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Transection of CA3 Does Not Affect Memory Performance in Rats

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Abstract

Longitudinal hippocampal pathways are needed for seizure synchronization, and there is evidence that their transection may abolish seizures. However, the effect of such transection on memory is unknown. In this study, we investigated the effect of transverse CA3 transections on memory function in Sprague-Dawley rats. Using a stereotactic knife, a single CA3 transection was made unilaterally (n=5) or bilaterally (n=5). Sham surgery was done in another group (n=4). Morris water maze (MWM) and Novel object recognition (NOR) tests were started 18 days later revealing no significant differences between transected animals and controls. Cresyl-violet brain staining confirmed the locations of transections in the CA3 region. We conclude that normal performance in MWM and NOR tests does not appear to require intact transmission throughout the whole length of CA3, supporting the hypothesis that CA3 transections may be used in temporal lobe epilepsy to interrupt seizure circuitry while preserving memory.

Keywords

Temporal Lobe Epilepsy; Hippocampus; Epilepsy Surgery; Memory Processing

INTRODUCTION

Mesial temporal lobe epilepsy (MTLE) is the most common cause of intractable focal epilepsy [1] and the most frequent indication for epilepsy surgery [2]. The current gold standard therapy of MTLE is surgical resection of the hippocampus [3], which may result in severe memory deficits, especially in patients with nonlesional brain MRI and with normal existing memory scores on neuropsychological testing. This underlies the need to pursue an intervention that will control hippocampal seizures without affecting memory.

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It is known that the hippocampus is a key neural substrate for memory processing, but the details of its involvement are not fully understood. Rodent experiments have constituted the basis for a number of theories about hippocampal memory processing because of remarkable structural and functional similarity between human and rat hippocampus [4]. Although tract tracing studies have identified longitudinal fibers that run parallel to the long axis of the hippocampus [5], most myelinated fibers in the hippocampus are organized in parallel loops with unidirectional connections [6, 7]. These loops originate and end in the adjacent entorhinal cortex, and are arranged in parallel lamellae, which possibly allows small strips of the hippocampal cortex to operate as independent functional units in memory processing [6, 8].

On the other hand, the longitudinal axonal circuits of the hippocampus have been shown to be important for seizure generation and propagation [9–12]. Not only is intrahippocampal connectivity known to be preserved in MTLE [13], but synchronization of ictal discharges across the whole length of the hippocampus is required for propagation of seizures outside of the hippocampus resulting in clinical seizures [14, 15]. Thus, preventing intrahippocampal seizure synchronization may control seizures in MTLE. *In vitro* studies suggest that hippocampal hyperexcitability is related to abnormal connectivity across CA3 [16], and that cuts in the CA3 prevent propagation of epileptiform activity in the longitudinal direction [12]. This effect could be attributed directly to the removal of recurrent excitation. Moreover, hippocampal transections have been shown to control kainic acid-induced seizures in rats [10] and mice [11], without assessment of the effect of such transections on memory. In this study, we intended to assess the effect of unilateral and bilateral CA3 transections on memory function in rats.

METHODS

CA3 TRANSECTIONS

The protocols used for this study were reviewed and approved by the Institutional Animal Care and Use Committee of Case Western Reserve University. Male Sprague-Dawley Rats weighing 247–285 g were anesthetized by intra-peritoneal injection of Ketamine 80mg/kg and Xylazine 10mg/kg, and received a cefazolin dose of 16mg/kg subcutaneously. The rats were monitored using the toe pinch reflex and maintained under sedation with a 30% maintenance dose of Ketamine. Their heads and upper backs were shaved and scrubbed with betadine and alcohol to prepare for the surgery. They were then mounted into a stereotaxic frame with their body temperatures maintained at 37°C with a heating pad. Along the planned incision line, 0.15 ml of bupivacaine – diluted by saline at a ratio of 1:5 – was injected subcutaneously. A small scalp incision was then made rostro-caudally to expose the skull for burr hole drilling.

A stereotactic knife (Kopf Instruments, Tujunga, California) was used for this study. This knife attaches to the stereotactic frame and has two calibrated knobs: one that controls the insertion distance of the sheath, and another that controls the extent of protrusion of the knife from the sheath. The sheath was implanted through the burr holes 3.3 mm posterior to bregma, 4.0 mm lateral to bregma and 4 mm ventral to the surface of the brain, targeting the CA3 region according to the rat brain atlas [17]. The knife was protruded from its sheath, extending for 1 mm in a plane perpendicular to the longitudinal axis of the hippocampus before the sheath was moved upwards by 0.5 mm to transect CA3. The knife was then withdrawn into its sheath, and the sheath was pulled out. Three groups were studied. Group 1 (n=5) underwent unilateral CA3 transection (n=2 left, and n=3 right), group 2 (n=5) underwent bilateral CA3 transections, and group 3 (n=4) underwent knife insertion through burr holes, but without transections. The scalps were then sutured using 4-0 prolene sutures.

All rats were housed in individual cages at room temperature (20–25°C) in a veterinarian-monitored animal care facility.

MEMORY TESTING

The Morris water maze (MWM) and novel object recognition (NOR) tests were started 18 days after surgery by experimenters blinded to the group membership of the rats. The MWM test used to detect spatial learning and memory deficits was adapted from other authors [18]. The rats were trained in a 182-cm diameter pool filled with water and a non-toxic black dye to a depth of 30 cm, and the water temperature was maintained at 26°C. A circular platform, 29 cm high and 15 cm in diameter, was placed in the center of the NW quadrant of the pool. A pretraining session was performed during which animals were introduced into the pool and guided to the platform where they were allowed to rest for 15 sec. Training sessions lasted 120 sec. during which, if the rat found the platform, it was allowed to remain on it for 15 sec., but if it didn't, escape was assisted. We performed 4 test trials divided in two sessions 30–40 minutes apart. All rats were released into the water randomly at one of the assigned starting positions, and facing distal cues consisting of black and white posters on the wall. Traveled distance, escape latency, and velocity were recorded using a video tracking system and software (Ethovision, Noldus Information Technology, Wageningen, The Netherlands). Testing was carried out for 4 consecutive days. The last trial on day 3 was a probe trial in which the platform was removed and animals were allowed to swim for 1 minute. In addition to the measurements done for training trials, we measured the time spent in each quadrant, the latency to reach the target quadrant, and the number of crossings over the location of the removed platform. Finally, proximal cue testing was carried out in order to ensure no visual acuity issues. For this test, all distal cues were removed and a flag was placed on the platform as a proximal cue. We performed 4 consecutive trials and escape latency was recorded. Animals with an average escape latency exceeding 30 sec were considered visually impaired.

The NOR test was performed in a manner identical to that described by other authors [19]. To acclimate the rats, we brought them one day before testing to the testing room for one hour, and allowed them to explore the empty 50 × 50 cm chamber for 15 min. On the testing day, animals were placed in the chamber with two identical objects 15 cm apart. The animals were allowed to explore the objects for 10 minutes and then returned to their home case for 3 hours. We then placed the rats in the chamber with two different objects, but with similar exploratory index, and allowed them to explore objects for 5 min. Exploration was defined as the rat nose being within 1 cm of the object and its whiskers moving. Discrimination scores were calculated as the percent time spent exploring the novel object over total exploration time. After behavioral testing, we confirmed location of the CA3 transection by cresyl violet staining of the rat brains.

RESULTS

For MWM, repeated measures ANOVA analysis for escape latency showed no significant difference across our experimental groups ($F = 0.812$; $p = 0.482$), and we confirmed successful animal training by finding a significant group difference across days ($p < 0.007$) (Fig. 1). To rule out swimming speed as a confounding factor, we studied differences in swimming velocity across groups. As expected, there was a significant difference in swimming speed across days ($F = 15.32$; $p < 0.001$), but no significant differences between groups ($p = 0.880$). For the probe trial, one way ANOVA analysis indicated no group difference in the time spent in the NW quadrant that had previously contained the platform ($F = 2.789$; $p = 0.129$) (Fig. 2). In addition, we found no difference in the latency to first enter the target quadrant among groups ($F = 0.812$; $p = 0.482$) (not shown).

For NOR, discrimination scores (mean and SEM) (calculated as time spent to explore novel object divided by total time spent to explore both familiar and novel objects) were also not different between controls and transected animals ($F = 1.633$; $p = 0.26$, ANOVA) (Fig. 3). Cresyl violet staining confirmed the locations of transections in the CA3 region (Fig. 4).

DISCUSSION

The efficacy of hippocampal transections in controlling seizures in animal models of temporal lobe epilepsy has been studied [10, 11], but the safety of such transections with regard to memory functioning has not been investigated. In this study, we have shown that unilateral and bilateral cuts of the CA3 layer did not affect NOR and MWM scores in rats, suggesting that memory processing necessary for these tasks does not require intact transmission throughout the whole length of this pyramidal cell layer.

MTLE is likely related to synaptic reorganization associated with the formation of new axon collaterals across the longitudinal axis of the hippocampus increasing recurrent excitatory circuitry [20–22]. Such circuitry has been demonstrated in the dentate gyrus [23, 24] and in the CA3 region [25]. The role of mossy fiber sprouting in epileptogenesis has been investigated extensively [24, 26–28], and transection of the dentate gyrus may reduce seizure discharges [11]. However, one study used intracellular recordings in the dentate granule cells to provide the strongest direct evidence that the mossy fiber sprouting is related to only weakly excitatory connections, and that the sprouted dentate gyrus does not normally generate epileptiform discharges [22]. Moreover, abnormal longitudinal hippocampal connectivity across the pyramidal cell layer has been associated with hyperexcitability and seizures [16], and CA3 transection is sufficient to abolish seizure discharges in a hippocampal preparation [12].

To study the effects of CA3 transections on memory, we used the novel object recognition (NOR) test to assess recognition memory and the Morris water maze (MWM) test to assess spatial memory. The hippocampus is important for performance in these tasks, and using both tests intended to provide a robust assessment of hippocampal function and increase the chances of detecting any impairment after CA3 transections.

The MWM is an excellent test for assessment of hippocampal function and is popular in studies that assess memory after manipulation of the hippocampus [29]. The effects on MWM performance after treatment have been more widely replicated than the effects observed with any other learning task [30]. The MWM is primarily a test of spatial learning and reference memory. The task is carried out across days to determine learning. Acquisition of the reference memory requires the hippocampus to form spatial associations between hidden platform location and surrounding environment (as in tasks of cued recall in humans), making the hippocampus necessary for this task [31, 32]. In this study, we found that all these functions appeared unaltered by CA3 transection.

The NOR test is popular for testing object memory in rodents. Selective hippocampal lesions have been reported to impair recognition memory performance in humans [33] as well as rodents [19, 34, 35]. The evidence that the hippocampus is important for recognition tasks comes from humans, monkeys, rats, and mice and is based on findings from permanent lesions, reversible lesions, and gene-knockout techniques [36].

Preserving the entorhinal cortex and its connections with the hippocampus is probably more indispensable for memory than preserving intrahippocampal longitudinal fibers. Declarative memory processing is known to be dependent on the entorhinal cortex and its bidirectional connectivity with the hippocampal lamellae [37]. The superficial layers (II and III) of the entorhinal cortex receive input from multimodal sensory associational areas and, project

perpendicularly to all subregions of the hippocampal formation [38]. Output stations of the hippocampus, including CA1 and subiculum, project back to the deep layers of entorhinal cortex (VI & V) [37, 39]. Thus, the entorhinal cortex serves as a bidirectional interface between the neocortex and hippocampal formation and as such forms a nodal part of the cortico-hippocampo-cortical loop that is the brain's hardware for the formation of declarative memories [38]. Indeed, entorhinal cortex lesions have resulted in learning and memory deficits in mammals [40], and hippocampal-dependent spatial learning only requires a minislabs of hippocampal tissue [41].

The small sample size is a limitation of this study. Post-hoc power analysis indicated that our sample size was able to detect a difference in Morris water maze (MWM) scores of at least 20% between sham and transection groups, with 80% power and 0.05 probability of Type I error (alpha). In other words, if the true difference between sham and transection MWM scores was less than 20%, our sample size may not have been sufficient to detect statistical significance. Nevertheless, a 20% decrease in memory score is less than what is expected after total hippocampal resection. Although our data are only preliminary and conclusions tentative, the results merit a larger adequately-powered study to investigate memory-related effects of hippocampal transections. Additionally, in order to further explore the translational implications of this work, future experiments should be done in epileptic animals and include assessment of both seizure control and memory function after hippocampal transections.

Highlights

- Hippocampal lamellae are believed to be functional units of memory processing
- Perpendicular cuts in the hippocampus do not seem to affect performance in memory tasks in rats.
- Hippocampal transections may disrupt seizures without interfering with memory processing in humans.

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Fig. 1. MWM training performance showed no differences between sham operated (n=4), unilaterally transected (n=5), and bilaterally transected rats (n=5).

Fig. 2.

Probe trial performance in the MWM test showed no significant differences in the percentage of time spent in the NW quadrant between groups.

Fig. 3. Discrimination scores in the NOR test. No differences were noted between sham operated (n=4), unilaterally transected (n=5), and bilaterally transected rats (n=5).

Fig. 3. Planned stereotactic lesioning using the rat brain atlas [17] (A) and performed CA3 cuts shown in cresyl violet staining (B). *DG*, dentate gyrus.