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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/6J 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.

Introduction

The relationship between epilepsy and autism has been discussed for over 5 decades (Creak and Pampiglione, 1969; Schain and Yannet, 1960). 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 and 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 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, resultes 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 (Holmes et al., 1998; Neill et al., 1996). In addition, rats that experience seizures during early development show defective hippocampal place cells involved in spatial learning (Karnam 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 and 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.

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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 cm^3/h 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 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 30 min. The experiments were conducted under artificial laboratory illumination (fluorescent lamps, 30 lx 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 were 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 are generally believed 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 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 × 40 × 30 cm) to investigate repetitive behavior. The lighting inside the test chamber was approximately 100 lx 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 home cage. 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 × 16.5 × 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 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 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 were 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 place in the other cylinder. The partner mice were initially habituated to being housed in the cylinder for 1 h 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 were 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 h 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 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 5 min (Psion computer