The Rostral Thalamic Reticular Nucleus: How Lesions to the Rostral TRN effect performance in rats on a Visual Discrimination Task

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Balleine and O'Doherty (2010) cite that the nucleus accumbens does not influence goal-directed learning, but that it may influence goal directed performance. They also state that the nucleus accumbens does not have a role in action-outcome learning. They do however point out that the nucleus accumbens may play a role in putting a "value" on a reward, therefore motivating or not motivating action. So, although the nucleus accumbens does not directly fit into the original hypothesis, there is still possibility that a lesion to the area might affect the rats' performance on the visual discrimination task. However, upon examining the data, the lesions had no noticeable effect on the rats' performance. Therefore, it can be concluded that the nucleus accumbens does not play a role in action selection nor does it affect their performance on a visual discrimination task.

INTRODUCTION

Francis Crick, the famous biologist, referred to the thalamic reticular nucleus as the "guardian of the gateway" (Crick, 1984). He saw the thalamic reticular nucleus (TRN) as a mediator for the "gateway" which connects the thalamus and the cortex; any communication between the two must pass first through the TRN. An abundance of sensory information travels between the thalamus, TRN, and the cortex before a decision is made. In fact, any and all of the axons passing between the cortex and the thalamus must pass through the TRN. Simply attenuating yourself to some stimuli requires many complex neural messages and connections. Research (eg: Weese, Phillips, and Brown, Yingling and Skinner) suggests that the TRN might play a role in attention and learning. With the number of children diagnosed with ADHD on the rise, information regarding a structure in the brain that may function in facilitating attention and learning could prove to be extremely useful.

Filled with thalamocortical and corticothalamic axons, the TRN is a thin layer of neurons that "cups" the dorsal and lateral parts of the dorsal thalamus (Sherman & Guillery, 2006; Landisman, Long, Beierlein, Deans, Paul, and Connors, 2002). Wilton Baird, Muir, and Aggelton, (2000) stated that the TRN sends inhibitory, GABAergic output to the thalamic nuclei and receives excitatory inputs from both the cortex and the thalamus. The TRN only sends inhibitory signals; however, it is important to note that the effect of those inhibitory signals may in the long run have an excitatory effect. For example, the TRN could send an inhibitory signal to the ventral lateral nucleus (VL) of the thalamus, which in turn could inhibit an inhibitory action on the VL. The final effect would then be excitatory. It can be thought of as a double negative type of effect.

A study by Weese et al. (1999) showed that the TRN plays a role in the orienting of attention. In his study, rats were trained on a visual discrimination task in which they were required to respond to a visual cue with either a "left" or "right" nose poke. Weese showed that unilateral lesions to the TRN of rats affected their reaction times on the visual task. This research provided empirical evidence for the assertion that the TRN had a role in attention. The next year, McAlonan, Brown, and Bowman (2000) provided evidence that the TRN is also involved in selective attention. Their experiment employed the use of Fos-protien, a marker of neuronal activity, and an appetitive, classical conditioning, blocking procedure. They found more Fos-protien stained neurons in the dorsal portion of the TRN, the area of the rats' brain associated with visual stimuli, in rats conditioned to the visual stimuli. Likewise, rats that were conditioned to the auditory stimuli had more Fos-positive neurons in the auditory area of the TRN, ventral and posterior to the visual sector. This provided evidence that the rats' TRN was involved in attention to a specific stimulus. These findings prompted further research on the function of the TRN in attention.

In 2002, Humphries and Gurney suggested that the TRN plays a role in "action selection," which is key to our ability to focus on a single task. Humphries and Gurney discussed how the TRN inhibits those inputs with less salience than others, allowing for the most salient input to be expressed and the actions necessary for carrying out that action to follow. So, even though there may be other noises or visual input in the room where you are, you are able to focus on this paper because it is providing the

most salient input and is the least inhibited by your TRN.

Humphries and Gurney reference the primitive importance of action selection by alluding to a problem a gazelle might face in the wild. They set up a situation in which a gazelle is grazing in grassland when a lion approaches. The Gazelle must select an action, either to continue grazing or the flee from the predator. Another example, which is more appropriate to current dilemmas, could occur in a classroom rather than grassland. Suppose there is a student in a classroom and he or she is provided with two options, pay attention to the teacher or doodle in his or her notepad. There must be action selection in order for the student to be able to direct his attention to his teacher. Understanding more about the TRN and its role in attention might provide the information necessary to aid children with ADHD and similar problems.

Many structures in the brain, an example being the cortex, are compartmentalized. The same type of compartmentalization is present in the TRN. Yingling and Skinner (1976) showed that the visual, auditory, and somatosensory regions in the cortex had "complimentary" regions in the TRN. In their paper they showed that stimulation of various parts of the TRN inhibited specific cortically evoked responses. Their experiment showed, for example, that stimulating the section of the TRN adjacent to the lateral geniculate body in the thalamus inhibited cortical evoked responses in the visual cortex. They also showed that similar suppression of cortically evoked responses occurred in the auditory cortex when the section of the TRN located lateral to the medial geniculate of the thalamus was stimulated. These examples give evidence that the TRN is indeed sequestered into functional sections, as are many other structures in the brain. Furthermore, this implies that these parts of the TRN, tied with specific functions, can be studied separately from one another.

Wilton et al. (2001) proposed that the rostral pole of the TRN might play a role in learning and memory due to its dense connections with the anterior thalamic nuclei. Wilton used spatial learning and memory tests to determine whether lesions in the rostral thalamus made a difference in rats' performance. Wilton used a T- maze test, radial arm task, and a version of the Morris water maze. None of these tests resulted in significant differences in the rats' performance due to the lesions in the TRN. He concluded his paper stating that there did not seem to be any evidence to suggest that the rostral TRN had any role in learning and memory.

Wilton commented on the fact that his findings contrasted the findings of another researcher, M'Harzi, Collery, and Delacour (1991). M'Harzi found that rostral TRN lesions did in fact cause significant differences between rats' performance on radial arm tasks, reporting that the lesion animals performed significantly worse. Wilton proposed a possible explanation for this might be the difference in the size of the lesions in each study. Wilton suggests that those lesions in M'Harzi's study might be too large; therefore, they could have possibly caused a lesion in other parts of the brain surrounding the target area. If the lesion is not limited to the target area, the rostral TRN, then the effects of the lesion cannot be attributed to the rostral TRN alone. The effects could be due to the lesioning of the other, surrounding tissue.

The current experiment was concerned with the rostral pole of the TRN and its possible role in rats' ability to perform on a visual discrimination task. The visual discrimination task differed from the spatial learning and memory tasks utilized by Wilton et al. (2001) in that there were specific cues, in the form of LED lights in a dark box, which gave the rat "cues" as to which type of decision to make. The cues provided something like a prompt, to which the rat was to answer. Ibotenic acid, rather than NMDA, was used to cause the lesions in the rats' brain. And, in light of Wilton's suggestions concerning lesion specificity, an attempt to keep lesions as concentrated and specific as possible were made.

METHODS

The experiment was carried out using 15 hooded rats. Rats were given food *ad libitum* but were deprived of water. The rats were held in a 12 hr day/night light cycle and were tested during the day section. The rats were caged in plastic boxes and given water during testing and for a period of 20 minutes post testing. Each rat was tested until 90 minutes had expired or until the rat had reached 128 trials. After the rat was finished testing, the data was recorded in a lab notebook and the rat was allowed water.

The testing box had 5 holes, each with a LED light inside. The holes were arranged like a "plus" sign, with one in the center, two to the left and right of the center hole, and two above and below the center hole. Water was administered at the rear of the box, opposite the front of the box where the LED lights and holes were located. The rat's task was to stick its nose in the center hole and wait for a visual cue (the "up" or "down" light to flash) they were then supposed to select a corresponding "left" or "right" hole selection.

The rats were slowly habituated to the training box through a series of computer programs. First the rats were trained to associate a flash of light with the administration of water at the rear of the cage. Then they were trained to put their noses in the center hole in order to receive water. Next they were trained to select either the left or right hole. As the training progressed the amount of time the rat was required to keep its nose in the hole increased. The rats were trained until they reached criteria, which consisted of making 52 correct selections for each side (left and right) 80% of the time. Once the criteria level was reached the rats were prepared for surgery.

Surgical Procedures:

Each rat was anesthetized using the same method. Ketamine and xylazine were used according to the specific rats weight at the time of surgery. The rats ranged in weight from 220g to 320g. All of the instruments were sterilized using both soap and water and a *Germinator 500* dry sterilizer immediately before surgery. The rat's heads were shaved using an electric razor. The rat's skull was then secured in position using a stereo-taxis. An incision was made on the rats scalp to expose the skull. Bregma and Lambda were located and the skull was set so that the two were on an even plane. Unilateral lesions were made to one side of the skull for each rat. A drill was used first. Bregma was located and the drill tip started at the bregma position; from there, the coordinates for the specific side and lesion were followed until a hole was drilled into the skull. Following the drilling, the needle, containing ibotinic acid with a phosphate buffer, was connected to the stereotaxic arm. The tip of the needle was placed at bregma and the specific coordinates were followed once again from the new starting point. From there the needle was lowered into the brain and the lesions were made. For the first lesion, 1.5 ul of the acid was administered over 3 minutes the co-ordinates for the lesion were AP 1.4, ML 1.9, and 6.7 mm deep. The needle was left in the brain for 3 additional minutes following the release of the acid. This allowed for the acid to kill the surrounding cells and to prevent the acid from following the needle tip up through the brain, possible killing non-targeted cells. For the second injection, 1 ul of acid was administered over 3-minutes, the co-ordinates were AP 1.8, ML 2.4, and 7.3 mm deep. Once again, 3 minutes was allowed to pass before the needle was removed. After the injections the rats' scalp was sutured and antibacterial cream was placed on the scar.

After surgery, the rat was placed in a separate cage under a heat lamp and was provided with water and food so it would be able to recover. The rats were under supervision until they woke from anesthesia. Each rat was given 3 days of post surgery recovery before they were tested again. They were deprived of water on the third day. The rats post-lesion testing was recorded for 10 days after the 3 days of rest.

Histology:

After testing was finished the rats were perfused using Ketamine. Next the rats were flushed with 50 mL of NaCl and then with 50 mL of Formaldehyde inserted through the aorta. A craniotomy was performed and the brain was kept in a jar containing 4% sucrose liquid. The brain was then sliced into 40-micron slices using a microtome. Next the slices of the brain were placed on a slide and dyed with cresly violet. After the brain tissue was dyed, coverslips were added.

Upon examining slices of the rat brain, it was found that lesions to the brain were made not in the thalamic reticular nucleus as was intended. The lesions were made more anteriorly in the nucleus accumbens. This may be a function of the new digital read-out for the stereo-taxis. Figures 1 – 3 show where the ibotenic acid caused lesions in the nucleus accumbens. The rats labeled BR and RB are considered the control rats. There were no signs of lesions in either rat.

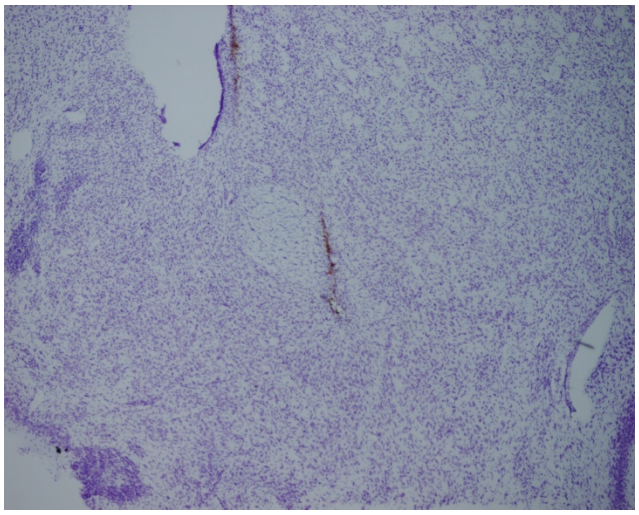


Fig 1

RESULTS

Movement time is defined as the rats' movement to a selected nose hole. It differs from reaction time in that reaction time is recorded once the rat has removed its nose from the hole. For

reaction time, it is presumed that the rat has already decided which hole to put its nose in before the rats' nose is in the other hole and while the rats' nose in the center hole. Movement time measure the time it takes for the rat to stick its nose in a peripheral hole after first sticking its nose in the center hole. The reason for this distinction is to discern whether there is a problem in the rats ability to make a decision on which hole to go to (which would be seen as an increase in the reaction time) or in the rats ability to physically select a hole (an increase in the movement time would suggest this).

Correct/Incorrect Responses

Data collected for Correct and Incorrect responses in the rats showed that there was no real effect for the rats with lesions. Figures 4 – 8 show graphical representations of the data. The largest difference in responses was seen in the rat labeled BR. This rat was a control and Fig. 4 shows the drop in Contralateral Correct responses as well as the increase in Contralateral Incorrect responses.

Movement and Reaction Time

Data collected for Movement Time showed that there was no effect on the reaction time of those rats with lesions. In BR (Fig. 9) there is an increase in the movement time it took for Contralateral Correct responses. This is different from the other rats. Figures 9 – 13 show the graphical representations of the data for movement times. There was no effect of the lesion on the rats' movement time. Figures 14 – 18 show the graphical representation of the data for Reaction Time. From reviewing the graphs, no trends suggesting the lesions had an effect were seen.

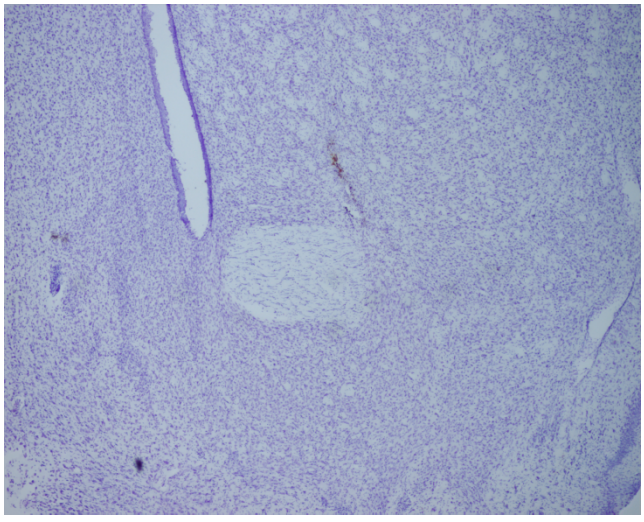


Fig 2.

DISCUSSION

The original target for the lesions was the rostral thalamic reticular nucleus. Because of a misuse of new instruments, that region of the brain was not hit; therefore, our original hypothesis that those rats with lesions in their TRN would exhibit less proficiency on a visual discrimination task could not be tested. Had the correct section been hit, there would be data relevant to the function of the TRN and its possible role in attention and learning. This might have had implications for those with ADHD.

However, the lesions were made anterior to the TRN in the nucleus accumbens. The nucleus accumbens is not thought to play a role in learning or attention. Instead, the nucleus accumbens is thought to play a large role in the reward pathway and in pleasure. Balleine and O'Doherty (2010) cite that the nucleus accumbens does not influence goal-directed learning, but that it may influence goal directed performance. They also state that the nucleus accumbens does not have a role in action-outcome learning. They do however point out that the nucleus accumbens may play a role in putting a "value" on a reward, therefore motivating or not motivating action. So, although the nucleus accumbens does not directly fit into the original hypothesis, there is still possibility that a lesion to the area might affect the rats' performance on the visual discrimination task. However, upon examining the data, the lesions had no noticeable effect on the rats' performance.

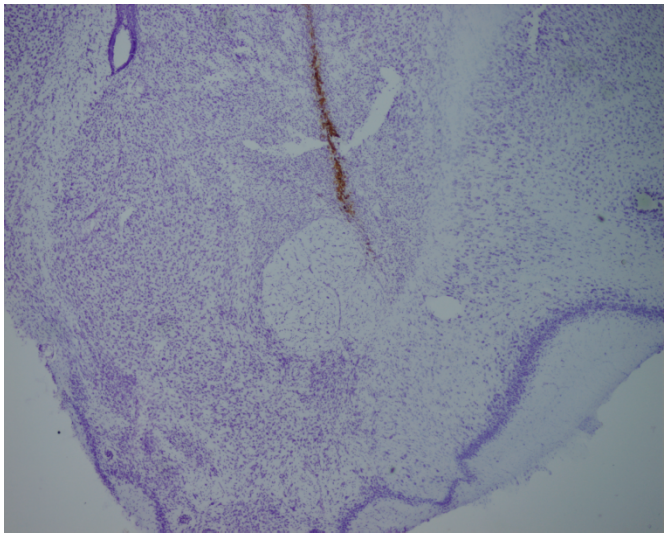
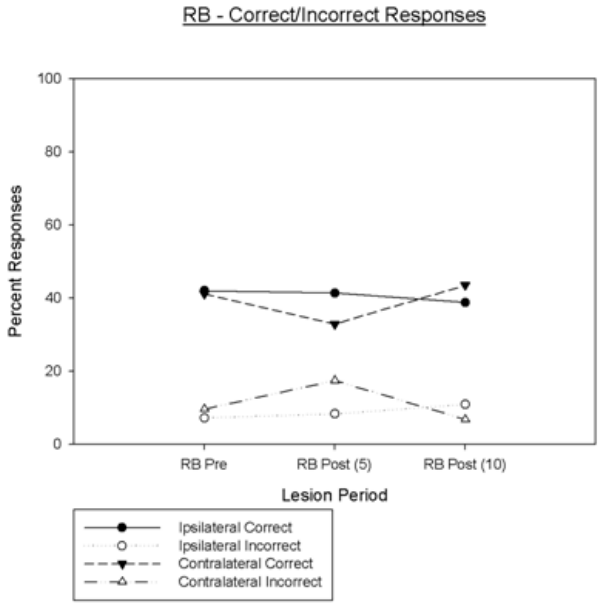
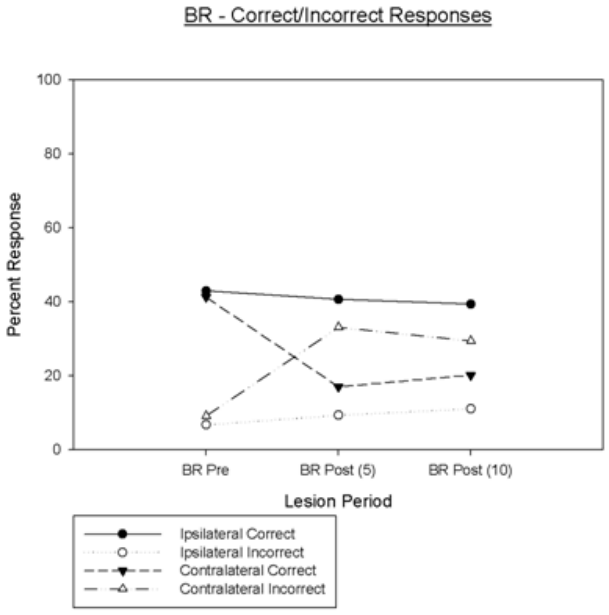


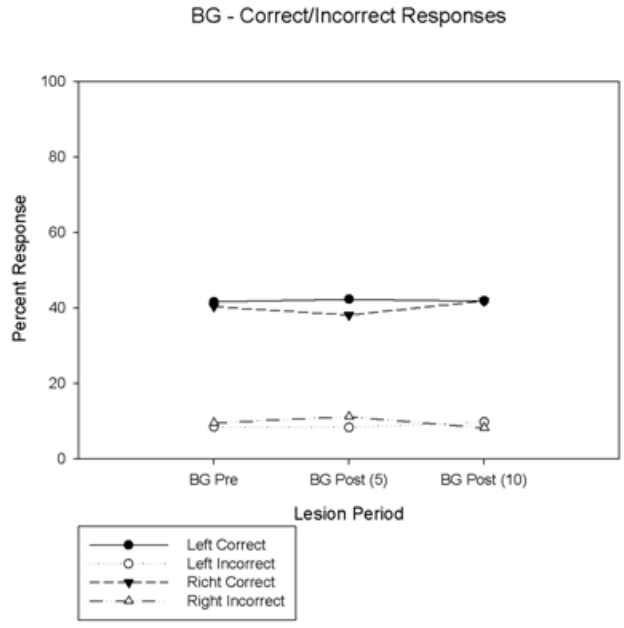
Fig 3.

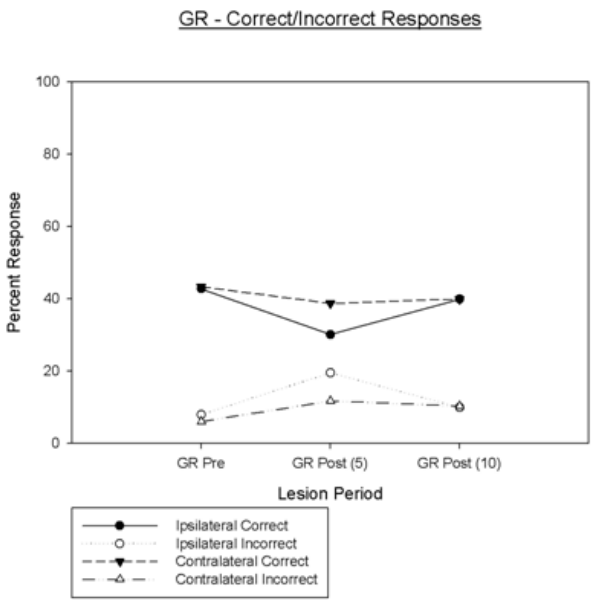
Therefore, it can be concluded that the nucleus accumbens does not play a role in action selection nor does it affect their performance on a visual discrimination task.

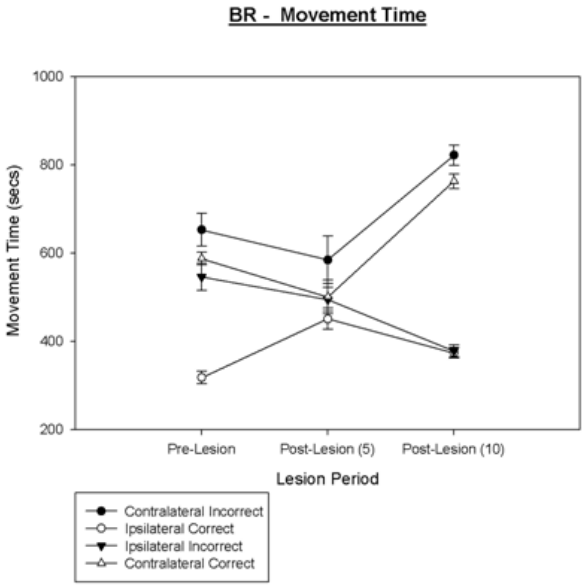
Although much research and training was done, and although each surgery and histology was completed very carefully, many improvements could be made to this experiment. First, a more thorough understanding of the instrumentation used could have eliminated the problem of hitting the wrong area in the brain. That knowledge may also have led to speedier surgeries. Second, the rats could have been stored in larger boxes or each rat could have been housed in a separate box. The ventilation in the room could have also been improved. Surgeries seemed extremely successful, however, a clear procedure for what to do if a rat does not wake from anesthesia needs to be created. Thirdly, more time to test the rats would have yielded a larger sample size of rats. That would have been better for data collection and subsequent analysis. In conclusion, even though a part of the brain different than the TRN was hit, and even though improvements could have been made, the experiment was useful.

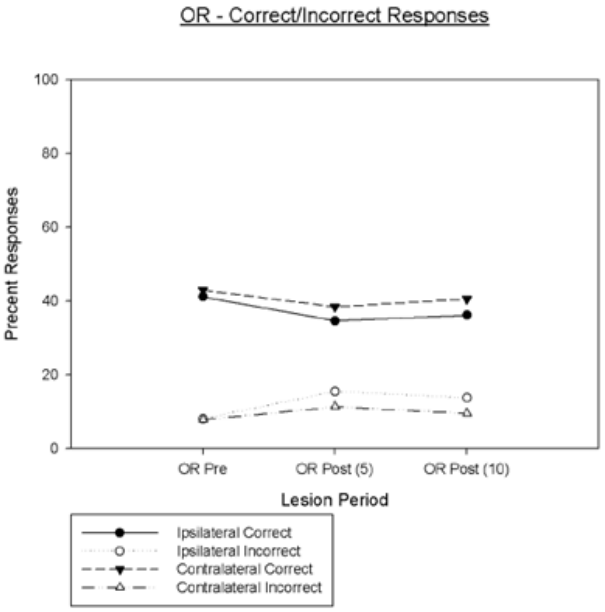


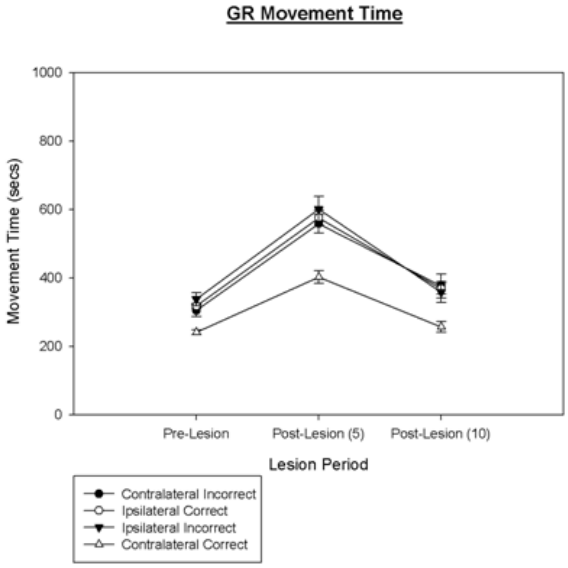


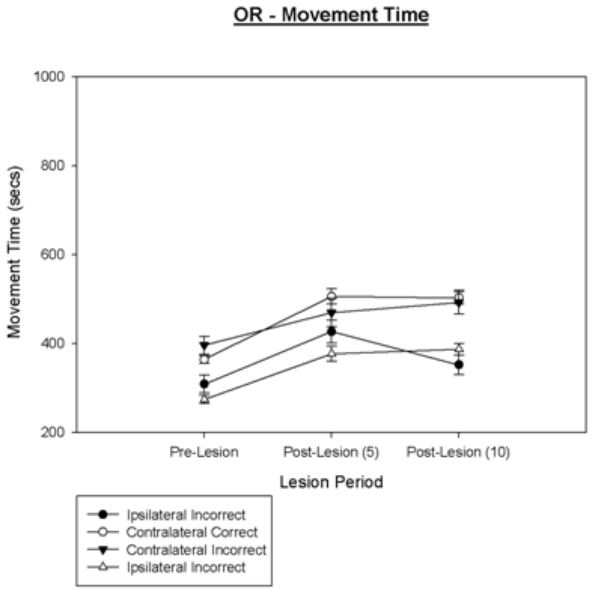


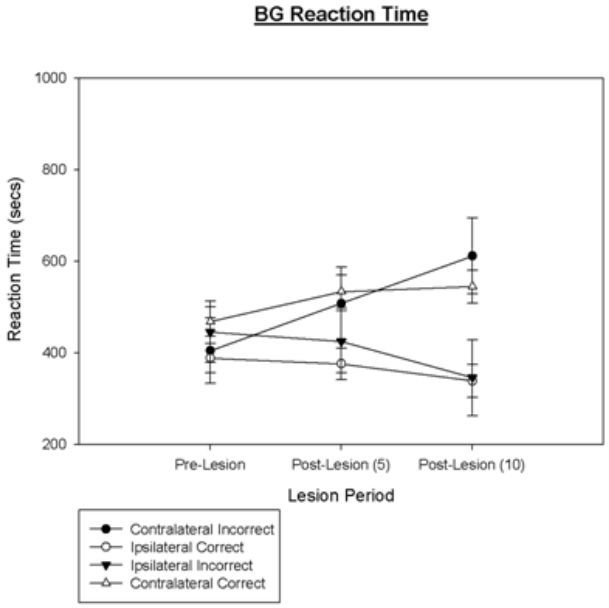

GR - Correct/Incorrect Responses
Percent Response
100
80
60
40
20
0
GR Pre
GR Post (5)
GR Post (10)
Lesion Period
Ipsilateral Correct
Ipsilateral Incorrect
Contralateral Correct
Contralateral Incorrect


BR - Movement Time
Movement Time (secs)
1000
800
600
400
200
Pre-Lesion
Post-Lesion (5)
Post-Lesion (10)
Lesion Period
Contralateral Incorrect
Ipsilateral Correct
Ipsilateral Incorrect
Contralateral Correct


OR - Correct/Incorrect Responses
Precent Responses
100
80
60
40
20
0
OR Pre
OR Post (5)
OR Post (10)
Lesion Period
Ipsilateral Correct
Ipsilateral Incorrect
Contralateral Correct
Contralateral Incorrect


GR Movement Time
Movement Time (secs)
1000
800
600
400
200
0
Pre-Lesion
Post-Lesion (5)
Post-Lesion (10)
Lesion Period
Contralateral Incorrect
Ipsilateral Correct
Ipsilateral Incorrect
Contralateral Correct





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