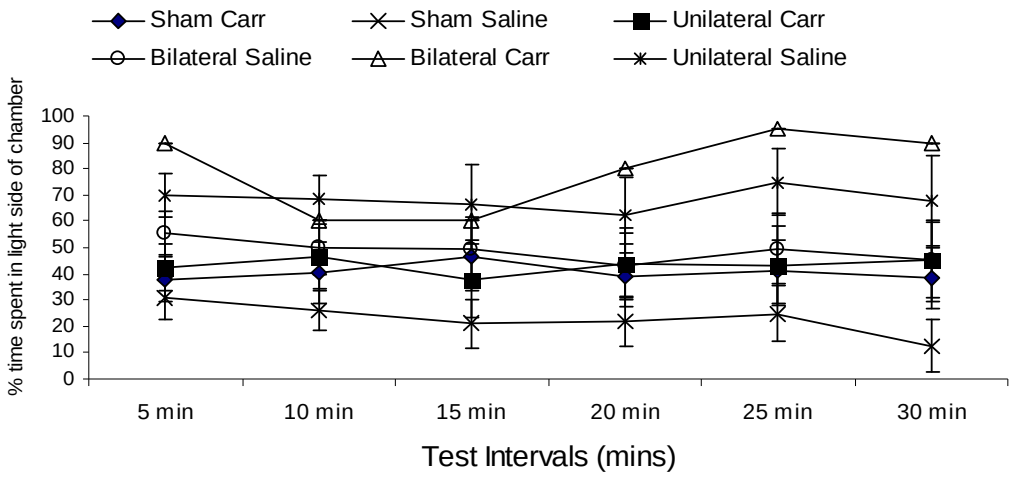


Figure 1. Mean Mechanical Paw Withdrawal Threshold difference (posttest – pretest) scores for animals that received PCC (A) or ACC (B) lesion type and Carrageenan or Saline injection type.

A.



B.

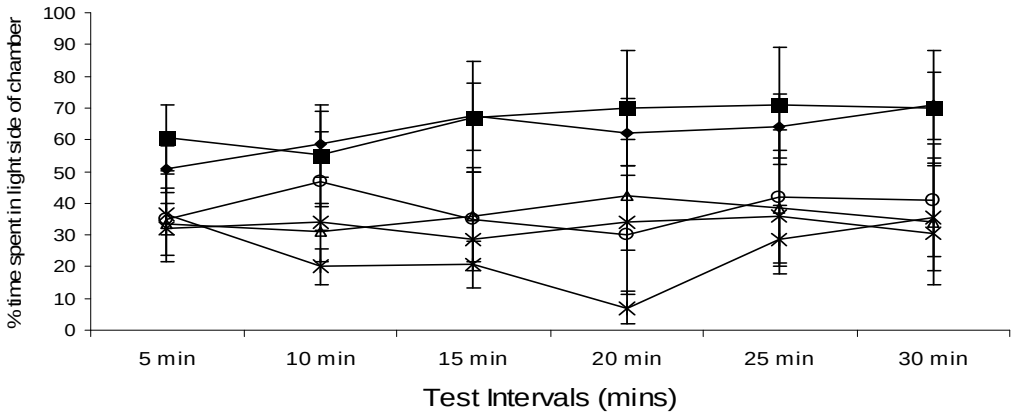


Figure 2. Mean percentage of time spent within the light side of the PEAP chamber across test intervals. PCC lesion groups (A) show higher percentage of time spent in the light side for Carrageenan injected animals compared to controls. The ACC lesion groups (B) shows a similar result except the Bilateral Carrageenan group which shows a decrease in avoidance/escape behavior.

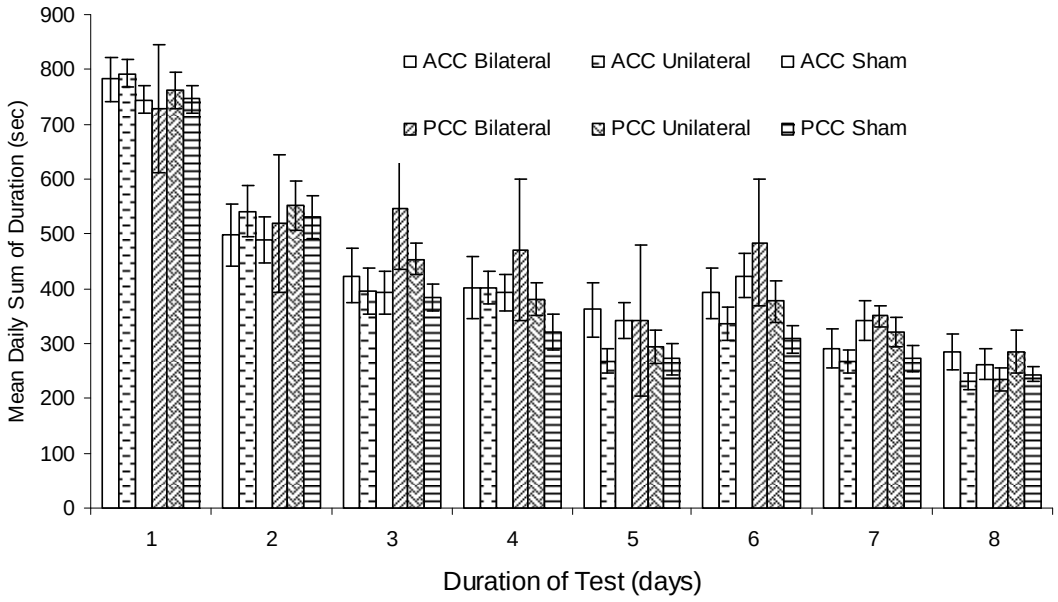


Figure 3. Mean daily sums of the each group's duration to escape to the platform in the Morris Swim Test.

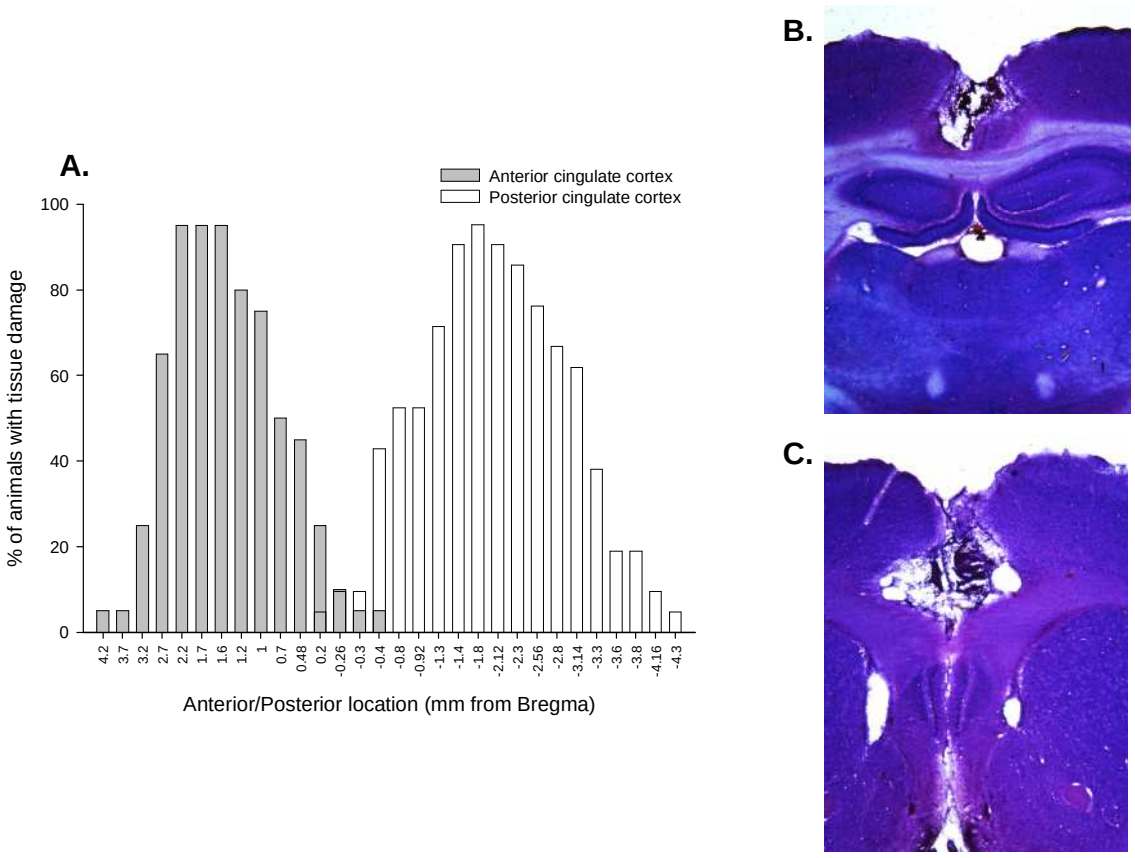


Figure 4. Histological results are shown for the percentage of animals (A) with tissue damage at the Anterior/Posterior location relative to Bregma for the Anterior then Posterior Cingulate Cortex lesion. An example of a Posterior Cingulate Cortex lesion is presented in figure B. An example of an Anterior Cingulate Cortex lesion is presented in figure C.

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BIOGRAPHICAL INFORMATION

Rebecca R. Weaver was born in Wisconsin later living in Arkansas and Illinois. She graduated with her Bachelor of Arts from Henderson State University in Arkadelphia, Arkansas in December of 2003. Upon completion of this project, she received her Master of Science in Experimental Psychology. Currently, she continues her education by pursuing a PhD in Experimental Psychology at the University of Texas at Arlington. Her current interests include mechanisms of sensory systems. Past projects include investigation of paternal loss during infancy and protective effects of DHA on hippocampal morphology against lead poisoning. In the future, she wishes to pursue topics such as sensory memory and sensory system disorders.