The Effect of Wheel Training on Cognition Following Unilateral Entorhinal Cortex Injury

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Traumatic brain injury (TBI) adversely affects an individual's memory, learning, and orientation. It is hypothesized that damage to the entorhinal cortex (EC) will lead to loss of sensory integration and cognitive dysfunction. Not all TBIs are symmetrical throughout the brain. If a car is sideswiped in a motor vehicle collision the sideways motion could cause the driver to hit their head on one side only. The impact could cause brain damage to the immediate area or damage to the other lobe. Damage to only one side of the brain could lead to different losses of cognitive and behavioral recovery. The loss of sensory integration, which is intimately tied to memory and learning, necessitates more study. The purpose of this study was to research the effect of wheel training on cognition following unilateral entorhinal cortex injury.

Sensory integration is the process of sorting external stimuli, such as vision, sound, smell, and touch, into discernible signals through the different levels of the brain. For example, a dog that first hears a rabbit in the grass turns to look at the rabbit and receives a visual stimulus of the rabbit that enhances the original sound. A blind dog would have a harder time locating the rabbit using only sound. Multisensory pathways integrate environment stimuli from different parts of the brain in order to form a memory or a motor response. In this process, the EC (located in the middle temporal lobe between the hippocampus (HPC) and the sibiculum) is used in conjunction with the HPC and dentate gyrus to integrate external stimuli [1].

Survivors with TBI continue to receive multiple stimulations from their environment, but often have difficulty integrating these stimuli to form memories. In 1994, Zola-Morgan related hippocampal damage to the severity of memory impairment and found that injuries to both HPC and EC showed a marked increase in memory impairment over HPC injuries alone. These results show that the EC is used in conjunction with the HPC for memory [1].

Background and Significance

The outside environment creates a multitude of simultaneous stimuli. The multisensory pathways that exist throughout the EC help to process these stimuli for the HPC. Information from the EC is then sent to various receptive fields in the brain according to the origin of the stimulation. The receptive fields then integrate the information and form the reaction and memory of the situation. Multiple stimulations in a normal brain excite these receptive fields within the HPC [2]. After injury, the presence of differentiated stimuli can lead to repression of the receptive fields due to missing integrating pathways. This repression will lead to difficulty forming new memories and controlling reactions in an intensely stimulated environment. For example, if a subject is unable to integrate or process multiple stimulations simultaneously, an overload will occur, because different receptive fields will be firing different signals [2]. If, for example, one noise occurs to the left while another occurs to the right, but if the subject is incapable of integrating the information, both stimulations would be depressed and neither would be acknowledged.

Every day people use multiple sensory stimuli to form memories. For a certain sound, a particular picture is brought to mind. This association is created through a process that involves the EC. The EC helps process information from sensory inputs to the HPC. Lavenex shows that the EC is not merely a relay of multisensory information to the hippocampus, but participates actively in the memory processes by further integrating information [3].

The HPC is associated with long and short-term memory. Different stimuli received from the secondary integration pathways of the EC help create both the long and short-term memory [3]. When the EC is injured, the paths of sensory integration to the HPC and memory formation are disrupted. Loesche

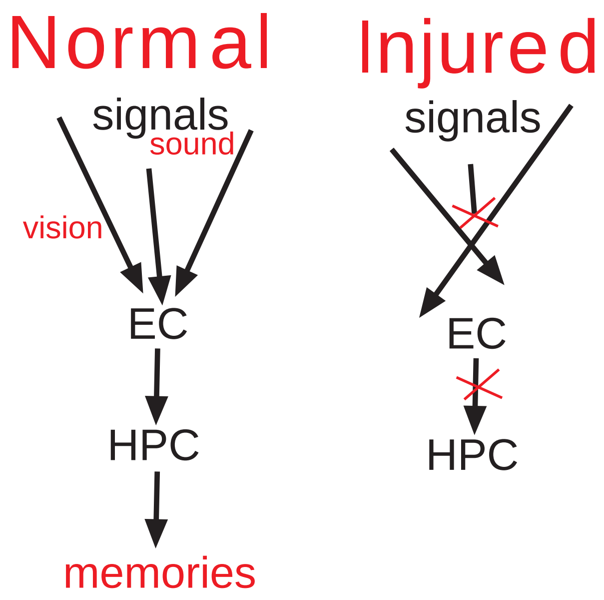

Figure 1

and Steward and Davis et al (2000) demonstrated this disruption through the deficit in the training and cognition of their bilateral and unilateral EC injured animals.

According to Loesche and Steward [4], subjects with unilateral EC damage were able to improve cognitive performance with time because of rewiring of the EC through the dentate gyrus. Specifically, Loesche and Steward hypothesized that cells in the dentate gyrus were reinnervated in part by nerve cell axons from the central lateral EC. This process, when the remaining uninjured neurons reconnect pathways, is known as plasticity. Although plasticity occurs after EC lesions, the degree of functional return is limited and is dependent on the difficulty or demand of a task [5]. A simple task may not require the animal to develop many projections of the EC into the dentate gyrus, but a more complex task can stimulate additional growth necessary for the animal to perform satisfactorily. For unilateral EC lesions, animals can partially reinnervate back into the dentate gyrus [5] so that complex motor stimulation of these animals should be able to increase the performance standard set by Loesche and Steward.

High frequencies of unilateral injuries necessitate plasticity of the injured area to help restore cognitive function. The specific aim of this study was to determine if a high demand, complex task wheel training would improve cognitive function in animals with unilateral EC injury. The hypothesis tested was that the wheel task helps increase the cognitive performance of the unilateral EC injury animals in a swimming maze.

Design

Experiments measured indicators of unilateral EC injury and cognition by varying the complexity of the wheel task. Research lasted 21 days with animals from the University of Michigan labs. A reverse 12-hour light-dark cycle was used so animals were at peak activity during testing and surgery. The animals were given four days to adjust to the new cycle before injury. Control and injured animals were selected randomly on day four. After injury, the animals were given two days of rest. After the two-day rest, the injured animals were again randomly divided into a continuous (group B) or intermittent wheel group (group A). The intermittent wheel group would run the first and last day of the 12 days of testing and the continuous group had six days of consecutive testing followed by six days of rest and retraining on day 12. Wheel testing started on the 4th day post-operation and continued until the 15th day post-operation. Each day the animals completed one round of testing (Fig. 2). After wheel testing, all animals were tested for cognition six days using a water maze over a period of six days.

Surgical Procedure

Unilateral lesions were created in the rats using specifically placed electrically pulsed leads as described by Loesche and Steward [4]. Post

Group	Test 1	Test 2	Test 3	Test 4	Test 5	Test 6	Test12
A	✓						✓
B	✓	✓	✓	✓	✓	✓	✓

Figure 2 : Wheel Training Schedule

experiment brains were removed and sliced horizontally in 80-micron sections. Brain slices were stained with cresyl violet and analyzed for extent and location of injury under a light microscope according to the parameter set forth in Paxinos [6].

Behavioral Testing – Three days following EC damage animals were tested using a wheel task [7]. The purpose of this task was to implement sensory integration and motor training for the animals. The motor task of the wheel helps to develop a sensitization to maintaining balance on the wheel and a habituation of wheel walking [7]. The number of times the wheel task was used depended on an animal's group. The wheel task consisted of one-minute total interval training where demand changes, from simple to complex, were made by increasing the speed of the wheel. A simple wheel task was to walk on top of a wheel that switched directions every 15 seconds. To remain on the wheel, animals had to learn to turn. The wheel task was a measure of sensory integration that to require that required the animals to use multiple senses including vision, hearing, balance, vestibule-motor, etc [7]. The wheel started slowly and increased its speed per certain number of trials. A complex wheel task built on the simple wheel task by increasing sensory stimuli. These sensory inputs included olfactory, visual, balance, and tactile stimuli used simultaneously.

Following the 12 days of wheel training, a swim task was used to measure the effects of the motor training upon cognitive orientation. The water maze was chosen as a reliable measure of spatial learning. The water maze was a 5-foot diameter by 3-foot deep pool. The water was shaded white with dry powdered milk to hide the white platform just barely beneath the water level. This platform was placed at the same location every day with cues around the pool, also in the same location every day. Animals were dropped at different locations around the pool and their swim paths, exploratory behaviors, and swim times recorded (Davis, 2001).

Twenty days from the injury, a probe memory test was conducted to see if the animals had learned the location of the platform after completing water maze training (Davis, 2001). For the probe test, the platform was removed completely from the pool. The animals were dropped into the water and allowed to explore the tub for 30 seconds. The path of exploration, including initial heading direction, was taken after the animal had been dropped into the water. Following the probe trial, cue tests were run to see if the animals could learn to swim towards a cued platform rather than the previously learned submerged platform. Cue tests consisted of removing the platform from the usual location and placing a novel platform with a visual cue in another location in the tub. The animals were dropped and their path, time, and exploratory behaviors were recorded just as in the water maze test in the 2001 Davis study [8].

Sample

The animals were Sprague-Dawley male rats. These animals were chosen specifically due to their increased ability to survive after lesion surgery, and because human males receive TBI 4:1 over human females.

Results

HISTOLOGY– Lesions produced damage to the entire medial and lateral EC. Animals with lesions

	n	Mean	SD
Uni B	6	33.1	57.2
Uni A	5	56.4	62.4
Con B	11	34.9	53.7
Con A	14	30	51.9

Table 1: Probe Directional Heading Error

Uni B = unilaterally injured with continual wheel training
Uni A = unilaterally injured with intermittent wheel training
Con B = control with continual wheel training
Con A = control with intermittent wheel training

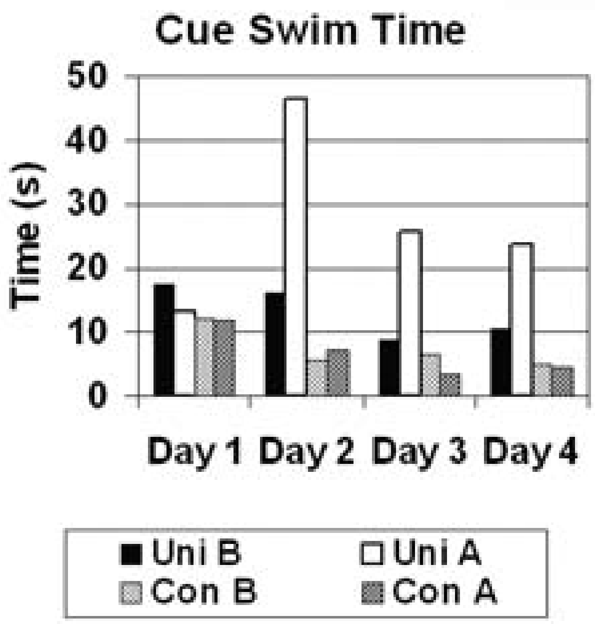

Figure 3

in the thalamus, superior colliculus, or cerebellum were eliminated from analysis, leaving 36 total animals. Lesions could not have damaged more than 20% of the hippocampus and more than 10% of the midbrain, thalamus, and basal ganglia. The minimum extent of damage and maximum to either side are available for comparison.

PROBE DIRECTIONAL HEADING ERROR: Unilaterally injured animals without wheel training had increased mean directional heading error in the probe task compared to controls with and without wheel training (Table 1). Unilaterally injured animals with wheel training had a mean heading error comparable to that of controls with and without wheel training. Error was greater than chance in these animals compared to injured animals with training and all controls. Overall, controls had no significant difference in mean heading error. Below, n is sample group size and SD is standard deviation for each mean.

CUE SWIM TIME EC: Injured animals and controls look similar on the first trial because all were unfamiliar with the task (Table 2). On trial 2 injured animals without wheel training took almost 9 times longer than controls and 3 times longer than injured animals with training to reach the cue. This trend continued in the animals without training, but gap diminished during the third and fourth trials. The between group effect in the injured groups was not significant ($p = .075$). Significance is $p < .05$. P values are statistical ways of showing significance in data, so the lower the number, the more significant the results are. No between group effect was found for controls

Discussion

In this study, animals with unilateral EC lesions exposed to a 7-day wheel-training task had improved cognitive function compared to animals without a 7-day wheel-training program. These results support the hypothesis that a wheel task helps increase the cognitive performance of the unilateral EC injury animals in the swimming maze. The results in this study also support the recommendations of Loesche and Steward [4], who set up a timeline for the cognitive recovery of unilateral EC injury. They suggested that modifying a post lesion testing/retraining program could improve performance and possibly shorten recovery time. Their hypothesis was tested in this experiment and the conclusions are: 1.Wheel training enhanced memory in animals with unilateral injury to the entorhinal cortex during a probe trial. 2.Wheel training enhanced learning in animals with unilateral injury to the entorhinal cortex during a cue trial. 3.Wheel training did not influence memory or learning in control animals. 4.A planned sensory stimulation and motor

Group	n	Mean 1 (SD)	Mean 2 (SD)	Mean 3 (SD)	Mean 4 (SD)	p
Uni B	6	17.3s(18.3s)	16s(16.5s)	8.8s(5.5s)	10.5s(7.5s)	.075
Uni A	5	13.2s(9.5s)	46.6s(51.1s)	25.8s(17.1s)	23.8s(25.6s)	
Control B	11	12.0s(6.8s)	5.5s(3.7s)	6.5s(3.2s)	5.1s(3.0s)	.859
Control A	14	11.8s(7.1s)	7.1s(3.3s)	6.2s(2.7s)	4.6s(2.0s)	

Table 2: Cue Swim Time

activity program influenced cognitive recovery from unilateral entorhinal cortex injury.

It is possible that the improvement comes from undamaged EC being forced to integrate several stimuli, thus increasing demand for multisensory integration through the EC. The more intense pattern of firing can help retrain the brain to use new pathways or create them through the damaged EC. Repression or excitement of the receptive fields of the animal may eventually have adjusted to the stimuli, integrating multisensory information through the injured EC. These are possible reasons for the development improved memory and learning after the wheel task.

Conclusion

The results provide some evidence that a post lesion retraining program may improve overall cognitive performance in unilaterally EC injured animals when compared to each other. Significance may not have been reached because this was a side study using accidental unilaterals. Future studies in this area should involve examining histological changes related to plasticity. The site and extent of plasticity needs to be examined and documented. Also, in this study there is no differentiation between left or right EC unilaterally injured animals. Therefore, planned use of unilateral injury to examine these same trends needs to include differences between left and right EC injury. Future studies could provide further information for increasing the cognitive performance of unilaterally injured animals by changing the post-training task. Eventually, a post lesion-retraining program to improve overall cognition in TBI humans may be developed.

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