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Research paper

# Early-life status epilepticus induces long-term deficits in anxiety and spatial learning in mice

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ABSTRACT

*Background:* One of the most devastating aspects of developmental epilepsy is the long-term impact on behavior. Children with epilepsy show a high co-morbidity with anxiety disorders and autism.
*Methods:* To examine whether early-life status epilepticus results in altered anxiety, repetitive behavior, social behavior, and learning and memory, we induced status epilepticus in male C57BL/6 mice on postnatal day (PD) 10. The mice received intraperitoneal injections of either kainic acid (2 mg/kg) or 0.9% normal saline. We also included a nontreated control group. Kainic acid induced status epilepticus for approximately 1.5 h. At PD60, the adult mice were then tested in a battery of behavioral tasks, including open field activity, elevated-plus maze, light-dark test, marble burying, social chamber, social partition, conditioned fear, novel object recognition, and Morris water maze.
*Results:* The early-life seizure group showed consistent increases in anxiety in the open field test ($p < 0.05$), elevated plus maze ($p < 0.05$), and light-dark task ($p < 0.01$). The seizure group showed significant ($p < 0.01$) impairment in the Morris water maze. There were no differences observed in marble burying, social partition, social chamber, novel object recognition, or delay fear conditioning tasks.
*Conclusions:* These results demonstrate that a single insult of status epilepticus during the neonatal period is sufficient to cause specific, long-term impairments in anxiety and spatial learning.


## 1. Introduction

Approximately 0.5–1% of children ages 0–16 are diagnosed with epilepsy.[1] The highest incidence of seizures occurs during the first year of life,[2] with prolonged seizures increasing overall seizure susceptibility in adolescence and adulthood.[3] One life-threatening type of seizure condition is status epilepticus (SE), which is characterized as a series of acute, prolonged seizures. SE is primarily observed in children,[4] but is also seen in the adult population. Although, the estimated mortality rate of SE is approximately 6–18%, children show significantly greater recovery than adults.[5,6]

Children experiencing SE during early life have been shown to exhibit long-term alterations in cognition and behavior,[7,8] and impairments in intellectual functioning.[7,9] Co-morbidity estimates between epilepsy and mood disorders, such as anxiety and depression, are approximately 50–60% of patients with epilepsy.[10] Further, mood disorders have a higher incidence rate in children with epilepsy than in children with any other long-term medical conditions.[11] There is also evidence indicating a high comorbidity between autism and epilepsy,[12–14] with approximately 30% of epileptic patients diagnosed with autism spectrum disorder.[12] Therefore, understanding the relationship between epilepsy and the development of its associated disorders has potential to improve the lives of many children.

Studies using animal models of epilepsy have confirmed many of the observations from human studies. SE during early development does not commonly result in spontaneous seizures, unlike when induced in adult animals.[15] There has also been less reported cell loss and synaptic alterations in animals with SE during early development than in adulthood.[16] Although many previous studies have examined the effects of early-life SE on cognition and anxiety, when taken together the overall long-term behavioral impact remains unclear. There have been reports that early-life SE in rodent models result in long-term elevation in anxiety,[17–22] while others have found no change in anxiety level.[23,24] There have also been studies that observed spatial learning deficits,[18,20–22] while others have found no deficits in

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spatial memory in rodents.[16,23] Several studies have found that early-life SE and early-life flurothyl seizures result in long-term changes in social behavior.[15,24,25]

One common concern with many of these studies is the use of only a single test to examine behavioral phenotypes. In the current study, we investigated long-term changes in anxiety, repetitive behaviors, social behavior, and learning following early-life SE on postnatal day 10. In order to improve the reliability of our results, as compared to previous studies, we included complementary measures for each behavioral phenotype. The results from this study could enhance our understanding of epilepsy, as well as provide strong foundations for future studies aiming to examine the cellular and molecular mechanisms for these deficiencies.

## 2. Material and methods

### 2.1. Animals

For the following studies we used C57BL/6J male mice. These mice were generated and housed at Baylor University at an ambient temperature of 22 °C, with a 14-h light and 10-h dark (20:00–6:00 h) diurnal cycle. The mice were given *ad libitum* access to food and water. All procedures to the animals were approved by the Baylor University Animal Care and Use Committee.

### 2.2. Design

On postnatal day (PD) 10, C57BL/6J pups received intraperitoneal injections of either 2 mg/kg (0.5 mg/ml) dose of kainic acid (Tocris, Bristol, UK) solution or 0.9% normal saline. The animals were subsequently monitored and behavioral seizures were scored using a modified Racine scale.[26] The seizure group experienced status epilepticus within thirty minutes of injection and continued for 1.5 h. The vehicle mice received an injection of saline and were isolated from their mother for an equivalent period of time as the seizure mice. The non-treated control pups remained with their mother throughout this period. Behavioral testing began on approximately PD 60. Throughout all behavioral testing animals were first acclimated to the room for 30 min. At the conclusion of each behavioral test we cleaned the behavioral apparatus with 30% isopropyl alcohol solution.

### 2.3. Assessment of behavioral parameters

#### 2.3.1. Open field activity

The mice were placed into the clear acrylic arena (40 × 40 × 30 cm) to investigate their activity and anxiety levels for 30 min using a computer-operated optical animal activity system (Fusion by AccuScan Instruments, Inc.; Columbus, USA). Total distance moved, distance in center, center time, activity count, and stereotypy count was measured. The sample size for the open field test was n = 17 for the controls and n = 19 for the SE group.

#### 2.3.2. Elevated plus maze

We used the elevated plus maze to evaluate differences in anxiety.[27] The apparatus consisted of two enclosed and two open horizontal perpendicular arms (30 × 5 cm) positioned 40 cm above the floor with a central square platform (5 × 5 cm) that forms from the connection of the four arms. We used video tracking software (Noldus: Ethovision; Netherlands) to score the time spent and frequency of visits in the open arms, center arms, and closed arms during a ten-minute test. We simultaneously video-recorded the test and later scored head-dips in the open arms and rearing in the closed arms manually. In the elevated plus maze video tracking data, the sample size for the control group was n = 15 and for the SE group was n = 8. For all data pertaining to the head dips and rearing n = 11 for the control group and n = 8 for the SE group.

#### 2.3.3. Light/dark test

A separate cohort of mice was tested in the light/dark exploration test as an additional measure of anxiety.[28] The mice were placed into a clear acrylic arena (40 × 40 × 30 cm) that was evenly divided into an open area and a black enclosed area in which the mice could freely enter and exit. Activity was collected by a computer-operated optical animal activity system (Fusion by AccuScan Instruments, Inc.; Columbus, USA). In the light/dark test the sample size for the control group was n = 7 and for the SE group was n = 10.

#### 2.3.4. Marble burying

The marble-burying test was used to examine repetitive behavior. The mice were placed in clean mouse cages (27 × 16.5 × 12.5) with sanichip bedding that had 20 black glass marbles placed in an equidistant 4 × 5 pattern. The number of marbles buried (>75%, 100%, or completely covered by bedding material) was recorded. The sample size for the control group was n = 18 and for the SE group was n = 19 in the marble burying test.

#### 2.3.5. Social chamber test

We used the three-chamber social behavior test to examine social behavior in mice using methods previously described.[24] There were two ten-minute phases to this test. In the first phase the animal was placed in the apparatus and we recorded the time the animal spent in the left chamber, center, right chamber, and corner cups. For the second phase, we placed an unfamiliar C57BL/6J (gender-, age-, weight-matched) mouse under one of the corner cups and a similarly sized black Lego® block at the other corner cup. We recorded the time spent in each chamber and at the cups for the trial. All behavior was recorded using a digital video recording program (Dazzle DVD recorder, Pinnacle; Ottawa, Canada) and was scored later by an individual blind to the condition of the mouse. The control group sample size was n = 18 and for the SE group was n = 19.

#### 2.3.6. Social partition test

We examined their behavior in the social partition test using methods previously described.[29] For this test each experimental animal was housed with an unfamiliar C57BL/6J (sex-, age, weight-matched) mouse for 24 h in a standard cage. A clear perforated (0.6 cm-diameter holes) partition separated the mice. The first five minute test consisted of the time and frequency the animal spent with the familiar mouse. The mouse was then removed and an unfamiliar mouse C57BL/6J was placed in the previously vacant part of the partition. We then measured time and frequency of the visits of the experimental mouse to the partition. After the 5 min test we then reintroduced the familiar mouse (same mouse as in trial 1) and measured the time and frequency at the partition. In social partition test the control group was n = 18 and the SE was group n = 19.

#### 2.3.7. Morris water maze

The Morris water maze (MWM) test was used to examine spatial learning and memory deficits as previously described.[30] Mouse movement was monitored by a video camera connected to a computer with digital tracking software (Noldus Ethovision; Netherlands). The mice were tested for their ability to locate a submerged square platform (14.5 × 14.5 cm). The mice were tested for 2 blocks per day for a total of 4 days. After the completion of the eighth block, each animal was given a probe trial for 60s. We conducted a visible platform test the following day to evaluate whether the mice had difficulty in locating a visible platform. In

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