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Early-life seizures result in deficits in social behavior and learning

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Abstract

Children with epilepsy show a high co-morbidity with psychiatric disorders and autism. One of the critical determinants of a child's behavioral outcome with autism and cognitive dysfunction is the age of onset of seizures. In order to examine whether seizures during postnatal days 7–11 result in learning and memory deficits and behavioral features of autism we administered the inhalant flurothyl to induce seizures in C57BL/6 mice. Mice received three seizures per day for five days starting on postnatal day 7. Parallel control groups consisted of similarly handled animals that were not exposed to flurothyl and naïve mice. Subjects were then processed through a battery of behavioral tests in adulthood: elevated-plus maze, nose-poke assay, marble burying, social partition, social chamber, fear conditioning, and Morris water maze. Mice with early-life seizures had learning and memory deficits in the training portion of the Morris water maze ($p < 0.05$) and probe trial ($p < 0.01$). Mice with seizures showed no differences in marble burying, the nose-poke assay, or elevated plus-maze testing compared to controls. However, they showed a significant difference in the social chamber and social partition tests. Mice with seizures during postnatal days 7–11 showed a significant decrease in social interaction in the social chamber test and had a significant impairment in social behavior in the social partition test. Together, these results indicate that early life seizures result in deficits in hippocampal-dependent memory tasks and produce long-term disruptions in social behavior.

Keywords

autism; epilepsy; recurrent seizures; flurothyl; social behavior; learning; memory; repetitive behavior; autistic; comorbidity; co-morbidity

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Introduction

The relationship between epilepsy and autism has been discussed for over 5 decades (Schain and Yannet 1960; Creak and Pampiglione 1969). Epidemiological studies suggest that perhaps as many as 25% of individuals with autism have some form of epilepsy (Tuchman and Rapin 2002). However, the reported rates of epilepsy in autism vary between 5–40% (Canitano 2007). This large range is likely attributed to the heterogeneity of the patient populations in individual studies. There are several risk factors that increase the probability of comorbidity of epilepsy and autism. Children who have a seizure in the first year of life have a higher risk for autism than those in the general population (Saemundsen, Ludvigsson et al. 2007). In addition, seizures are more frequently found in individuals with autism and intellectual disability. Even though there is an elevated risk of epilepsy in individuals with autism and intellectual disability, it is not clear whether seizures directly influence the development of autistic features and cognitive dysfunction.

Studies in animal models could help elucidate the potential interplay between these comorbidities. For instance, normal animals without neuropathology can be induced to have seizures to determine if seizures themselves contribute to autistic and cognitive disabilities. Indeed, results from numerous laboratories have reported that early-life seizures result in learning and memory deficits. Rats that experience brief but recurrent seizures during the first weeks of postnatal life have visual and auditory spatial learning and memory deficits during later adolescence and adulthood (Neill, Liu et al. 1996; Holmes, Gairsa et al. 1998). In addition, rats that experience seizures during early development show defective hippocampal place cells involved in spatial learning (Karnam, Zhou et al. 2009).

Even though there is significant evidence that early-life seizures result in learning and memory deficits in later life, the influence of seizures on autistic-like behaviors such as social and repetitive behaviors has received less attention. In the experiments reported here, we examined whether flurothyl-induced seizures during postnatal days 7–11 affect social, repetitive behaviors, and learning and memory deficits. Given the comorbidity between epilepsy and autism and the background studies presented above, we hypothesized that early-life seizures would lead to impairments in autistic-like behaviors and learning and memory.

Materials and methods

Animals

Starting on postnatal day 7, mice were placed in an acrylic container located in an exhaust hood. A gauze located in the chamber but above the pups received a flurothyl (bis-2,2,2-trifluoroethyl ether, Aldrich Chemical Co, USA) solution that was infused into the chamber at a rate of 3 cc/hr through a Hamilton syringe pump. Flurothyl was administered until all the mice displayed tonic extension of forelimbs and hindlimbs. The animals were then removed from the chamber and allowed to recover before being returned to their home cage with their respective litters and dams. The chamber was then opened to flush out the solution and cleaned between each seizure induction with 30% isopropanol. They received three seizures per day with a two hour interval between each seizure for five days. We chose an

early time period since children who have a seizure in their first year of life have a higher prevalence rate of Autism Spectrum Disorder (ASD) than during a later developmental period (Saemundsen, Ludvigsson et al. 2007). “Handled control” pups were placed in a chamber for an equivalent amount of time but did not receive flurothyl and then returned to their home cages. The naïve control pups remained with their mother throughout this period. On postnatal day 60, male and female mice were used for behavioral experiments.

Elevated Plus maze

The elevated plus maze is a commonly used test to measure anxiety in rodents and consists of two open arms and two closed arms that are elevated above the ground. Mice that are less anxious will spend more time in the open arms compared to control mice and mice that are more anxious will spend less time in the open arms compared to controls. This test provides a sensitive measure of anxiety and we used the test to evaluate whether seizures on postnatal days 7–11 result in a change in anxiety. The elevated-plus maze apparatus consisted of two open and two enclosed horizontal perpendicular arms (30×5 cm) positioned 40 cm above the floor. There was also a central square platform (5×5 cm) that forms from the connection of the four arms. The mice were initially allowed to acclimate to the room for thirty minutes. The experiments were conducted under artificial laboratory illumination (fluorescent lamps, 30 lux in the open arm) and a white noise generator produced a 60 dB background white noise. The sessions were scored by an investigator blind to the experimental condition using a handheld Psion from Noldus Information Technology and later transferred to a computer with the Noldus Observer 5.0 program. The number of entries into the open and closed arms and the average time spent in each of the arms was recorded during the 10 min test. The higher number of entries into the open arms and the time spent in the open arms compared to the closed arms is generally believed to be an index of lowered anxiety. The number of entries into the closed arms is a rough index of overall animal activity. Between each trial, the maze was thoroughly cleaned with 30% isopropyl alcohol solution and then dried with paper towels.

Nose poke and open field activity

Nose poke and locomotor activity were measured simultaneously. The test consists of 16 holes and mice that have an increase in repetitive behavior will produce more nose pokes. One additional benefit of this test is that locomotion can be measured simultaneously, which provides a measure of activity levels in rodents. Locomotor activity was evaluated as previously described (Paylor, Spencer et al. 2006). The mice were weighed and allowed to acclimate to the testing room for 30 min. The nose poke test consisted of a board insert that was placed into the clear acrylic arena ($40 \times 40 \times 30$ cm) to investigate repetitive behavior. The lighting inside the test chamber was approximately 100 lux and a white noise generator produced approximately 55 dB inside the test arena. Activity in the open field was collected by a computer-operated Digiscan optical animal activity system (Versamax by AccuScan Instruments, Inc.; USA). The hole board had 16 equidistantly-spaced holes. A nose poke was counted whenever the nose extended into the hole as far as the eyes. Between each trial, the area was thoroughly cleaned with 30% isopropyl alcohol then dried with paper towels. Data were collected in 2-min intervals over the 10-min test session and analyzed using independent-samples t-tests. During this time the number of nose pokes was also measured.

Marble burying

In order to further examine repetitive behavior, the marble burying test was used. The test measures the natural tendency of mice to dig objects by placing 20 marbles in a cage similar to their homecage. This test has been shown to be a sensitive measure of compulsive-type behaviors in mice and is a rapid way to measure repetitive behavior with a large group of mice. The mice were placed in clean Allentown mouse cages ($27 \times 16.5 \times 12.5$) with 4.5 cm corncob bedding that had 20 black glass marbles (15 mm diameter) placed in an equidistant 4×5 pattern (Thomas, Burant et al. 2009). The mice were tested for 30 min in a room with a background white noise generator (55 dB). The number of marbles buried (>50% marble covered by bedding material) was recorded.

Social chamber

The three-chamber social approach task for mice was first described by Dr. Jacqueline Crawley and is accepted as a sensitive measure of social behavior in mice (Nadler, Moy et al. 2004). The test can be used to distinguish the mouse preference for a novel object vs. an unfamiliar mouse. Mice were placed in a clear acrylic box with removable partitions into three chambers and mice were tested in two conditions. In the first condition, a mouse was placed in the center chamber. The partitions were removed and the animal was allowed to freely explore the chamber. The corners of the two side chambers housed empty black wire-mesh cylinders. A tall plastic cylinder was placed on top of the wire-mesh cylinders to prevent the animal from climbing on top of the cylinder. The time and frequency of the animal in the three chambers and at the cylinders was recorded. The mouse was then placed back in the center chamber after 10 min. In the second condition, an unfamiliar C57BL/6J mouse (gender-, age-, weight-matched) was placed in one cylinder and a similar sized black Lego® block object was placed in the other cylinder. The partner mice were initially habituated to being housed in the cylinder for one hour for two days prior to testing. The side where the novel partner mouse was placed was alternated to correct for possible side-bias. The mouse undergoing testing was then allowed to explore the chamber, and the time and frequency in the three chambers and at the cylinder was recorded. The mouse was then removed after the 10 min testing period and the chamber was cleaned with 30% isopropyl alcohol.

Social Partition test

We used the social partition test to determine whether the mice respond differently to a familiar mouse than to an unfamiliar mouse. This test can be used to measure whether the mouse generally responds less to another mouse or only when presented with an unfamiliar mouse. This test complements the three-chambered social approach task. The experimental animals were individually housed for 24 hrs by placing a mouse into one side of a standard cage that was divided by a clear perforated (0.6 cm-diameter holes) partition as previously described (Spencer, Alekseyenko et al. 2005). In the other half of the partition a partner C57BL/6J mouse (gender-, age-, and weight-matched) was placed. The next day (day of testing) the approaches and time spent at the partition by the experimental mice were measured for five minutes (Psion computer with the Noldus Observer® program). The first observation was with the partner that it had been housed with overnight (familiar condition

one). The partner mouse was then removed and an unfamiliar C57BL/6J (gender-, age-, and weight-matched) mouse was added and the behaviors recorded (unfamiliar condition). The unfamiliar mouse was then removed and the original partner mouse was reintroduced (familiar condition two). This resulted in three conditions of five minutes of testing per experimental mouse. For this test only male mice were used. The female control mice did not show an increase in time at the partition with an unfamiliar animal. Since this increase in time due to the unfamiliar mouse is a critical component of this test only male mice were used.

Conditioned Fear test

The conditioned fear test was used to determine whether the mice with seizures on postnatal days 7–11 had deficits in their ability to associate an aversive stimulus to a tone. This test is considered an amygdala-dependent test and complements the Morris Water Maze, which is a hippocampal-dependent spatial learning and memory test. Performance in a conditioned fear task was analyzed as previously described (McIlwain, Merriweather et al. 2001), using the freeze monitor system (San Diego Instruments: CA). The test chamber (26×22×18 cm high) was made of clear acrylic and was placed inside a sound attenuated chamber (Med Associates: St. Albans, VT). Two sides of the box were acrylic, two sides were metal, and a grid floor bottom was used to deliver a mild foot shock. On the training day, mice were placed in the test chamber and allowed to explore for 2 min. The conditioned stimulus (CS) was presented for 30 s (a white noise 80 dB sound) and followed immediately by a mild foot shock (2 s, 0.7 mA) that served as the unconditioned stimulus (US). After 2 min, the mice received a second CS–US pairing. The FreezeFrame monitor system was used to control the timing of CS and US presentations. Freezing behavior was measured through the computer program. During the administration of the shock, responses to the foot shock such as run, jump, or vocalize were also recorded to verify that the animal received the shock. After each testing session the chamber was cleaned with 30% isopropyl alcohol.

Mice were tested for cued fear conditioning 24 h after conditioning. For the cued fear test, mice were tested for responses to the auditory CS in a new environment. For the CS test, white acrylic inserts were placed on the sides and floor of the chamber to alter the shape, texture and color of the conditioning chamber. The odor in the chamber was changed by vanilla extract that was placed in a cup located behind the wall insert. In addition, 70% ethanol was used to clean the chamber between test sessions. Transfer cages were altered (paper towels instead of bedding) and the lights were dimmed in the room with the conditioning chamber to change the entry context. Mice were placed into this new chamber and freezing was recorded for 3 min during this phase preceding the CS in this new context. The auditory CS was then presented for 3 min and freezing was recorded as described. New context and CS test data were analyzed using an independent-samples t-test.

Morris water maze

The Morris water maze (MWM) test was used to examine spatial learning deficits in mice. It is a test to determine disruptions in hippocampal-dependent learning and memory. In this test the animal learns to navigate to a hidden platform by using spatial cues on the surrounding area across multiple trials. We used a testing scheme previously described

(McIlwain, Merriweather et al. 2001). The test used a 1.3 m diameter circular white pool, which was filled with water made opaque by the addition of white non-toxic paint. The mouse movement was monitored by a video camera connected to a digital tracking device (Noldus: Ethovision; Netherlands). The mice were first allowed to acclimate to the room for thirty minutes then were tested for their ability to locate a hidden square platform (14.5×14.5 cm). The mice were tested for a total of 4 days, two blocks of trials per day, four trials per block for their ability to locate a hidden platform that was located approximately 1.5 cm beneath the surface of the water. Thirty minutes after the completion of the eighth block, each animal was given a probe trial. In the probe trial, the platform was removed and each animal was allowed 60 s to search the pool for the platform. The amount of time that each animal spent in each quadrant was recorded (quadrant time). The following day a visible platform test was conducted to evaluate whether the mice had difficulty in locating a visible platform. An inability or reduced ability to find the visible platform could be due to a visual or motor deficit.

Data Analysis

All data were analyzed by using SPSS 17.0 for PC (SPSS, Chicago, IL). For all comparisons, the level of significance was set at $p < 0.05$. For all analyses naïve controls and handled controls were combined since no statistically significant differences were found between the groups. Males and females were also combined per group since no statistically significant differences were found between them. Animals were monitored for normal weight gain and no significant differences were found between groups.

Results

To determine if early-life seizures lead to deficits in social behavior we used the social partition and social chamber tests

Mice that underwent seizures during postnatal days 7–11 displayed a significant decrease in social behavior in the social partition test compared to control animals [$F(1,29) = 6.627$, $p < 0.05$]. We found a significant interaction between group and testing conditions [$F(2, 58) = 14.75$, $p < 0.001$], and thus we used repeated-measures ANOVAs to analyze the groups separately over the three conditions. The control group showed the expected pattern of social interaction for a normal rodent. There was some baseline interaction with the familiar mouse at the partition. The interaction at the partition significantly increased when an unfamiliar mouse was presented in the opposite side of the partition. Subsequently, there was a reduction in time the animal spent at the partition when presented with the familiar mouse. A repeated measured ANOVA provided statistical support for this observation across these three conditions in the control group [$F(2, 28) = 61.31$, $p < 0.001$]. However, animals that experienced seizures did not show a change in social interaction when presented with an unfamiliar mouse. A repeated-measures ANOVA revealed no statistical difference in the time to interact at the partition across the three conditions [$F(2,30) = 2.61$, $p = 0.09$] (Figure 1). These results suggest that the seizure group did not show increased social behavior when presented with an unfamiliar mouse. However, the seizure group did show similar levels of interaction with a familiar mouse compared to controls (Figure 1).

The decrease in social interaction with an unfamiliar mouse was not due to an overall decrease in interaction or movement. The seizure animals entered the chamber that housed the animal more frequently than the control mice [$t(1, 32) = 2.89$, $p < 0.01$; seizure: 49.45 ± 2.27 ; control: 41.52 ± 1.58] and entered the middle chamber more frequently than the control animals [$t(1,32) = 3.92$, $p < 0.001$; seizure: 29.48 ± 2.0 ; control: 20.00 ± 1.36]. It appeared that the seizure mice had more transitions between the center chamber and the chamber that housed the mouse. However, the seizure animals showed a significant decrease in the time at the cup with a mouse. There were no differences between the control and seizure mice in the time they spent in the left, middle, or right chamber in the baseline phase of the chamber test (Figure 2A). Additionally, in the second phase of the social partition test there was no difference in the time spent in the chamber that housed the mouse compared to the chamber that housed the object (Figure 2B). These results demonstrate that there was no preference for chamber side in the training portion of the social chamber test (Figure 2A) and that the groups were similar in their preference for the chamber that housed the mouse. It appeared that the seizure group demonstrated a specific deficit in interaction with another mouse.

Mice that experienced seizures during postnatal days 7–11 also displayed deficits in social behavior when examined in the social chamber test. The seizure mice compared to controls showed a significant reduction in the amount of time spent with the mouse versus the inanimate object [$t(1,32) = 2.19$, $p < 0.05$] (Figure 2C), and in the average (mean) time spent with the mouse [$t(1,32) = 3.0$, $p < 0.01$] (Figure 2D). Taken together, these results and those from the social partition test support the conclusion that seizures early in life result in abnormal social behavior later in life.

Analysis of seizures during postnatal days 7–11 on repetitive behavior

Because autism is associated with abnormal repetitive behaviors we assessed this phenotype in our mice using the following: hole-board nose poke and marble burying tests. There were no differences between the groups in the number of holes poked when allowed to explore the hole-board for five minutes (control: 15.43 ± 1.08 ; seizure: 16.45 ± 1.55) or ten minutes (control: 25.17 ± 1.19 ; seizure: 27.0 ± 2.29) of testing between control and seizure animals, respectively. We also examined whether there were differences in the holes poked in corners, along different walls, consecutively examining holes repeated or whether there was a difference in the number of different holes examined, and no differences were found between the groups. The results suggest no alterations in repetitive behavior in the seizure group. However, the seizure group had a significant increase in movement (number of lines crossed in open-field chamber) [$t(1,32) = 2.775$, $p < 0.01$], in the open field compared to the control group, 1886 ± 77.4 ; 1577 ± 67.1 , respectively. Even though this may suggest increased activity, there was not an increase in the number of holes explored.

In addition, the seizure animals did not show an alteration in repetitive behavior as measured in the marble burying test. The number of marbles buried during a thirty minute testing period was analyzed for control and seizure groups. No difference was found in the number of marbles buried [$t(1,31) = 0.072$, $p = 0.943$] for control and seizure mice.

The impact of seizures during postnatal days 7–11 on anxiety

Since, our results have shown deficits in socialization following early-life seizures, it was important to evaluate whether the mice also demonstrated increased anxiety, as enhanced anxiety has been reported in many animal models of autism (Kwon, Luikart et al. 2006; Brodtkin 2007; Chahrour and Zoghbi 2007). We used an elevated plus maze to measure anxiety. There was no difference between control and seizure groups in the frequency in the open, center, or closed arms, $p > 0.05$ (Figure 3A). There were no differences between seizure and control groups in terms of time spent in the open arms, center, or closed arms (Figure 3B). These findings suggest that there is no change in anxiety following early-life seizures in the mice.

Impact of seizures during postnatal days 7–11 on learning and memory

Next we examined the effects of early-life seizures on learning and memory using the Morris water maze (MWM) to assess spatial learning and conditioned fear to assess more global learning and memory impairments. In the MWM training, seizure mice showed a significant increase in the time to find the hidden platform compared to the control group [$F(1,24) = 11.38$, $p < 0.01$] (Figure 4A). The seizure group also showed a significant memory impairment in the probe trial. In the probe trial the hidden platform is removed and the animal is allowed to explore the maze for 60s. The control animals showed a significant preference for the quadrant where the hidden platform was previously located during the training phase [$F(3,79) = 17.72$, $p < 0.001$] (Figure 4B). The seizure animals did not show a preference for any quadrant in the MWM probe trial ($p > 0.05$). The control mice spent more time in the target quadrant than the seizure mice [$t(1, 27) = 2.206$, $p < 0.05$]. The seizure animals were not different in their time to find a visible platform compared to the control group (10.33 ± 1.27 s; 11.83 ± 1.32 s, respectively), suggesting they had no significant motor or visual impairments. The MWM training and probe trial provides evidence that early-life seizures significantly impair spatial learning and memory.

The fear conditioning test was used to examine whether mice with early-life seizures had more global learning and memory deficits. The seizure mice exhibited similar freezing to the conditioned stimulus compared to the control mice (Figure 4C Right Graph). The seizure mice also showed no difference in freezing in a new context compared to controls (Figure 4C Left Graph). The data from the conditioned fear test suggest that the seizure animals did not have global learning and memory deficits. Since the seizure animals have spatial learning and memory deficits in the MWM, their memory impairments are most likely localized to the hippocampus.

Discussion

Individuals with autism show behavioral abnormalities in three core areas: social, stereotypic/repetitive, and communication. There is a high comorbidity with autism and cognitive impairment as well as seizures and epilepsy. In these situations it is unclear whether seizures themselves contribute to the autistic phenotype and cognitive impairments. In the present study we demonstrate that mice that experience brief seizures during postnatal

days 7–11 have a significant reduction in social behavior. Furthermore, these mice have significant spatial learning and memory deficits compared with controls.

Our results complement recent studies that found a decrease in social behavior after status epilepticus (SE) in immature rats (Waltereit, Japs et al. 2010; Bernard, Castano et al. 2013; Castelhamo, Cassane Gdos et al. 2013). Waltereit et al. (2010) used kainic acid to induce status on postnatal day 7 and induced continuous seizures again on day 14. They examined social behavior using a social anxiety test and found that animals with prior seizures have a decrease in social interaction. In the current study we found that flurothyl-induced seizures result in impairment of spatial learning behavior and socialization. One reason for the differences in experimental outcome between the two studies may be that multiple recurrent seizures daily may induce more dramatic alterations in behavior than 2 isolated episodes of SE early in life. Another consideration may be due to species differences in terms of the effect of seizures on developing mouse brain versus the developing rat brain. Nonetheless, despite the differences between our results and those of Waltereit et al. (2010), both demonstrate that early-life seizures decrease social behavior later in life.

Since one of the core features of autism is an alteration in stereotyped behavior, we initially hypothesized that mice with early-life seizures will show a change in repetitive behavior. Rats that received 75 flurothyl-induced seizures during postnatal days 1–10 have impaired behavioral flexibility, which may be due to alteration in repetitive behavior (Kleen, Sesque et al. 2011). We did not find a change in repetitive behaviors in the marble burying test or the hole-board test. These two tests have been validated to measure repetitive behaviors in mice (Moy, Nadler et al. 2008; Thomas, Burant et al. 2009). A potential reason why we did not find alterations in repetitive behavior may be due to the type of tasks we used to measure this behavior. One study has suggested that there are many different types of repetitive behaviors, which may fall into two clusters (Lewis, Tanimura et al. 2007). One cluster consists of a “lower-order” type which is more often observed and easily measured since it consists of stereotyped motor actions. The second cluster consists of more complex “higher-order” type of behaviors such as rituals and compulsions. This second cluster would be more influenced by alterations in cognition. The behavioral tests we used measure the “lower-order” type of repetitive behavior. Since we demonstrate learning and memory deficits in our study it is possible that seizures are influencing the “higher-order” repetitive behaviors and not the “lower-order” type. Future experiments could utilize behavioral tests that have been shown to measure both features of the repetitive behaviors seen in autism (Moy, Nadler et al. 2008; Pearson, Pobbe et al. 2010).

We did not find alterations in anxiety in the mice with recurrent seizures. The mice did not show a change in the number of entries or duration in the open arms of the elevated plus maze. The impact of alterations in anxiety may be another difference between flurothyl-induced recurrent seizures and kainic acid-induced or pilocarpine-induced SE animal models. One study using pilocarpine to induce SE found altered anxiety after seizures induced by SE (Castelhamo, Cassane Gdos et al. 2013). Another report found that kainate-induced SE results in enhanced anxiety (Sayin, Sutula et al. 2004). It may be that SE results in different behavioral outcomes than recurrent seizures. In addition, Walderit et al., (2010) and Castelhamo et al., (2013) used a social behavior test that measures social anxiety (File

and Hyde 1978). It is possible that flurothyl may be used to examine the effects of seizures without alterations in anxiety, while animal models of SE can investigate the influence of seizures on social behavior with enhanced anxiety. These studies may be useful to investigate different mechanisms that underlie the differences in behavior.

There has been some discussion of the comorbidity between enhanced anxiety and autism. Previous studies have found that the rate of anxiety is higher in children with autism than in the general population (Simonoff, Pickles et al. 2008; Ozsivadjian and Knott 2011). However, a review of the relationship between anxiety and autism found that many clinical studies did not use the appropriate control groups and examined mixed types of individuals with autism (White, Oswald et al. 2009). The authors concluded that even though those with autism appear to have a higher rate of anxiety disorder, further research is needed to establish a firm relationship between anxiety and autism.

We expected to find learning and memory deficits in mice with early-life seizures due to previous findings in several labs. One group has found that one episode of early-life SE by kainic acid results in spatial learning and memory deficits (Cornejo, Mesches et al. 2007; Cornejo, Mesches et al. 2008; Bernard, Castano et al. 2013). Another group has shown that early-life flurothyl seizures results in learning and memory deficits and spatial map deficits (Holmes, Gairisa et al. 1998; Karnam, Zhou et al. 2009). However, Waltereit et al. (2010) did not find deficits in spatial learning and memory in the Morris water maze. There may be some differences in the induction of SE or in the strain of rat used in this study. Future studies will investigate how seizure number and duration influence social behavior with and without changes in learning and memory, and if there is a critical neurodevelopmental period when these effects are most easily produced. Such studies may help to delineate the effects of seizure load and timing on different aspects of behavior.

One difference in our study is that we found that mice with early-life seizures have long-term learning and memory deficits. One benefit of using mice is that there are many mouse models of genetic disorders such as tuberous sclerosis complex, fragile X syndrome, and cortical dysplasia that demonstrate autistic-like behaviors. By using such mice one can investigate the influence of recurrent seizures on genetic phenotypes and examine the question whether recurring seizures, which occur clinically in many neurodevelopmental syndromes, exacerbate the autism phenotype in relevant mouse models.

One future direction for experimentation is to examine molecular mechanisms underlying deficits in learning and memory and social behavior following early-life seizures. It has previously been shown that early-life seizures decrease PSD95 and the NMDA receptor subunit NR2A protein levels in the hippocampus (Swann, Le et al. 2007). Furthermore, mice that have genetic deletion of NR2A have spatial learning and memory deficits (Tsien, Huerta et al. 1996; Nakazawa, Quirk et al. 2002). A recent series of experiments extended the biochemical experiments in the flurothyl model by showing that the developmental-dependent alterations in biomarkers for glutamatergic synapses is likely explained at least in part by seizure-induced suppression of dendrite growth (Nishimura, Gu et al. 2011). Thus, the molecular substrates for learning and memory thought to be present at glutamatergic synapses would be diminished in animals that experience early-life seizures.

The decrease in the expression of molecules present at glutamatergic synapses may also provide some clues to the mechanism for the social behavior deficits. There have been suggestions that autism may be due to aberrant synaptic homeostasis (Kelleher and Bear 2008; Bourgeron 2009). A leading genetic candidate for autism is SHANK3, a protein present in the postsynaptic density of glutamatergic synapses. Genetic deletion of SHANK3 and haploinsufficiency of SHANK3 have been found to result in social behavior deficits, cognitive impairment, and epilepsy (Bozdagi, Sakurai et al. 2010). A future direction will be to examine whether SHANK3 protein levels are altered in association with early-life seizures.

In addition to examining molecular changes in the hippocampus it would be interesting to examine changes in the prefrontal cortex. A recent paper found that inducing 5–6 flurothyl seizures per day from postnatal days 6–16 alters short-term plasticity in particular layers of the prefrontal cortex (Hernan, Holmes et al. 2013). Alterations in the prefrontal cortex could underlie some of the learning and memory deficits observed after early-life seizures and contribute to some of the other behavioral abnormalities we observed in our study. For instance, the medial prefrontal cortex has a critical role in the formation and regulation of fear memories and requires NMDA receptor transmission (Gilmartin and Helmstetter 2010; Gilmartin, Kwapis et al. 2013). It would be interesting to determine whether there are alterations in the levels of NMDA receptors, AMPA receptors, and other synaptic proteins in the prefrontal cortex after early-life seizures.

Our results support the growing body of literature that seizures during early-life result in long-term consequences in behavior. Our observation that seizures on postnatal days 7–11 may appear to provide support that there is a relationship between seizures and autism. However, our observations are limited in that we only observed changes in one of the three core behavioral features of autism. It may be that social behavior and learning are more susceptible to early-life seizures than repetitive behaviors. There are many instances where only one of the three behavioral core features of autism is found in a rodent model, which then leads the researchers to claim that they found a model of autism. This is not a criticism of the work but a limitation of finding a behavioral change in only one of the three core features of autism. Future studies could examine the “reciprocity chain” between animals believed to be autistic with normal animals (Bishop and Lahvis 2011). Our study only examined the social behavior of seizure and control mice with normal mice. It would be interesting to examine the direct interactions between seizure and normal mice. Additional studies would be needed to more thoroughly address the connection between seizures and autism in animal models.

Conclusions

Our work demonstrates that flurothyl seizures induced in mice on postnatal days 7–11 result in long-term deficits in social behavior and spatial learning and memory. Our results provide evidence that seizures during early-life produce behavioral deficits in social behavior. These alterations are specific to one of the three core features of autism, since there was no difference in repetitive behaviors.

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Highlights

We induced seizures during early development in mice.

Early-life seizures results in social behavior deficits.

Early-life seizures results in learning and memory deficits.

Early-life seizures did not produce alterations in repetitive behaviors.

Figure 1. Mice with early-life seizures show deficits in social behavior in the social partition test Time spent at the partition is shown in seconds for the control and mice with early-life seizures (seizure) during three sequential 5 minute tests with their familiar partner (white bars) and an unfamiliar partner (black bars). The values represent the mean $\pm$ SEM. *** = $p < 0.001$ $n = 15$ control; $n = 18$ seizure.

Figure 2. Mice with early-life seizures show deficits in social behavior in the three chamber partition test

During the testing day wire mesh cups were placed at the corner of the chamber test. The control and seizure mice were allowed to explore the chamber for ten minutes. (A). During the first part of testing, time the mice spent in the chamber was recorded. The control and seizure mice spent similar amount of time in each of the three chambers. (B). During the second part of the test, one cup contained a mouse and the other contained an object. The seizure animals spent similar amount of time in the chamber with the object, center, and side with the mouse compared to the control animals. (C) The seizure mice spent less time at the cup with the mouse than the control group ($p < 0.05$), while they spent a similar amount of time at the cup with the object. (D) The seizure mice also spent significantly less time per visit to the cup with the mouse than the control group ($p < 0.01$). The seizure group spent similar time (mean duration) at the object and the mouse, while the control group spent more

time at the cup with the mouse than the object ($p < 0.05$). The values represent the mean $\pm$ SEM. * $p < 0.05$, ** = $p < 0.01$. n=23 control; n=11 seizure.

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Figure 3. Mice with early-life seizures do not show abnormal anxiety in the elevated plus maze task

Control and seizure mice were examined in a test used to measure anxiety. (A) The control and seizure mice explored the open, center, and closed arms a similar amount in the ten minute test. (B) The mice also spent similar amount of time in each arm. Data are shown as mean $\pm$ standard error of the mean; n=24 control; n=12 seizure.

Figure 4. Mice with early-life seizures show impairment in spatial learning and memory
Control and seizure mice were examined for differences in acquisition and retention of spatial memory through the Morris water maze. The mice were trained over eight blocks to find the hidden platform. (A) The seizure mice show impairment in the ability to learn to find the hidden platform, $p < 0.01$. After 8 blocks of learning were completed, the animals were tested for spatial memory retention by the probe trial. (B) The seizure mice show less time spent in the target quadrant compared to the WT mice, $p < 0.05$, $n=20$ control; $n=9$ seizure. (C) Mice were also tested in a fear conditioning test. The control and seizure mice

show no difference in their freezing to a conditioned stimulus (tone) and show no differences in freezing in a new context, n= 21 for control; n=9 seizure. Data are shown as mean $\pm$ SEM; * = $p < 0.05$; ** = $p < 0.01$;