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Early Life Seizures Cause Long-Standing Impairment of the Hippocampal Map

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Abstract

Children with seizures are at risk for long-term cognitive deficits. Similarly, recurrent seizures in developing rats are associated with deficits in spatial learning and memory. However, the pathophysiological bases for these deficits are not known. Hippocampal place cells, cells that are activated selectively when an animal moves through a particular location in space, provides the animal with a spatial map. We hypothesized that seizure-induced impairment in spatial learning is a consequence of the rat's inability to form accurate and stable hippocampal maps. To directly address the cellular concomitants of spatial memory impairment, we recorded the activity of place cells from hippocampal subfield CA1 in freely moving rats subjected to 100 brief flurothyl-induced seizures during the first weeks of life and then tested them in the Morris water maze and radial-arm water maze followed by place cell testing. Compared to controls, rats with recurrent seizures had marked impairment in Morris water maze and radial-arm water maze. In parallel, there were substantial deficits in action potential firing characteristics of place cells with two major defects: i) the coherence, information content, center firing rate, and field size were reduced compared to control cells; and ii) the fields were less stable than those in control place cells. These results show that recurrent seizures during early development are associated with significant impairment in spatial learning and that these deficits are paralleled by deficits in the hippocampal map. This study thus provides a cellular correlate for how recurrent seizures during early development lead to cognitive impairment.

Introduction

Childhood epilepsy is associated with a significant risk for cognitive impairment. The distribution of IQ scores of children with epilepsy is skewed toward lower values than children without epilepsy (Farwell et al., 1985; Neyens et al., 1999) and the number of children experiencing difficulties in school because of learning disabilities is greater than in the normal population (Williams et al., 1998; Sillanpaa et al., 1998; Wakamoto et al., 2000; Bailet and Turk, 2000). While most children with epilepsy maintain stable IQ scores, some slow in their mental development (Neyens et al., 1999) or even have progressive declines of IQ on serial intelligence tests (Bourgeois et al., 1983). Children with medically refractory epilepsy are at

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particularly high risk for cognitive impairment (Bjornaes et al., 2001; 2002; van Rijckevorsel, 2006).

Animal studies have also shown that recurrent seizures result in cognitive deficits. Using a variety of techniques to induce seizures, investigators have found that rats subjected to a series of recurrent seizures during the first weeks of life have considerable learning and memory impairment in the water maze when studied during adolescence or adulthood (Holmes et al., 1998; Huang et al., 1999; Liu et al., 1999; Sogawa et al., 2001; de Rogalski Landrot et al., 2001; Huang et al., 2002; Chang et al., 2003). The mechanisms responsible for these deficits have not been well elucidated.

To directly address the cellular concomitants of spatial memory impairment, we recorded the activity of single hippocampal neurons in freely moving rats subjected to recurrent seizures during early development and compared this activity to that of control rats. We studied place cells, cells that are activated selectively when an animal moves through a particular location in space (the 'place field'). Firing fields are stable over days to weeks as long as the environment remains constant, suggesting that place cells retain information about location rather than creating it *de novo* each time the rat enters the environment (O'Keefe and Conway, 1978; Muller and Kubie, 1987; Thompson and Best, 1989; Thompson and Best, 1990; O'Keefe et al., 1998). Based on the finding that a considerable fraction (25–50%) of the cell population of the hippocampus is place cells (Muller, 1996; Vazdarjanova et al., 2002), the hippocampus is proposed to function as a spatial map (O'Keefe and Nadel, 1978). Hippocampal place cells have also been shown to code non-spatial information (Ranck, Jr., 1973; Hampson et al., 1993; Young et al., 1994; Wood et al., 1999), suggesting that the hippocampal map stores experiences associated with certain locations in the environment (Colgin et al., 2008). As shown by studies showing the association between place cell firing patterns and spatial performance (Liu et al., 2003; Zhou et al., 2007a; Lenck-Santini and Holmes, 2008; Dube et al., 2008), place cell function appears to be a robust surrogate biological marker for spatial memory.

Prior studies from our laboratory have shown that status epilepticus (Liu et al., 2003; Zhou et al., 2007b; Lenck-Santini and Holmes, 2008) and prolonged experimental febrile seizures (Dube et al., 2008) are associated with aberrant place cell firing patterns and stability and impaired spatial cognition.

We now report for the first time that rats experiencing recurrent seizures during early development show deficient performance in tasks of spatial learning and memory and have an impaired hippocampal map as evidenced by defective place cells. These results demonstrate that recurrent seizures during brain development have consequences that are reflected at the cellular level in the hippocampus, the brain region implicated in episodic memory in humans and spatial memory in rodents.

Materials and Methods

The experimental procedures were approved by the Animal Care and Use Committee of Dartmouth College and were performed in accordance with NIH guidelines for the humane treatment of animals.

Animals

Male Sprague-Dawley rats ($n = 41$) were used in the study. The Recurrent Seizure (RS) group ($n = 19$) received 100 flurothyl-induced seizures from P15-P37; Controls (Cont.) ($n = 22$) were handled in the same manner but were not exposed to flurothyl.

Flurothyl-induced seizures

Serial seizures were induced by the flurothyl inhalation method previously described in our laboratory (Huang et al., 1999; McCabe et al., 2001; Sogawa et al., 2001). For each trial 4–5 rats were placed into a plastic chamber (length = 28 cm, width = 18 cm, height = 26 cm). Liquid flurothyl (bis-2,2,2-trifluoroethyl ether, Aldrich Chemical Co) was delivered through a plastic syringe and dripped slowly (3 cc/hr) onto filter paper in the center of the chamber where it evaporated and inhaled by the rats. The chamber was flushed with room air and cleaned between trials. Experimental rats were exposed to flurothyl until all had tonic extension of both the forelimbs and hindlimbs. Rats were then quickly removed from the chamber and allowed to recover before being returned to their cages.

Animals were subjected to 5–6 seizures per day, spaced two hours apart. Control rats were placed in the chamber but not exposed to flurothyl. The controls were separated from the dams for a time similar to the recurrent seizure group.

Following the recurrent seizures, rats were housed with 2–3 rats per cage in approved animal facilities until water maze testing was conducted at P42.

Water maze

To assess spatial memory function, we used the Morris water maze (Morris et al., 1982a; 1986; Morris, 1989; Morris et al., 1989). It is a test of hippocampal-dependent spatial memory (Morris et al., 1982b; Morris, 1984), the closest parallel to episodic memory in humans (Jeltsch et al., 2001; Spiers et al., 2001a; 2001b). This test measures both working and reference memory. Working memory is measured by the ability of the rat to find the escape platform during a single testing session whereas reference memory is a measure of how well the rat does on subsequent testing days. Working memory is primarily served by frontal cortex (Ragozzino et al., 1998; Jones, 2002) and reference memory by the hippocampus (Morris, 2006; 2007). Rats underwent Morris water maze testing between P42–P46 using techniques previously described in our laboratory (Rutten et al., 2002; Liu et al., 2003).

Apparatus—A stainless-steel circular swimming pool (2 m in diameter, 50 cm high) was filled to a depth of 25 cm with water. 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.

Behavioral procedures—On the first day 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 during the four days of locating the hidden platform provides a measure of spatial reference memory, while the probe trial is a measure of the strength of spatial learning (Jeltsch et al., 2001).

Radial-arm water maze

An 8-arm radial-arm water maze was used to measure spatial learning and memory (Sayin et al., 2004; Mortazavi et al., 2005; Cornejo et al., 2007). The test allows assessment of working and reference memory performance simultaneously (French et al., 2006) and is considered to require a greater memory load than the Morris water maze (Hyde et al., 1998). Its utilization also omits the necessity of a food reward and is hippocampal dependent (Mesches et al., 2004).

Apparatus—The radial-arm maze consisted of eight stainless steel arms (length - 50 cm, width - 15 cm) extending radially from a central area (diameter 40 cm) placed in a stainless-steel circular swimming pool filled with water. One arm was chosen as the target arm, in which a clear plexiglass escape platform was placed that allowed the rat to climb atop it, thus exiting the water, and rest. The escape platform remained in a constant position for the duration of the experiment. White paint was added to make the water opaque and prevent the rats from seeing the platform. Cue cards were distributed around the maze and remained constant throughout the experiment.

Behavioral procedures—Testing in the radial-arm water maze was conducted between P60-P80. Rats received trials with 30-minute intertribal intervals. Each rat was placed in the center of the maze and the trial continued until the escaped platform was found or until 2 minutes had elapsed. A visit to an arm was scored if all four limbs of the rat were within an arm. On mounting the platform the rats were given a 30-second rest period, and were taken to the home cage for a 30-minute intertrial interval. If the rat did not find the platform in 120 seconds, it was manually placed on the platform for a 30-second rest before being taken to the cage. Rats were placed in the maze at randomly selected different start arms with the same goal arm position. Time to completion was measured as the time taken to reach the escape platform and complete the trial. It was recorded for each trial and the observer also manually recorded the arms visited by the rat before it found the platform. The rats were trained until they reached the criteria of no more than one error in a single trial and no more than two errors in total for three consecutive trials. If the rat entered into an incorrect arm the error was coded as a reference error. Re-entrance into an arm during a trial was coded as a working memory error.

Place cells

Place cell recordings were performed between P100 and P140 following all other behavioral measures. Subsets of the rats ($n = 6$) from the recurrent seizure ($n = 8$) and control groups ($n = 8$) were studied. We used methods described previously (Zhou et al., 2007a). The techniques are briefly described below.

Recording chamber and training—The recording area was a gray cylinder 76 cm in diameter and 51 cm high placed on a piece of gray paper that was replaced between each session. A sheet of white cardboard occupied 90° of inside arc of the cylinder and was the only polarizing stimulus. Food deprived rats were trained to travel to all areas of the cylinder by

chasing 20 mg food pellets dropped randomly about the cylinder from an overhead feeder at an average rate of about 3/minute. Training was completed when the rat spent a minimum of 12 of the 16 minutes walking.

Electrode implantation—Following training, electrode microdrives were surgically implanted with stereotaxic techniques previously described. We used microdrives (Rivard et al., 2004) to implant eight tetrodes (a group of four 25 μ m nichrome electrode wires twisted together) and four EEG electrodes in CA1. Electrode tips were placed in the dorsal CA1 region of the right hippocampus. The tetrodes could be individually advanced. The initial placement of the electrode tips was 3.8 mm posterior to bregma, 2.5 mm lateral to midline and 1.5 mm below dura, directly above the dorsal hippocampus (Paxinos and Watson, 1998). Tetrodes allowed excellent signal detection and cell discrimination and allowed us to record from several cells simultaneously. Each electrode was checked for waveforms of sufficient amplitude. If none were detected, the tetrode was advanced 20 μ m and the rat was returned to its home cage for 4 to 6 hours. This sequence was repeated until one or more pyramidal cells with waveforms of >150 μ V were isolated.

Four nichrome, formvar isolated 100 μ m EEG electrodes were stereotaxically positioned so that two crossed the pyramidal cell layer and the other two remained in the stratum oriens. Differential EEG recordings were made above and below the cell layer using a bandwidth of 1–475 Hz. For single unit recording, the signal coming from the headstage preamplifiers was differentially amplified 10,000 times, acquired at 32 kHz, and recorded (Cheetah® Recording System; Neuralynx, Inc.). The acquired waveforms were sorted off-line using a computer program (Plexon®, Inc.).

Tracking position—The position of a light-emitting diode (LED) on the head was tracked with an overhead TV camera at 60 Hz in a 64 $\times$ 64 array of square pixels 2.7 cm on a side. The total time the LED was detected in each pixel and the number of spikes fired in each pixel was accumulated. A time-averaged firing rate distribution was calculated by dividing the number of spikes in each pixel by the dwell time in that pixel. Color-coded firing rate maps were used to visualize positional firing distributions. Pixel rates were coded in the sequence: yellow, orange, red, green, blue and purple, in order from lowest to highest firing frequency. The firing rate was exactly zero for yellow pixels. Unvisited pixels in the cylinder and pixels outside the cylinder were coded white.

Electrophysiological Recording—All of the rats reported here underwent a minimum of four recording sessions of 16 minutes each. All rats were visually observed or videotaped to determine if any spontaneous seizures occurred in the four hours prior to place cell recordings. The intervals between sessions 1 and 2 and between sessions 3 and 4 were 2–3 minutes, and between sessions 2 and 3 5–6 hours. After recording, discriminated waveforms were classified as arising from pyramidal cells if the action potentials fired in complex spike bursts (decrementing spike sequences with interspike interval <10 ms), had a negative initial phase >300 μ s, and showed silent intervals lasting at least 1 second. We required that all cells have >100 action potentials per session. The following parameters were measured for pyramidal neurons:

- i. Overall firing rate: The firing rate averaged over all pixels in the field.
- ii. Field center rate: For each pixel in the field, the average of its rate and the rate in its 8 nearest neighbors is calculated. The peak rate is that in the pixel for which this average is greatest.
- iii. Coherence: This is a two-dimensional nearest-neighbor autocorrelation. It is calculated by listing the firing rate in each pixel and the average firing rate in its eight

nearest neighbors. Coherence is the z-transform of the correlation between these lists and estimates the local smoothness of the positional firing pattern.

- iv. Field size: Size of the place cell field in pixels
- v. Information content: This is a measure of the amount of information conveyed about spatial location by a single action potential emitted by a single cell (Skaggs et al., 1993).
- vi. Total number of APs: Total number of APs over the 16 minute recording session.

All measurements of place cell and interneuron function were done using software developed by Robert U. Muller, PhD, State University of New York-Health Science Center at Brooklyn, New York. Based on positional firing rate distributions, CA1 pyramidal cells were categorized as place cells or pyramidal cells without spatial firing characteristics. To be classified as a place cell the coherence was required to be >0.28 and the area of the largest field had to be $< 70\%$ of the apparatus. To be classified as an interneuron, the unit had to: 1) never fire complex bursts; 2) have a negative initial phase $< 300 \mu\text{s}$; 3) never show silent intervals $> 0.2 \text{ s}$; 4) approximately double its discharge rate when the rat was walking compared to when it was standing still.

Histology

Following completion of the place-cell recordings all rats were sacrificed, the brains removed, sectioned and stained using thionin and Timm stain for mossy fibers using methods previously described from our laboratory (Holmes et al., 1998; 1999; Rutten et al., 2002). Rats were perfused transcardially with: 1) 200 ml of normal saline; 2) 200 ml sodium sulfide medium (2.925 g Na_2S , 2.975 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ in 500 ml of H_2O); 3) 200 ml of 4% paraformaldehyde (PFA). The brains were removed, postfixed in 4% PFA for 24 hours and placed in 30% sucrose for 24 hours or longer until the brains sank. Coronal sections along the entire extent of the hippocampus were cut at $40 \mu\text{m}$ on a freezing microtome and stored in phosphate-buffered saline (PBS). Every third section was stained with thionin and every fourth section was Timm-stained for mossy fibers.

A Timm score was obtained by using a semi-quantitative scale for mossy fiber sprouting in the CA3 and the supragranular region. This scale is a modification of one proposed by Cavazos et al. (Cavazos and Sutula, 1990; Cavazos et al., 1991) which has previously been used in our laboratory (Holmes et al., 1998; 1999; Huang et al., 1999) and ranges from 0 to 5. Timm scores in the CA3 region and the supragranular region were assessed on each section from the septal area where the two blades of the dentate were equal and formed a V shape (2.8 mm posterior from the bregma) to a point approximately 3.8 mm posterior to the bregma. Assessment of the Timm score in the supragranular region was done in the infrapyramidal blade of the dentate gyrus, avoiding the tip and crest of the gyrus. Five sections on both sides per rat were scored. A mean score for both the supragranular region of the dentate gyrus and pyramidal cell layer of CA3 were obtained.

Thionin staining was used to verify electrode location and assess cell loss. A total cell loss score was obtained by averaging the scores from three hippocampal regions. Cell loss in CA1, CA3 and the hilus were graded separately on the following scale: No cell loss = 0; cell loss $< 25\% = 1$; cell loss $25\text{--}50\% = 2$; cell loss $50\text{--}75\% = 3$; no remaining cells = 4 (Schmid et al., 1999).

Statistical analysis

The Kolmogorov-Smirnov goodness-of-fit test was used to assess normality (Gaussian-shaped distribution) for all continuous variables. Mean escape latency to water maze platform was

compared using the repeated measures ANOVA. For other parametric measures the t test was used to compare the groups. Histological scores were compared using the non-parametric Mann-Whitney test. Results are presented as means±standard errors and a $p < 0.05$ was used to defined statistical significance.

Results

Flurothyl-induced seizures

The flurothyl-induced seizures were stereotyped and consisted of a sequence of agitation, swimming movements, followed by extension of the fore- and hindlimbs. The rats recovered quickly from the seizures. Within 5–10 minutes of the end of the seizure the rats were upright and walking in the cage. Within 30 minutes they were behaving normally.

Morris water maze

The results of the water maze are presented in Figure 1A. Both groups learned to find the escape platform over the 24 trials conducted over four days with decreased latency to the escape platform over trials ($F_{23} = 23.55$; $p < 0.001$). Significant differences were noted between the controls and the recurrent seizure animals ($F_1 = 17.53$; $p < 0.001$). There was also a time/group interaction ($F_{18} = 7.86$; $p < 0.001$). No significant difference in swimming speed between the two groups was observed (Controls: 39.00 ± 0.59 cm/sec; RS: 39.60 ± 0.64 ; $t = 0.6926$, $p = 0.4943$). In the probe test the control rats spent more time swimming in the target quadrant than the recurrent seizure rats ($t_{28} = 2.087$, $p = 0.046$) (Fig. 1A, insert), indicating that the rats with recurrent seizures did not have a strong memory for the previous location of the platform.

The Morris water maze findings demonstrate that recurrent seizures during development result in long-standing deficits in spatial learning and memory.

Radial-arm water maze

A significant difference in trials to criteria between the two groups was seen ($t_{20} = 4.35$, $p < 0.001$) with the controls (5.42 ± 0.40) reaching criteria faster than the rats with recurrent seizures (10.40 ± 1.17) (Fig. 1B). There was a significant difference in groups in number of incorrect arms entered during the trials with the recurrent seizure group having more incorrect arms than the controls ($F_1 = 13.20$, $p = 0.002$). The mean number of reference errors per trial was significantly different in the two groups ($t_{268} = 5.23$, $p < 0.001$) with higher references errors in the recurrent seizure group (2.64 ± 0.24 errors/trial) than the controls (1.03 ± 0.19 errors/trial). No differences were noted between the controls (0.95 ± 0.24 errors/trial) and recurrent seizures (1.28 ± 0.54 errors/trial) groups in working memory errors ($t_{57} = 0.633$, $p = 0.529$).

The water radial-arm water maze results show that reference memory is impaired in rats with recurrent seizures.

Place cells

An example of action potentials from a place cell and a rate map is shown in Figure 2. According to the spike classification criteria outlined in Methods, we recorded 123 place cells and 33 interneurons in the recurrent seizure group and 203 place cells and 78 interneurons in the control group.

CA1 pyramidal cells judged as place cells in recurrent seizure rats appeared normal in several ways. Specifically, we saw no differences between control place cells and place cells from the recurrent seizure rats in overall firing rate ($t_{(324)} = 1.65$, $p = 0.101$) or total action potentials ($t_{(324)} = 1.211$, $p = 0.247$) (Fig. 3). However, in other measures the place cells in the two groups differed strikingly. There were significant differences in coherence ($t_{(324)} = 8.68$, $p < 0.0001$)

which measures the local smoothness of spatial firing patterns, center firing rate ($t_{(324)} = 5.08$, $p < 0.001$), a measure of the intensity of firing in the center of the field, and information content ($t_{(324)} = 8.98$, $p < 0.0001$), a measure of the amount of information conveyed about spatial location by a single action potential. The field size was smaller in the recurrent seizure rats than in the controls indicating each cell covered a smaller area of the recording apparatus than the control. In addition, place cells from rats with recurrent seizures were “noisier” than cells from control animals showing a higher out of field firing rate (Cont: 0.33 ± 0.06 , RS: 0.50 ± 0.03 ; $t_{(324)} = 2.92$, $p = 0.004$).

Examples of place cell firing maps are shown in Figure 4A. Corresponding coherence histograms are shown in Figure 4B where a clear shift to lower values is visible for place cells from the recurrent seizure rats. Thus, firing field organization was weaker in adult rats subjected to recurrent seizures during early development. On the assumption that place cell activity is essential to normal navigational behavior, the decreased coherences helps account for the poorer water maze performance shown by the recurrent seizure rats.

We next asked if the positional firing patterns of the place cells from the recurrent seizure rats are stable over time. To this end, we ran four recording sessions for each control cell and cell from the recurrent seizure groups within a 7-hour period. Place cells in the control rats appeared stable (Fig. 5); their fields remained in approximately the same position and retained their shapes across the four recording sessions. In marked contrast, the positional firing patterns of recurrent seizure rats were less stable during each of the recording sessions.

Stability was assessed numerically by computing rotational cross-correlation profiles for pairs of recording sessions. We evaluated six session pairs from the 4 recording sessions: 2 pairs (S1S2, S3S4) with 2–3 min intersession intervals and pairs (S2S3, S1S4) with 5–6 hour intersession intervals. After the recurrent seizures, place cell firing fields were significantly less stable as measured by change in position than the controls at all intervals (Short intervals: S1S2 - $t_{75} = 5.262$, $p < 0.001$; S3S4 - $t_{75} = 6.054$, $p < 0.001$; Long intervals: S2S3 $t_{75} = 5.191$, $p < 0.001$; S1S4 - $t_{75} = 5.906$, $p < 0.001$)(Fig. 6).

These findings indicate that the positional firing patterns for the place cells from the recurrent seizure rats drift away from their initial states. Somewhat surprisingly, the reduced stability in the recurrent seizure group was similar at both the short and long intervals, indicating that decay of memory was rapid and not time related. The reduced stability of the place cells from the recurrent seizure rats at both long and short intervals provides an additional basis for explaining observed deficits in complex spatial tasks.

We also examined the firing rate of interneurons in the two groups. There was a significant difference between the recurrent seizure and control groups in interneuron firing with the recurrent seizure group having significantly lower firing rates (3.27 ± 0.67) than the controls (10.07 ± 1.60) ($t_{(109)} = 2.683$; $p = 0.008$).

Taken together, our results demonstrated that recurrent seizures result in long-standing changes in place cell function with recurrent seizure rats having impaired precision and stability compared to control animals. In addition, interneuron firing also differed between groups suggesting recurrent seizures resulted in impairment in inhibition.

Histology

No cell loss was seen in either the recurrent seizure or control groups. Using the Timm stain, rats with recurrent seizures were noted to have a modest increase in sprouting in the pyramidal cell layer of CA3 (1.09 ± 0.38) compared to the controls (0.07 ± 0.02) (Mann-Whitney - 158.0; $p = 0.031$).

Discussion

We report here that recurrent seizures during early development are associated with pronounced long-term deficits in spatial learning and memory in the Morris water maze and radial-arm water maze. In parallel, we found substantial deficits in action potential firing characteristics of place cells. In the rats with the recurrent seizures there were two major defects of place cell activity: i) the coherence (local smoothness and precision), information content, center firing rate, and field size were reduced compared to control cells; and ii) the fields were less stable than those in control place cells. The reduced coherence, firing rates and information content indicate that the place cells are providing inadequate information to rats with a history of recurrent seizures compared to the controls. The instability of the place cell firing fields indicates field reproducibility decays, implying a forgetting or poorly learned process. This is first report showing that recurrent early-life seizures results in long-standing changes in single cell firing patterns in the hippocampus.

The observation that place cell representation normally is stable over time makes it an important indicator of memory processes in hippocampus, complementary to studies of synaptic plasticity and behavioral data (Muller, 1996). It is known that there is a close functional relationship between the spatial firing patterns of place cells and the spatial behavior of the rat (O'Keefe and Speakman, 1987; Lenck-Santini et al., 2001; 2002; 2005). A number of studies have shown that place cell abnormalities are associated with impaired spatial learning and memory supporting the spatial mapping theory of hippocampal function which predicts that place cell defects must lead to deficits in the learning, performance or recall of complex spatial tasks (O'Keefe and Nadel, 1978; O'Keefe, 1979; 1993). Destruction of the hippocampus and its place cells (O'Keefe and Nadel, 1978; Olton et al., 1978; Sutherland et al., 1982; Morris et al., 1982b), abnormal place cells in genetically modified mice (Rotenberg et al., 1996; 2000; Kang et al., 2001), and partial disruption of place cell activity due to blockade of muscarinic cholinergic transmission (Brazhnik et al., 2003) lead to spatial memory impairment. We have previously shown that seizure-induced injury in adult rats results in abnormal place cell firing and aberrant spatial learning memory (Liu et al., 2003; Zhou et al., 2007a). Here we show that recurrent seizures during early development result in long-standing changes in single cell firing patterns. As predicted from the hippocampal map theory (O'Keefe and Nadel, 1978), rats with abnormal place cell firing had impaired spatial performance, as shown in both the Morris water maze and the radial-arm water maze.

Epilepsy is a chronic condition characterized by recurrent seizures. We elected to use recurrent flurothyl seizures in this study since the seizures mimic the clinic situation of a child with poorly controlled epilepsy. Flurothyl inhalation results in brief, generalized seizures and are associated with a low mortality rate (Zhao and Holmes, 2006). Recurrent flurothyl seizures in immature rats do not lead to spontaneous recurrent seizures. However, animals subjected to multiple flurothyl-induced seizures demonstrate a kindling phenomenon with a decrease latency to forelimb clonus (Liu et al., 1999). Furthermore, recurrent flurothyl seizures are associated with a reduced seizure threshold when examined at an older age (Holmes et al., 1998; Sogawa et al., 2001). The flurothyl model can be contrasted with models of status epilepticus using agents such as kainic acid, bicuculline, or pilocarpine in which a single prolonged seizure is induced with a chemoconvulsant and the animal has a prolonged seizure with a subsequent number of animals going on to develop spontaneous recurrent seizures (Ben-Ari et al., 1981; Stafstrom et al., 1993; Ben-Ari and Cossart, 2001; Cilio et al., 2003). The consequences of recurrent seizures and status epilepticus in the developing brain are therefore quite different (Holmes and Ben-Ari, 2001; Holmes, 2005; Ben-Ari and Holmes, 2006).

Previous studies have shown that rats with status epilepticus during pubescence (Liu et al., 2003), prolonged experimental febrile seizures in pups (Dube et al., 2008), and recurrent flurothyl-induced seizures in adult rats (Zhou et al., 2007a) have impaired performance in the water maze and aberrant place cell function. Rats with status epilepticus, prolonged febrile seizures, or recurrent seizures exhibited two defects in place cell activity: the coherence of firing fields was lower and fields were less stable than those in control place cells. However, some differences in place cell firing patterns were found between rats with recurrent flurothyl seizures during early life and a single prolonged seizure induced by hyperthermia at P10 (Dube et al., 2008). Rats experiencing 100 short seizures had more impaired place cells as demonstrated by smaller mean field size, lower coherences, lower central firing rates, and lower information content than rats with experimental febrile seizures. Likewise, performance in the Morris water maze was significantly worse following 100 short seizures than a single prolonged febrile seizure. These findings suggest that place cells serve as a powerful surrogate marker of spatial cognition. Moreover, the abnormalities in place cell firing patterns suggest that both prolonged and recurrent seizures have a final common mechanistic pathway in seizure-induced impairment in spatial memory as reflected by similarities between the two conditions in place cell firing characteristics and stability.

There is increasing evidence that recurrent seizures early in life result long standing changes in synaptic function and organization. As seen in our rats, repetitive seizures in the first weeks of life result in synaptic reorganization with sprouting seen in the pyramidal cell region of the CA3 subfield (Holmes et al., 1999; de Rogalski Landrot et al., 2001). Kainic acid-induced repetitive seizures in the neonatal period result in reduced long-term potentiation (LTP) and enhanced long-term depression (LTD) and memory impairment (Cornejo et al., 2007).

Recurrent neonatal seizures have been shown to reduce the membrane pool of α -amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) subunits (GluR1), decrease the total amount of N-methyl-D-aspartate subreceptors (NR2A) and increase the post-synaptic density protein 95 (PSD-95), the primary subsynaptic scaffold protein (Cornejo et al., 2007). The seizure-induced derangements in glutamate receptor expression and PSD-95 presumably lead to impaired synaptic function.

Alterations in synaptic efficiency could inhibit experience-dependent strengthening of synaptic connection and contribute to the impairment seen in the behavioral spatial deficits and impaired place cell function seen in our rats (Muller et al., 2002; Jeffery and Hayman, 2004). In unpublished results we have found that recurrent flurothyl seizures in rat pups result in impaired LTP when the rats are studied as adults. In addition, we have shown that recurrent seizures in adult rats result in impaired LTP, spatial memory impairment, and altered place cell firing (Zhou et al., 2007a).

We also found that rats with recurrent seizures had a reduction in interneuron firing. Recurrent seizures during the early life have been previously shown to be associated with long-standing enhanced excitability (Holmes et al., 1998; Liu et al., 1999; Huang et al., 1999; Villeneuve et al., 2000). Flurothyl-induced early-life seizures (P15–20) result in reduction of the spike frequency adaptation and afterhyperpolarizing potential following a spike train in CA1 (Villeneuve et al., 2000). Long-standing reductions in GABAergic currents have been found in hippocampal slices obtained at P15–P17 from rats with recurrent flurothyl seizures during the first week of life (P0–P5) (Isaeva et al., 2006). The significantly faster theta frequency seen in the rats with recurrent seizures compared to the non-controls is another indication that inhibition was impaired in the recurrent seizure rats (Halonen et al., 1992). Since the interplay between principal cells and interneurons plays an important role in timing the activity of individual cells (Marshall et al., 2002), altered inhibition may also contribute to the deficits seen in the recurrent seizure rats.

This study adds to the increasing evidence that seizures during early development have long-term adverse effects on cognitive function and these cognitive changes are reflected at the single cell level. However, it must be emphasized that the current study is preliminary but provocative. We have not demonstrated a causal relationship between seizure-induced aberrant place cell firing and spatial learning and memory. It should be emphasized that our study indicates there is a strong relationship between place cell abnormalities and impaired spatial ability. It should also be acknowledged that hippocampal place cell firing patterns can also be altered by abnormalities outside the hippocampus (Markus et al., 1994; Paz-Villagran et al., 2002; Muir and Bilkey, 2003). Nevertheless, this study, and others (Rotenberg et al., 1996; 2000; Liu et al., 2003; Zhou et al., 2007a; Lenck-Santini and Holmes, 2008; Dube et al., 2008), confirms that abnormalities in place cell firing patterns can be associated with adverse cognitive consequences. The challenge is to determine which of the myriad of pathological changes that occurs following seizures mechanistically lead to place cell changes with associated spatial learning and memory disturbances. Understanding these mechanisms will be a critical step in designing novel therapeutic interventions.

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Figure 1.

Morris water maze and radial-arm water maze performance. A. Morris water maze. Mean time to the escape platform is plotted against trial number. Although both groups of rats reduced latencies to the escape platform across trials, rats with recurrent seizures (RS) were significantly slower in reaching the escape platform than the controls across all trials. The insert shows results from the probe test. Recurrent seizure rats spent significantly less time in the target quadrant than the controls. B. Radial-arm water maze. Trials to completion, working memory and reference memory errors.

Figure 2.

Firing pattern and rate map of place cell recorded in a cylinder with cue card. A. Example of action potential firing recording from tetrodes from a single place cell. B. Rate map of place cell. Pixel rates were coded in the sequence: yellow, orange, red, green, blue and purple. The firing rate was exactly zero for yellow pixels. Unvisited pixels in the cylinder and pixels outside the cylinder were coded white. Maximum firing of the field is seen at 10 o'clock (arrow).

Figure 3. Place cell firing characteristics. A. Overall firing rate; B. Coherences; C. Information content; D. Field size; E. Center rate; F. Total spikes. Significant differences were noted in all of the parameters except overall firing rate and total spikes.

Figure 4.

A. Examples of place cells and interneurons from controls (Cont.) and recurrent seizure (RS) rats. Color-coded firing rate maps were used to visualize firing distributions. Note that the place cell firing fields were smoother and more precise in the controls as evidenced by higher coherences than in the recurrent seizure rats. Cells from the recurrent seizure groups were “noisier” with greater out of field firing than the controls. The interneurons did not show preferential place firing. B. Histogram of coherences in the recurrent seizure and control groups. Note the distributions were different with the recurrent seizure groups having lower coherences than the controls.

Figure 5.

Stability of the place cells. Each line represents a single cell recorded on four occasions. The time between sessions S1 and S2 and S3 and S4 was 2–3 min while the time interval between S2 and S3 was 5–6 hours. Note that in the controls the place cells were quite stable and remained in a similar position from session to session. In the recurrent seizure rats PC1 was stable whereas PC2 and PC3 were not stable, changing positions between sessions.

Figure 6. Stability of place cells across recording session. Graph shows the change in position of the highest firing pixel in the recurrent seizures (RS) and controls. At all intervals tested the recurrent seizure group showed reduced stability compared to the controls (* = $p < 0.0001$).