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Spatial Cognition Following Early-Life Seizures: Performance Deficits are Dependent on Task Demands

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Abstract

Cognitive impairment is a common co-morbidity in childhood epilepsy. Studies in rodents have demonstrated that frequent seizures during the first weeks of life results in impaired spatial cognition when the rats are tested as juvenile or adults. To determine if spatial cognitive deficits following early-life seizures are task-specific or similar across spatial tasks we compared the effects of early-life seizures in two spatial assays: 1) The Morris water maze, a hippocampal-dependent task of spatial cognition; and 2) The active avoidance task, a task that associates an aversive shock stimulus with a static spatial location that requires intact hippocampal-amygdala networks. Rats with early-life seizures tested as adults did not differ from control rats in the water maze. However, while animals with early-life seizures showed some evidence of learning the active avoidance task, they received significantly more shocks in later training trials, particularly during the second training day, than controls. One possibility for the performance differences between the tasks is that the active avoidance task requires multiple brain regions and that inter-regional communication could be affected by alterations in white matter integrity. However, there were no measurable group differences with regard to levels of myelination. The study suggests that elucidation of mild cognitive deficits seen following early-life seizures may be dependent on task features of active avoidance.

Graphical Abstract

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Keywords

epilepsy; Morris water maze; active avoidance; epilepsy; myelin

1.0 Introduction

Although seizures are the most striking clinical manifestation of the epilepsies, individuals with epilepsy are at risk not only for seizures, but also for a myriad of co-morbid health problems that occur in people with epilepsy at a higher rate than would be expected by chance [1]. Among the co-morbidities associated with epilepsy in children, cognitive abnormalities are among the most common and troublesome [2, 3]. The distribution of IQ scores of children with epilepsy is skewed toward lower values [4, 5] and the number of children with epilepsy experiencing difficulties in school because of learning disabilities is greater than children without epilepsy [6-12]. While etiology of the epilepsy clearly plays a major role in cognitive development, there are indications that early-life seizures (ELS) independent of etiology can lead to cognitive impairment [13, 14]. In a prospective study of children with new-onset epilepsy (< 8 years), pharmacoresistance was associated with an 11.4 point decline in full scale IQ [15]. The decline in IQ was highly correlated with age of onset, with children with an early onset of the epilepsy at greatest risk.

The cognitive deficits seen in children with frequent seizures have been modeled in rat pups. Using the recurrent flurothyl seizure model of recurrent ELS, we have shown cognitive impairment when animals are tested as adolescents or adults. Cognitive deficits seen following ELS include deficits of spatial cognition in the Morris water maze (MWM) and radial-arm water maze [16-19], non-match to sample task [20], auditory discrimination [21] and behavioral flexibility [22].

To further characterize the adverse cognitive effects of ELS we compared performance in same cohort of rats in two spatial cognitive tasks, the MWM, which is primarily a hippocampal-mediated task, and the active avoidance task, which involves a fear component. The MWM is one of the most commonly used tests of spatial cognition. It is a test of hippocampal-dependent spatial memory [23, 24], and a close parallel to episodic memory in humans [25-27]. While the task is hippocampal-dependent, dorsomedial striatum and medial prefrontal cortex support acquisition and recall in the task [28, 29]. As opposed to the water maze, the active avoidance task is a systems-level task requiring coordination between different brain regions that allow for associating a region of space with an aversive stimulus [30-32]. In this task, animals learn to associate an unmarked region of space with a mild shock on a constantly rotating arena. The rats must attend to their ever-changing position on the rotating arena lest they be rotated into a static shock zone in the room frame where they receive a mildly painful electrical shock.

While the histological sequelae of flurothyl-induced seizures has been well studied there have been no studies examining myelination following ELS. An additional goal of this study was to therefore assess myelination following ELS. Since myelination in the cortex is not complete until age six month [33] we evaluated the rats in the MWM and active avoidance task at age six months.

We hypothesized that following ELS, rats would have deficits in both the MWM and active avoidance task. Furthermore, we hypothesized that the deficits would be greater in active avoidance due to task performance dependence on multiple brain regions, such as the amygdala in the conditioned emotional response, as well as the need to calculate position with more than one frame of reference. In addition, we hypothesized that putative differences in task performance could be due to ELS affects on levels of axon myelination that could ultimately affect communication between relevant brain regions.

2.0 Methods

2.1 Overview

From postnatal (P) day 5 to P14 rat pups received 70 flurothyl-induced seizures. At age 6 months rats with ELS and controls were first tested in the MWM and then 20 days later in active avoidance. Following completion of the active avoidance test, rats were sacrificed and the brains analyzed for putative differences in white matter integrity that might interfere with communication between brain regions. In all studies, the investigator was blinded as to the indemnification of the group.

2.2 Animals

All experiments were performed in accordance with the guidelines provided by the National Institute of Health and University of Vermont for the humane treatment of animals. The animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Vermont.

Two litters of 10 male Sprague-Dawley pups were used for the study. Ear holes were used to identify individual rats. Ten rats randomly selected from the two litters were subjected to 7 flurothyl-induced seizures daily from P5 to P14 for a total of 70 seizures using previously described methods [18, 20, 34]. A 10% flurothyl solution (Bis(2,2,2-trifluoroethyl) ether), an inhaled convulsive agent, was delivered to the pups, which were placed in a plastic container located in an airflow hood. Flurothyl (0.1 ml) was injected slowly onto filter paper placed on the inside of the container where it evaporated. Pups were removed from the flurothyl chamber after when tonic extension of both forelimbs and hindlimbs was observed. Littermate control pups ($n = 7$), randomly selected from the two litters, were handled and removed from the dam during the time of the seizure to control for the effects of maternal separation stress.

2.3 Behavioral Studies

2.3.1 Water maze—Rats were first tested for spatial memory function using the MWM at age 6 months using techniques previously described in our laboratory [35, 36]. We used video tracking (ANY-maze, Stoelting Co., Wood Dale, Ill) to automate the testing.

A stainless-steel circular swimming pool (2 m in diameter, 50 cm high) was filled to a depth of 25 cm with water at room temperature (70° F). Non-toxic white paint was added to make the water opaque and prevent the rats from seeing the platform. Room cues visible from the water surface were constant from day to day. Four points on the perimeter of the pool were

designated north (N), east (E), south (S), and west (W), thus dividing the pool into four quadrants (NW, NE, SE, SW). A clear plexiglass escape platform 8 cm in diameter was positioned in the center of one of the quadrants, 2 cm below the water surface.

On the first day of testing each rat was placed in the pool for 60 seconds without the platform present; this free swim enabled the rat to become habituated to the training environment. Starting three hours after habituation the rats began the hidden escape platform portion of the test. The rats underwent 6 timed, hidden platform trials with the platform in the same quadrant across days, for four days (Days 1-4). The point of immersion into the pool varied between N, E, S, and W, in a random order for each trial, so that the rat was not able to predict the platform location from the point at which it was placed in the pool. The latency from immersion into the pool to escape onto the platform was recorded for each trial, and the observer also recorded the route taken by the rat to reach the platform. On mounting the platform, rats were given a 30 second rest period, after which the next trial was started. If the rat did not find the platform in 120 seconds, it was manually placed on the platform for a 30 second rest. At the start of each trial, the rat was held facing the perimeter and dropped into the pool to ensure immersion.

On day 5 the platform was removed and animals underwent the probe test for 60 seconds when the time spent in the quadrant where the platform had previously been located was recorded. The test began with the rat in the quadrant opposite to the trained platform location. The path and time spent in the quadrant where the platform had previously been placed was recorded. In this part of the water maze, termed the probe test, normal animals typically spend more time in the quadrant where the platform had been previously located than in the other quadrants. The testing procedure used with the platform present provides a measure of spatial learning and reference memory, while the probe trial is considered to measure the strength of spatial memory [27].

2.3.2 Active avoidance—Twenty days following water maze testing animals underwent testing in the active avoidance task (Biosignal; Brooklyn, New York). In this task, animals learn to associate an unmarked region of space with a mild shock on a constantly rotating arena. The rats must attend to their ever-changing position in the room frame lest they be rotated into a pre-determined shock zone where they receive a mildly painful electrical shock (Fig. 1).

Rats were lightly anesthetized and implanted with a stainless steel swivel in the skin between the shoulders. This allowed attachment of a cable with an LED at the end that allows for automated tracking and also the delivery of shock. Experimenters were blind as to which animals were control and ELS.

The arena consists of a steel disc 82 cm in diameter and is lighted from both above and below. The arena is centered in a room where it is approximately 50 cm from black curtains on the S and E sides and 50 cm from white walls on the N and W sides. The N and W walls have an 11 cm gray power-strip that forms a continuous line 50 cm above the floor of the arena. Two rectangular spatial cues (30 cm high $\times$ 43 cm wide) depicting a red star (centered at W position) and a black circle (centered at N position), both on a white background, were

placed 18 cm above the arena floor. An additional rectangular polarizing cue (53 cm high × 84 cm wide) made of white paper (Color-Aid 2.5 grey) with five 2.5 cm wide diagonal black stripes (Color-Aid 9.5 gray) was centered at the N position, 5 cm above the gray power strip.

On day one of training, the animal was connected to the cable and allowed to explore the arena for 10 min. On the second day, a clear plexiglass barrier was placed around the perimeter of the arena that rotated at a rate of one revolution per minute. The rat was again allowed to explore the arena for 10 min. On all subsequent days, rats received a 0.4 ma shock in an unmarked 876 cm² wedge-shaped sector covering a 60° arc in the NE sector of the arena. The shock zone was stable in the room frame while the arena rotated. The entrance latency of the shock was 1ms, the shock duration was 0.5 sec and the inter-shock latency was 2 sec.

Mixed groups of ELS and control rats were trained 8 ten-minute sessions per day for two days (16 sessions) and the number of shocks were recorded. Animals that received 5 or fewer shocks in 2 consecutive sessions met task criterion. Animals that did not were designated as non-learners and were excluded from analysis [31].

2.4 Histology

Following the behavioral testing, rats were sacrificed and the brains stained for myelin to determine whether inter-regional communication could be affected by alterations in white matter integrity. Prior studies in this model have examined volume and cell number, using unbiased stereological estimations, of the prefrontal cortex and hippocampus and found no differences between controls and rats with ELS [37]. However, myelination following ELS has not yet been done. Alterations in the integrity of white matter projections could explain cognitive deficits as well as disruption to normal electrophysiological communication between brain regions previously described in this model [37]. A gold chloride stain was used here to assess myelination [38, 39].

After deep anesthesia with isoflurane the rats were transcardially perfused with 0.1 M phosphate-buffered saline (PBS, pH 7.4) followed by 4% paraformaldehyde. The brains were removed immediately, post-fixed for 24 hours in 4% PFA and then immersed in 30% sucrose (w/v) at 4° C until the brains sank to the bottom of the chamber. Frozen coronal sections through the entire extent of the hippocampus were cut at 40 µm with a freezing microtome and then air dried for 30 min at room temperature. The sections were fixed with 10% formalin for 10 min then air dried for 30 min. After rinsing the slides were stained with 0.2% gold chloride in phosphate buffer (1.8 g crystalline gold chloride, 0.33 g sodium phosphate monobasic monohydrate, 3.6 g sodium phosphate dibasic anhydrous, 9.0 g sodium chloride, 1000 ml distilled water) in an oven at 37° for 40 min. Following a rinse in tap water the slides were placed in 2.5% sodium thiosulfate anhydrous (25 g in 1000 ml distilled water) for 5 min. Sections were then cover slipped after dehydration in graded alcohols and clearing in xylene.

Myelin was examined both qualitatively and quantitatively. Degree of myelination was qualitatively assessed blindly in the frontal, parietal, occipital, temporal, and piriform cortex, thalamus, amygdala and hippocampus by examining the number of myelin fibers seen. As

shown in Fig. 2, length and number of fibers crossing from cortex into the corpus callosum were compared between animals. Corpus callosum volume quantification was performed using the Nikon Eclipse E600 microscope with a digital video camera fitted with a 3D motorized stage. Using the atlas of the rat brain from Paxinos [40] the corpus callosum area was calculated from sections between plates 11-13 and plates 21-23 for the frontal cortex and dorsal hippocampus, respectively. The areas measured in the frontal and dorsal hippocampus are shown in Fig. 2.

2.5 Sample size and statistical analysis

Group size was determined by the goal to detect an 8 second difference in mean time in the target quadrant during the probe test with a standard deviation of 6 seconds. A total of 12 rats (6 per group) provided a power of 0.90 and an alpha of 5 to detect such a difference. Because of concern about loss of animals during the study, the group sizes were increased and 7 controls and 8 rats with ELS completed all the testing.

The repeated measure ANOVA was used to compare performance in water maze and active avoidance tests. A two-way ANOVA was used to determine if there were differences in time in the target quadrant during the probe test. To satisfy parametric test assumptions, number of shocks in the active avoidance was log-transformed. The generalized linear model, utilizing the gamma log link model (SPSS, V22; Armonk, NY: IBM Corp) was used to analyze the cumulative number of shocks per animal and group on each training day. Data is expressed throughout as the mean $\pm$ sem. The t-test was used to compare white matter area between controls and rats with ELS.

3.0 Results

All of the rats had tonic seizures with administration of the flurothyl. Two rats died during the period of time the flurothyl was administered while none of the controls died.

3.1 Behavioral Testing

3.1.1 Water maze—There was a significant group $\times$ trial interaction ($F_{(6,161)} = 3.278$, $p = 0.005$) but no differences in trials ($F_{(6,276)} = 1.329$; $p = 0.251$) or groups ($F_{(6,276)} = 0.7665$; $p = 0.597$). A two way ANOVA showed no interaction or quadrant or group effect (Fig. 3). There were no differences in swimming speed in the controls and ELS groups (Cont: 0.249 ± 0.022 meters/sec; ELS: 0.225 ± 0.007 meters/sec; $p = 0.269$).

3.1.2 Active avoidance—There were significant difference in groups $F_{(6, 105)} = 10.87$, $p < 0.001$ as well as a group trial interaction $F_{(6, 105)} = 17.40$, $p < 0.001$). We used the gamma log link model to analyze cumulative time spent in each quadrant of the active avoidance arena each day and found a significant group effect for the cumulative time spent opposite the shock zone between ELS animals (Mean = 1201.12 ± 120.22) and controls (Mean = 1787.35 ± 191.25 ; $p = 0.007$). We did not find a group $\times$ day interaction ($p = 0.71$). In a complimentary manner to this result, we also found a significant group effect for the cumulative time spent clockwise to the shock zone between ELS animals (Mean = 2375.65 ± 214.31) and controls (Mean = 1778.48 ± 171.51 ; $p = 0.028$). Again, we did not find a

group $\times$ day interaction ($p = 0.61$). These results indicate that the avoidance strategy of the control animals involved spending more time as far as possible from the shock zone while the avoidance strategy of the ELS animals involved spending more time adjacent to the shock zone.

Three ELS animals and two control animals were eliminated from analysis due to failure to reach criterion. As all animals were able to learn the water maze task, this suggests that the active avoidance task is more difficult for these animals. No significant group difference was found with regard to the cumulative number of shocks accrued during the 8 training sessions on the 1st day of training between control (Mean = 115.60 ± 33.88), and ELS rats (Mean = 119.40 ± 34.99 ; $p > 0.05$). However, a significant group effect was found with regard to the cumulative number of shocks accrued during the 8 training sessions on the 2nd day of training by control rats (Mean = 53.8 ± 15.54), and ELS rats (Mean = 132.20 ± 38.19 ; $p = 0.028$).

In the ELS animals there was no significant improvement between the 1st and 2nd day of training with regard to the number of cumulative shocks ($p > 0.05$). On the contrary, control animals did demonstrate significant improvement with a reduction in the number of shocks on the 2nd day ($p = 0.022$). The ELS animals that reached criterion, as suggested by Fig. 4, showed signs of performing better than controls on session 4 in the first day of training and on session 9 during the second day of training. However, toward the end of each training day, the ELS animals tended to get more shocks than controls. This difference was even more pronounced on the second day when the control animals continued to improve their performance. There was no difference in locomotion speed (Cont: 4.364 ± 0.002 meters/sec; ELS: 4.465 ± 0.263 meters/sec; $p = 0.767$).

3.2 Histology

In both the controls and ELS rats there were no qualitative differences in the degree of myelination in the frontal, parietal, occipital, temporal, and piriform cortex, thalamus, amygdala and hippocampus. There were also no differences between groups in corpus callosum area in the frontal lobe ($t_{(28)} = 1.405$, $p = 0.170$) or hippocampus ($t_{(28)} = 0.2280$, $p = 0.775$) (Fig. 5).

4.0 Discussion

In this study we found that while rats with ELS performed as well as controls in the MWM, they received more shocks than controls in the active avoidance task, indicating that deficits in spatial cognition may be task-dependent following early seizures.

We chose to study the MWM and active avoidance tasks since while both tests provide a measure of spatial cognition, the two tasks involve different networks and levels of cognitive demand. As opposed to the MWM, which is primarily hippocampal-dependent, the active avoidance task is a systems-level task that requires coordination between frontal cortex for attention and short-term memory, hippocampus for reference memory and amygdala for fear. Inactivation of the hippocampus [41] and basolateral amygdala [42] have shown these two structures to be necessary for task performance. Active avoidance also has several other

remarkable aspects. The to-be-avoided sector is defined solely by its room-frame coordinates and the animals thus cannot learn the task by simple associative learning; instead they probably use allothetic strategy [41]. The idiothetic mode of navigation, based on the collection and integration of self-motion signals, and substriatal exteroceptive cues such as scent marks, are irrelevant since the shock sector is stable [43]. The observation that rats were impaired in active avoidance but not the MWM suggests that detecting differences in spatial cognition following ELS may require a task with a higher cognitive demand, as suggested by the fact that there were animals in both groups that did not reach criterion in active avoidance while all rats learned to reach the platform in the MWM. Another important difference is that the active avoidance task has been shown to require coordination between multiple brain areas, particularly the amygdala [42].

While the ELS animals could learn the task, they would make more errors during the later training sessions each day. This may have been secondary to cumulative task demands or learned helplessness. However, we did not carry out an evaluation of depression such as the forced swim test, in these rats. Analysis of cumulative time spent in each quadrant revealed that while the control animals spend more time opposite the shock zone, the ELS animals spend more time adjacent to the shock zone. In other words, the control animals exhibit a more efficient strategy by spending more time at the furthest point from the shock zone. The ELS animals exhibit a less efficient strategy as they had a higher probability of being rotated into the shock zone the more time they spend next to it. Remarkably, our group has reported a similar result in an animal model of cortical dysplasia [44].

Somewhat surprisingly, we found no deficits in the MWM whereas prior studies using this model from our own laboratory found impairments in this task [19, 34, 45, 46]. Of note, the animals tested here were older (6 months) compared to our other studies. In P35 rats subjected to kainic acid-induced status epilepticus in P35 rats, it was observed that when rats were tested in the MWM at (P46-49) or (P60-63) spatial deficits were noted, whereas at older ages (P74-77 and P91-94) no deficits were seen [47]. Likewise, following lithium-pilocarpine-induced status epilepticus at P20 rats performed showed increasing improvement in the MWM with age [35]. Similarly, flurothyl seizures induced in adult rats showed that adverse seizure effects on the stability of place cell activity dissipates with time [48]. It thus appears likely that WMM performance shows recovery from the ELS with time.

The histological sequelae of flurothyl seizures has been well studied [19, 37, 46, 49, 50]. While sprouting of mossy fibers into CA3 has been seen following flurothyl-induced seizures [49, 50], there is no cell loss [37, 46, 51]. We add here the observation that there is no long-term effect of ELS on myelination with the key interpretation that white matter degradation interfering with communication between brain regions likely does not account for cognitive deficits shown in the model here and in other work [16, 34, 37, 46] or in alterations in the synchrony of electrophysiological signals between different brain regions (i.e., [37]).

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Highlights

- We did not find MWM task spatial deficits in 6 month old ELS animals
- ELS animals exhibited performance deficits on an active avoidance task
- ELS animals accrued more shocks than controls on the second training day
- ELS animals tended to do worse than controls on later training trials each day
- Performance deficits are not due to alterations in white matter integrity

Fig. 1.

Rotating arena for active avoidance. The gold wedge-shaped area, which is not visible to the rat, is the shock zone. The arena rotates clockwise and all clues within the arena rotate with the rat. Static cues are placed on the walls outside the arena. The pink lines represent the movements of the rat. The rat is tethered to a recording cable and the location of the rat is marked by the green cross. The red circles represent the shocks the rat receives when entering into the shock zone.

Fig. 2. Myelin stain in the cortex and corpus callosum. Note the vertical myelinated tracts traversing the cortex and corpus callosum in a control (A) and ELS (B) rat. Degree of myelination was compared in the controls and ELS rats. Area of myelin measured in the frontal lobe (A) and dorsal hippocampus (B). The area measure is marked by the dotted yellow line.

Fig. 3.

Water maze results showing latency in seconds to the escape platform. No group differences were found. The insert shows results of the probe test where there were also no differences noted in time in the target quadrant.

Fig. 4.

Active avoidance results showing number of shocks versus trial across trials. Rats with early-life seizures had significantly more shocks than controls. Note that towards the end of each of the training days (Day 1 = sessions 1-8; Day 2 = sessions 9-16) the ELS animals tended to receive more shocks than controls. This difference was even more pronounced on the second day when the control animals continued to improve their performance.

Fig. 5.

Corpus callosum areas in hippocampus and frontal lobe in controls and rats with ELS. No differences were note between groups in either brain region.