Epilepsy & Behavior 98 (2019) 249–257

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Cognitive and behavioral effects of brief seizures in mice

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ARTICLE INFO

Article history:
Received 13 May 2019
Revised 3 July 2019
Accepted 4 July 2019
Available online 6 August 2019

Keywords:
Electroshock
Seizure
Epilepsy
Memory
Cognition

ABSTRACT

Comorbidities associated with epilepsy greatly reduce patients' quality of life. Since antiepilepsy drugs show limited success in ameliorating cognitive and behavioral symptoms, there is a need to better understand the mechanisms underlying epilepsy-related cognitive and behavioral impairments. Most prior research addressing this problem has focused on chronic epilepsy, wherein many factors can simultaneously impact cognition and behavior. The purpose of the present study was to develop a testing paradigm using mice that can provide new insight into how short-term biological changes underlying acute seizures impact cognition and behavior. In Experiment 1, naïve C57BL/6J mice were subjected to either three brief, generalized electroconvulsive seizure (ECS) or three sham treatments equally spaced over the course of 30 min. Over the next 2 h, mice were tested in a novel object recognition paradigm. Follow-up studies examined locomotor activity immediately before and after (Experiment 2), immediately after (Experiment 3), and 45 min after (Experiment 4) a set of three ECS or sham treatments. Whereas results demonstrated that there was no statistically significant difference in recognition memory acquisition between ECS and sham-treated mice, measures of anxiety-like behavior were increased and novel object interest was decreased in ECS-treated mice compared with that in sham. Interestingly, ECS also produced a delayed inhibitory effect on locomotion, decreasing open-field activity 45-min posttreatment compared to sham. We conclude that a small cluster of brief seizures can have acute, behaviorally relevant effects in mice, and that greater emphasis should be placed on events that take place before chronic epilepsy is established in order to better understand epilepsy-related cognitive and behavioral impairments. Future research would benefit from using the paradigms defined above to study the effects of individual seizures on mouse cognition and behavior.

1. Introduction

People who suffer from epilepsy comprise 1–2% of the global population [1]. The hallmark feature of epilepsy is spontaneous, recurring seizures; however, comorbidities can be just as detrimental to patient quality of life [1]. These include cognitive comorbidities (e.g., memory impairments and migraines) [2] and behavioral/psychological comorbidities (e.g., anxiety, depression, autism spectrum) [1]. Currently, there are a host of antiepilepsy drugs (AEDs) that effectively reduce seizure frequency, but these therapeutics demonstrate mixed results in treating associated comorbidities, and in some cases, even worsen the symptoms [3]. Thus, there is a need for treatments that address comorbidities common to epilepsy.

To treat epilepsy-related comorbidities, the mechanisms by which they occur must be better understood. Whether epilepsy-related comorbidities are causative of epilepsy [4–6], sequelae of epilepsy, or whether epilepsy and comorbidities share an upstream etiology [7], remain a topic of debate [2]. Epilepsy-centric theories posit that comorbidities arise from seizures (i.e., active epilepsy). While these theories have gained traction, the way by which seizures bring about comorbidities remains unclear. Seizures can affect an individual's instantaneous and short-term cognitive state as well as trigger more distant biological events, such as neuronal degeneration and mossy fiber sprouting. The epilepsy-centric view has been supported strongly by cross-sectional studies in humans examining the relationship between duration of epilepsy and cognition [8] as well as by animal studies wherein researchers introduce epilepsy-related neurological insults through treatment with drugs such as pilocarpine [9–11], kainic acid [12], and other chemoconvulsants [13–16].

Although seizures can cause cognitive dysfunction, nonseizure-related factors tied to epilepsy can also play a role in comorbidities. Thus, some studies have analyzed genetically engineered, seizure-susceptible strains of mice and rats, reporting that these animals display diminished capacity to learn and remember tasks even before experiencing seizures [17]. Such results suggest that genes associated

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https://doi.org/10.1016/j.yebeh.2019.07.015
1525-5050/

with epilepsy progression contribute to the etiology of epileptic comorbidities before active epilepsy occurs. In addition to genetic differences, cognitive impairments and other common epilepsy comorbidities can be attributed to the effects of AEDs and the presence of unrelated comorbidities and treatments [2]. Most epilepsy, memory, and behavior research has focused on chronic, advanced models of epilepsy (e.g., [2, 18]), wherein one or more aforementioned factors can simultaneously affect cognition and behavior. This is problematic because of the challenge of determining the role that each factor plays in the etiology/progression of epilepsy-tied comorbidities.

Although a substantial body of research has examined changes in learning, memory, and behavior in either chronic epilepsy or status epilepticus, there has been less focus on understanding how these factors are impacted by individual seizures outside the context of epilepsy. However, a few recent studies have started to examine the effects of individual brief seizures on associative and spatial learning and memory. In these cases, chemoconvulsants flurothyl [18] and pentylenetetrazol (PTZ) [19] were administered to previously naïve rodents to induce acute seizures lasting less than a minute. This is in contrast to earlier studies that used higher dosages or more frequent injections of chemoconvulsants to induce status epilepticus [12–16]. While certain studies have focused on reducing the influence of confounding factors, the behavioral tests employed appeared to be impacted by differences in locomotion or confounded by stress-related effects [18,19].

The purpose of this study was to develop a testing paradigm using mice that can provide new insights into how short-term molecular events following acute seizures impact cognition and behavior in the absence of other confounding variables. To evaluate the cognitive and behavioral consequences of intermittent seizures, this study used the novel object recognition (NOR) paradigm and open-field locomotor activity analyses. Patterns of locomotor activity in an empty open-field arena were analyzed to determine whether intermittent electroconvulsive seizure (ECS) produced detectable behavioral disruptions that could confound interpretations of NOR task performance, and as an additional measure of anxiety-like behavior. We hypothesized that ECS would negatively affect measures of short-term cognition and cause changes in locomotor activity patterns.

2. Materials and methods

2.1. Animals

Male C57BL/6J mice (60–100 days of age) were used for all studies. Mice were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed in a barrier vivarium facility at Cooper Medical School (Camden, NJ). Mice were grouped four or five per cage and maintained on a 12-h light/dark schedule. Food and water were available ad libitum throughout the course of the study. Following their arrival to the laboratory from the vendor, mice were equilibrated for at least 2 weeks in the vivarium prior to the initiation of experiments. All experimental procedures were performed during the light phase of the light/dark cycle. All studies were approved by the Rowan University Animal Care and Use Committee, and all procedures performed were compliant with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

2.2. Electroconvulsive seizures

All seizures were induced using a constant current electroshock unit (model #7801, Ugo Basile, Varese, Italy). Stimuli were transmitted through ear-clip electrodes at a current level of 50 mA, frequency of 60 Hz, pulse width of 0.4 ms, and shock duration of 0.2 s, values that reliably elicit a generalized seizure phenotype in male C57BL/6J mice in our laboratory [20]. The sequence of events that defines a generalized seizure includes facial, forelimb, and hindlimb clonus without tonic hindlimb extension, as tonic hindlimb extension demarcates the maximal seizure phenotype. In each experiment, 3 single generalized electroconvulsive stimuli were administered at 15-min intervals over a 30-min period (i.e., at 0, 15, and 30 min). A control group received 3 sham treatments in which electrodes were attached but no stimulus was delivered. Electrodes were disconnected and mice returned to their home cage immediately after seizure or sham treatments. The ECS and sham treatments were performed within a ventilated hood in the animal housing room; following these treatments, animals were immediately moved to a behavioral suite in the adjoining room. The ECS and sham groups were randomly assigned.

2.3. Open-field test

Locomotor activity, as assessed by distance traveled and time spent in various zones of a gray plastic open-field arena (40 cm × 40 cm × 40 cm), was measured using an overhead mounted camera connected to AnyMaze software (Stoelting Co., Wood Dale, IL). The level of light in each test arena was 650–750 lx. The field was divided into 16 equal zones—the four centermost zones occupied a 20-cm × 20-cm Center Region in the middle of the open field and the remaining zones were grouped together as the Outside Region—and comparative activity in these two regions was used to create a measure of anxiety-like behavior. Since mice and rats are known to spend more time in the outside zones of an arena when they are anxious [21–23], the time spent by mice in the outside zones of the arena was divided by the total time of the experimental phase in question. Thus, higher fractional values were indicative of a greater anxiety-like response.

2.4. Novel object recognition (NOR) test

Novel object recognition testing was performed within the aforementioned gray plastic open-field arenas (40 cm × 40 cm × 40 cm) with overhead mounted cameras. Before this experiment was conducted, pilot tests were performed to optimize experimental parameters. These studies determined that mice showed no intrinsic preferences for the objects used in testing. Mice showed a general preference for the two quadrants of the open field furthest away from experimenter access, so these quadrants (named Zone C and Zone D) were used for all NOR testing.

Two object types were used for object exploration: metal binder clips and metal hooks. Object types used in the training and test phases were of similar material, size, and shininess, differing predominantly on the basis of shape. In addition, both objects were difficult for mice to climb and were unmovable, as they were secured to the arena by adhesive squares. Between phases, the objects and the arena were cleaned with water and wiped with 70% ethanol to reduce olfactory cues. Preliminary tests revealed that mice did not prefer one object type to the other.

2.5. Experiment 1

The pattern of training and testing for Experiment 1 is shown in Fig. 1. Prior to any treatment, mice (n = 16 ECS; 16 sham) were acclimated to the open-field arena for 15 min per day on three consecutive days (days 1–3). In the context of the NOR test, this was called the habituation phase. The purpose of habituation was to acclimate the mice to both the arena and to being handled, such that test day stress and noise in the training phase could be minimized.

On the test day (day 4), the mice received three ECS or sham treatments over 30 min. Following the last treatment, the mice were moved into the behavioral suite and, after 15 min of rest in the home cage, underwent a 15-min habituation session in the empty open-field test arena. The mice returned to the home cage for 15 min of rest, then participated in a 15-min training session in the open-field test arena with two identical objects (i.e., metallic binder clips) placed in two adjacent corners (Zones C and D) furthest from experimenter

access. After a 15-min rest period, in which the mice were returned to their home cages, there was a 5-min test phase, otherwise known as the NOR test phase. This phase was identical to the training phase except that the zone C training-phase objects were replaced with a novel object (i.e., metallic hook).

Object exploratory behavior was defined by the nose of the mouse being oriented toward an object, within a 5-cm radius from the object center. Sitting/climbing on objects was not scored as exploratory behavior. Mouse exploratory behavior for each object was manually assessed from recorded videos using keystrokes in the AnyMaze software. Behavioral scoring was performed by an observer blinded to treatment (ECS or sham) in order to prevent accidental bias from occurring. Exploration was operationalized in "seconds of exploration." These values were also used to calculate the measure of object interest (time exploring both objects/total time of phase). In the training phase, a C/D ratio (i.e., time spent exploring the object in zone C divided by the time spent exploring the object in zone D) was used to determine baseline spatial preferences. Preliminary tests demonstrated that mice did not collectively display a significant preference between zones C and D.

Object type and location in the test phase were not counterbalanced because test C/D ratios were never analyzed in isolation. Rather, they were compared to the training-phase C/D ratios, which functioned as a baseline and a screening tool. That is, if the training C/D ratio for an animal was >1.86 or <0.54, which is equivalent to >65% or <35% zone C exploration preference, recognition data were excluded from analysis as mice exceeding these values would demonstrate extreme spatial preferences (2 ECS and 3 sham animals were ultimately excluded based on these criteria).

The purpose of this experiment was to determine whether ECS or sham treatment differentially affected object recognition memory. Interpretation of NOR test data is based upon the observation that mice are naturally curious and show preference toward novelty; in the context of this test, mice must remember the *familiar* object in order to show a preference for the *novel* object. A test C/D ratio significantly greater than 1 indicates a preference for exploration of the novel object and is interpreted as demonstrating preserved memory, whereas a C/D ratio close to 1 represents no object preference and is interpreted as memory impairment. Training phase C/D ratios (baseline) were compared with test C/D ratios in order to determine whether memory was impaired or preserved. Test C/D ratios higher than training C/D ratios demonstrate memory preservation, whereas equivalent test and training C/D ratios demonstrate memory impairment.

2.6. Experiments 2, 3, and 4

The purpose of Experiments 2, 3, and 4 was to comprehensively assess the effects of intermittent ECS on locomotor activity in an open-field arena. The patterns of testing for Experiments 2, 3, and 4 are shown in Fig. 1.

In Experiment 2 (n = 8 ECS; 8 sham), mice participated in a 15-min open-field test immediately preceding 3 ECS or sham treatments and an additional 30-min open-field test immediately following the treatments. The purpose of this experiment was to determine the rate and degree that mice would recover from ECS (or sham) treatments. Data from this experiment were used to provide insight into whether significant effects from Experiment 1 would be more likely due to insufficient recovery from prior seizures or to short-term biochemical and physiological changes induced by the seizures.

Experiment 3 (n = 8 ECS; 8 sham) was identical to Experiment 2 with the exception that there was no pretreatment open-field test. The purpose of this experiment was to compare the data to that of Experiment 2 in order to establish the effect of pretreatment open-field test exposures on posttreatment locomotor activity.

In Experiment 4, mice (n = 7 ECS (1 excluded due to maximal seizure); 8 sham) participated in a 30-min open-field test 45 min after having received their third treatment (the same time that mice started the training phase in Experiment 1). In this experiment, objects from the Experiment 1 training phase were placed in adjacent corners of the arena. The purpose of this experiment was to replicate the significant finding from Experiment 1 that ECS treatments had a delayed inhibitory effect on locomotor activity compared to sham treatments, however, to do so under a slightly different context (i.e., no pretreatment open-field exposures and no handling or participation of mice in experimental phases shortly after the third treatment). We reasoned that comparable findings in Experiments 1 and 4 with respect to differences in locomotion between the ECS and sham groups would further strengthen conclusions.

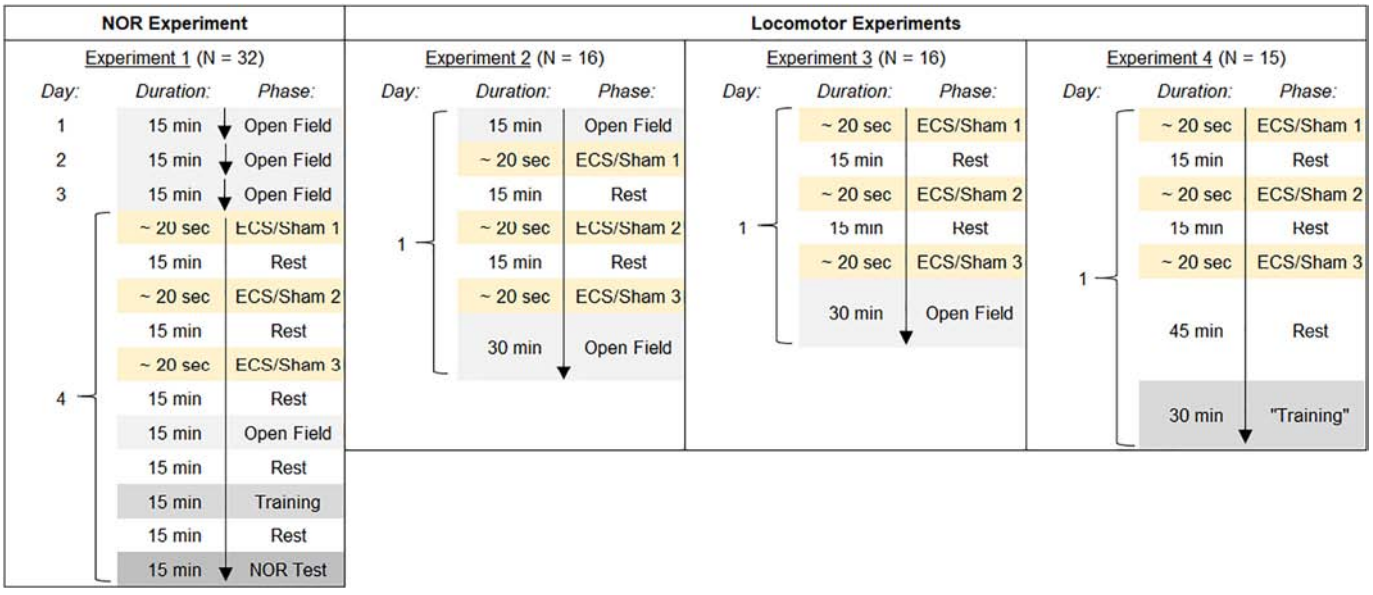

Fig. 1. Timelines for the novel object recognition experiment (Experiment 1) and the locomotor experiments (Experiments 2, 3, and 4). Note that Experiments 1, 2, 3, and 4 were started on different days within the same month. Each round of data collection for Experiment 1 was conducted over the course of 4 consecutive days, whereas each round of data collection for Experiments 2, 3, and 4 were conducted in a single day.

2.7. Statistical analyses

All data were analyzed using Microsoft Excel and GraphPad Prism. Most of the data were analyzed using a series of two-factor analyses of variance (ANOVAs) with replication that examined the relationship between treatment type (ECS, sham) and various experimental phases. For the instances that had different sample sizes due to the exclusion of mice, 2-sample unequal variance *t*-tests were performed. In all cases, alpha level was set to $p < 0.05$.

3. Results

3.1. Electroconvulsive seizures

Seizures induced by ECS were brief, lasting less than 10 s and usually no longer than 5 s. Seizures induced by the first, second, and third treatment were generally similar in duration and character, involving instantaneous loss of postural control and rhythmic contraction of the limbs. In all but one case, in which a maximal seizure was elicited unexpectedly (this mouse was ultimately excluded from Experiment 4), the stimulus elicited the predicted generalized seizure phenotype as described in Section 2.2.

3.2. Experiment 1

Table 1 shows summary data for Zone C/Zone D exploration ratios, outside region percentages (time spent in outside region/total time) and object exploration percentages (time spent exploring both objects/total time). Table 2 shows summary statistics for distances traveled (cm).

3.2.1. Recognition (nonassociative) memory acquisition

Fig. 2A shows the results of the NOR test. A two-way repeated measures ANOVA, with ECS or sham as a between-subjects factor and training/test C/D ratios as a within-subjects factor, showed a significant increase in C/D ratio as a result of an increased proportion of time spent with the novel object ($F(1, 25) = 16.13$, $p = 0.0005$), but no significant effect of ECS vs. sham treatment ($F(1, 25) = 0.20$, $p > 0.65$) and no significant interaction overall ($F(1, 25) = 0.09$, $p > 0.76$).

3.2.2. Object interest

Fig. 2B shows the proportion of object exploration during the training and testing phases of the NOR test. A two-way repeated measures ANOVA, with ECS or sham as a between-subjects factor and training/testing exploration time as a within-subjects factor, showed a near-significant increase in overall object exploration following the introduction of the novel object ($F(1, 25) = 4.05$, $p = 0.055$), and significant effect of ECS vs. sham treatment ($F(1, 25) = 10.50$, $p = 0.0034$), and no significant interaction overall ($F(1, 25) = 0.69$, $p > 0.41$).

3.2.3. Anxiety-like behavior

Fig. 3A shows the proportion of time spent within the outside region of the open field across habituation, prior to the training and testing phases of the NOR test. Evaluating ECS and sham groups prior to any ECS testing, a two-way repeated measures ANOVA, with ECS or sham as a between-subjects factor and habituation day phase as a within-subjects factor, showed a significant increase in overall proportion of time spent within the outside region of the open field as habituation training progressed ($F(1, 25) = 14.53$, $p = 0.0008$), no significant difference between groups ($F(1, 25) = 0.01$, $p > 0.90$), and no significant interaction overall ($F(1, 25) = 1.35$, $p > 0.25$). Importantly, because of computer errors, data for three animals were lost for habituation day 2; thus day 2 was excluded from this analysis overall to maintain the integrity of the repeated-measures analysis.

Fig. 3B shows the proportion of time spent within the outside zones of the open field during the training and testing phases of the NOR test. A two-way repeated measures ANOVA, with ECS or sham as a between subjects factor and habituation day phase as a within subjects factor, showed no significant difference in overall proportion of time spent within the outside region of the open field during the progression of the testing on the NOR test day (habituation, object training, novel object test) ($F(2, 50) = 1.33$, $p > 0.27$), a significant increase in the proportion of time spent within the outside region of the open field for the ECS group over the sham treatment ($F(1, 25) = 16.19$, $p = 0.0005$), and no significant interaction overall ($F(2,50) = 0.11$, $p > 0.89$). Bonferroni posthoc tests revealed a significant difference in the proportion of time spent within the outside zones of the open field between ECS and sham animals during all three phases of the test day.

3.2.4. Locomotor activity

Data presented in Table 2 document that treatment type ($F(1, 150) = 10.82$, $p = 0.001$) and experimental phase ($F(4, 150) = 67.68$, $p < 0.0001$) influenced locomotor activity. As expected, there was no significant difference in total distance traveled between mice destined to be in the ECS and sham treatment groups on pretreatment days 1 and 3 ($F(1, 60) = 1.04$, $p = 0.313$). However, mice collectively demonstrated significantly less locomotor activity on day 3 compared to day 1 ($F(1, 60) = 22.93$, $p < 0.0001$). When day 3 and test day (day 4) habituation phases were compared, there still was no significant difference in locomotion between the ECS and sham groups ($F(1, 60) = 0.14$, $p = 0.711$); however, mice across both treatment groups displayed a significant decrease in locomotor activity in the test day habituation relative to the day 3 habituation ($F(1, 60) = 5.90$, $p = 0.018$). When the training and test phase data were compared, the ECS group demonstrated significantly less locomotor activity relative to the sham group ($F(1, 60) = 22.23$, $p < 0.0001$).

3.2.5. Relation between measures of locomotor activity and anxiety

Given that ECS-treated mice demonstrated both higher anxiety-like behaviors and lower locomotor activity than sham-treated mice, the possibility exists that reduced locomotor activity led directly to fewer incidents where mice were present in the middle of the arena, thereby inflating measures of anxiety. This possibility was further investigated by comparing values of each mouse to the mean values across both treatment groups. Most ECS-treated mice in the training and test phases displayed below-average (<3014 cm training, <1040 cm test) locomotion (12/16) and above-average (>94% and >95% of time spent in outside regions during training and test) anxiety-like behaviors (13/16). Conversely, most sham-treated mice in the training and test phases displayed above-average locomotion (11/16) and below-average anxiety-like behaviors (12/16 during training, 10/16 during test).

Table 1
Memory, object interest, and anxiety-like behavior of mice in novel object recognition experiment (Experiment 1).

Nonassociative memory acquisition			Object interest		Anxiety-like behavior					
Treatment	Zone C/D time exploration		Percent time exploring objects		Percent time spent in outside region					
Type	Training	Testing	Training	Testing	D1 Hab	D2 Hab	D3 Hab	TD Hab	Training	Test
ECS	0.99 (0.08)	2.35 (0.45)	3.71 (0.40)	4.20 (0.05)	82.75 (1.16)	84.81 (1.92)	86.57 (1.94)	96.17 (1.29)	97.20 (0.86)	97.89 (0.73)
Sham	1.22 (0.07)	2.38 (0.38)	6.02 (0.89)	7.20 (0.78)	81.53 (1.51)	82.65 (1.95)	86.73 (1.46)	91.44 (1.69)	91.31 (1.36)	91.77 (1.32)

Means listed with SE in parentheses.

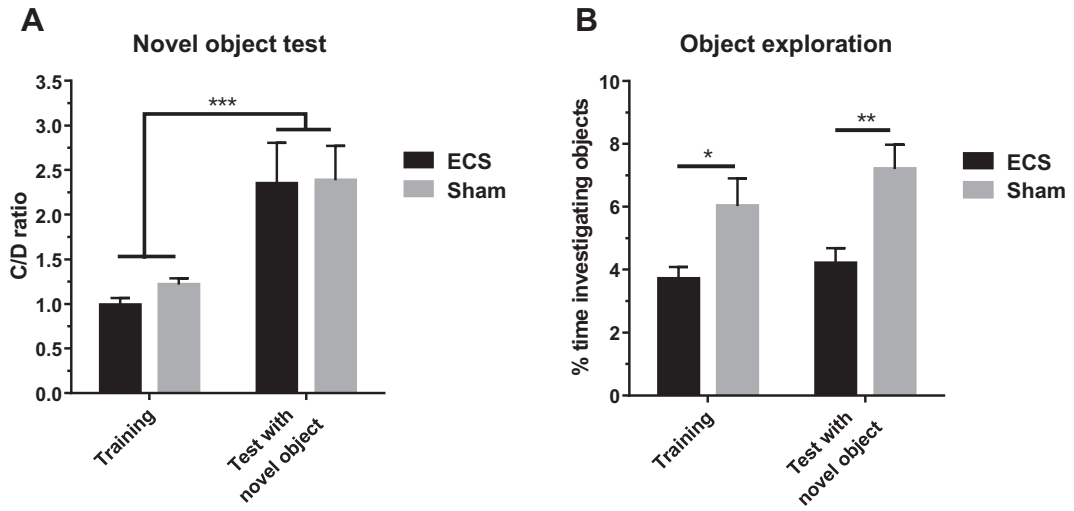

Fig. 2. A. Zone C/Zone D object exploration ratios as a function of experimental phase and treatment type. Both ECS and sham groups showed no preference for either training object, but a distinct preference for the novel object when it was introduced. There was no statistical difference between ECS and sham groups in their relative object preference. B. Overall object exploration as a function of experimental phase and treatment type. Compared to sham controls, ECS-treated animals spent a significantly smaller proportion of their time investigating objects during the training and testing phases. Data are presented as means ± SE. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.3. Post-ECS or sham locomotor activity: experiments 2, 3, and 4

Experiments 2 and 3 differed only with respect to the inclusion of a 15-min open-field locomotor trial prior to ECS or sham treatment. Experiment 2 included the pretreatment open-field period whereas Experiment 3 did not. For both experiments, a 30-min post-treatment open-field locomotor trial occurred immediately after ECS or sham treatment. Experiments 3 and 4 differed mainly as to whether the 30-min open-field locomotor trial occurred immediately after treatment (Experiment 3) or after a 45-min rest in the home cage (Experiment 4). In addition, training-phase objects were added to the open-field arena for the Experiment 4 posttreatment open-field phase in order to fully simulate conditions of the training phase from Experiment 1. These data are presented in Fig. 4.

Data from the 30-min post-treatment open-field locomotor trials from Experiments 2 and 3 were compared using a two-way repeated measure ANOVA, with ECS or sham as a between-subject factor and Experiment 2 or 3 as a within-subject factor. These tests revealed that mice demonstrated greater locomotor activity in Experiment 3 than in Experiment 2 ($F(1, 28) = 4.34$, $p = 0.046$) but that both ECS and sham treatment groups displayed equivalent locomotor activity in both experiments ($F(1, 28) = 0.005$, $p = 0.944$). There was no overall interaction effect ($F(1, 28) = 0.120$, $p = 0.732$).

Mice in the Experiment 4 posttreatment open-field phase appeared to demonstrate decreased locomotor activity (effect approaching statistical significance) compared with those in the Experiment 2 posttreatment open-field phase ($t(1, 21) = 1.95$, $p = 0.064$), yet a comparable locomotor activity with those in the Experiment 3 posttreatment open-field phase ($t(1, 22) = 0.52$, $p = 0.605$). In Experiment 4, however, there was a significant decrease in locomotor activity in the ECS-treated group compared to sham controls during the 30-min open-field locomotor trial ($t(1, 12) = 3.82$, $p = 0.002$). Further, it was found that the difference in locomotion between the ECS and sham-treated groups was more pronounced in the second half of the 30-min

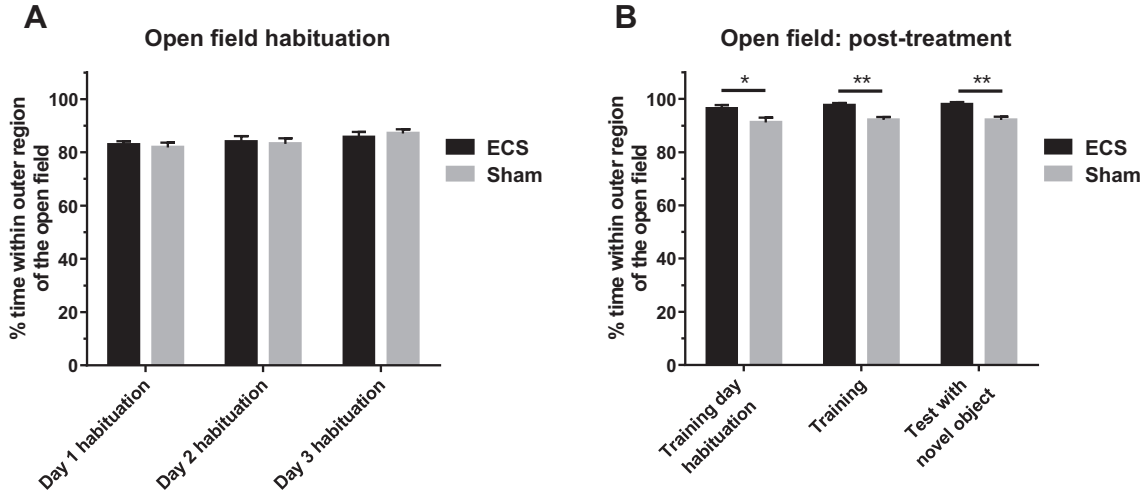

Fig. 3. Anxiety-like behavior as a function experimental phase. A. During the three open-field habituation training periods in Experiment 1, prior to ECS or sham treatments, there was no difference between ECS and sham groups in the time spent in the outside region of the open field. B. Following ECS or sham treatment, ECS-treated animals displayed persistent avoidance of the center region compared to sham controls. Data are presented as means ± SE. * $p < 0.05$, ** $p < 0.01$.

Table 2
Locomotor activity in novel object recognition experiment (Experiment 1)

Treatment	Distance traveled (cm)					
Type	D1 Hab	D2 Hab	D3 Hab	TD Hab	Training	Test
ECS	4944.80 (283.02)	3593.90 (268.54)	3567.40† (181.68)	3309.70†† (277.40)	2186.40 (232.89)	743.81††† (102.99)
Sham	5089.20 (298.73)	3926.90 (238.20)	3957.50† (268.62)	3091.60†† (178.37)	3843.40 (386.82)	1346.10 (123.20)

Means listed with SE in parentheses.
† $p < 0.0001$ compared to day 1.
†† $p < 0.05$ compared to day 3.
††† $p < 0.0001$ vs. Sham (2-way ANOVA, See Methods).

open-field period ($t(1,13) = 4.49, p < 0.001$) than in the first ($t(1,11) = 2.96, p = 0.013$). This assertion was further verified by determining the change in locomotor activity between the first and second 15-min segments of the Experiment 4 posttreatment open-field period and then conducting a two-tailed one sample *t*-test comparing the values of ECS and sham-treated mice. Treatment type significantly influenced the change in locomotor activity between the first and second half of the Experiment 4 habituation phase ($t(1,9) = 2.26, p = 0.049$). Specifically, the mean difference between the first and second 15-min segments of ECS-treated mice in the open field ($\overline{X} = 13.78$, SE = 2.51) was almost twice as great as that of sham-treated mice ($\overline{X} = 7.45$, SE = 2.31).

4. Discussion

The purpose of this study was to evaluate the short-term effects of individual electroshock seizures on nonassociative memory acquisition, object interest, anxiety-related behavior, and locomotor activity in mice. Electroshock was chosen as a method to induce seizures because it is highly reproducible and well-tolerated, having no major adverse effects on the general health of mice when administered intermittently over relatively short time intervals [20]. On the other hand, chronic ECS in rodents has been reported to cause structural changes in the brain including mossy fiber sprouting [19]. In human neuroimaging studies, no structural differences were detected in the brain before and after electroconvulsive therapy (ECT) [24]. This in contrast to changes found after status epilepticus [25] in which seizure activity is more intense and of longer duration. Procedural differences between ECS in rodents and ECT in humans result in the need for caution when comparing results between preclinical and clinical studies. In rodents, ECS stimulation is usually administered via bilateral corneal or ear clip electrodes once a day over the course of a week or longer, usually for up to 14 treatments in total (e.g., [26,27,28,29]). Electroconvulsive therapy in humans is typically administered via scalp electrodes, either unilaterally or bilaterally, 2–3 times per week for a total of 4–12 sessions for depressive disorders and 12–20 sessions for other disorders such as schizophrenia [26]. Another important difference is that humans receiving ECT are pretreated with anesthetic agents and muscle relaxants, whereas ECS studies in rodents do not typically employ these agents. Thus, although the exact relationship to ECT is unclear, ECS treatments used in the present study may be considered an effective means of inducing seizures without causing significant brain damage or introducing confounding factors present in chronic epilepsy.

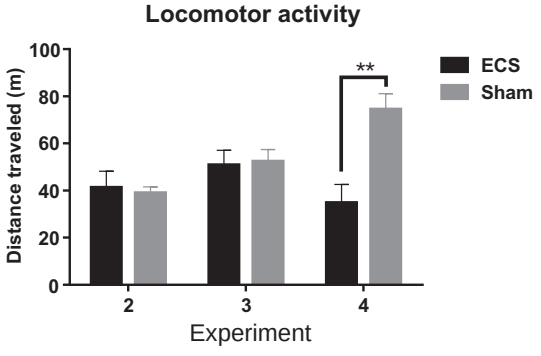

Fig. 4. Locomotor activity in the 30-min posttreatment open-field periods in Experiments 2, 3, and 4. Locomotor activity did not differ between ECS and sham treatment groups in Experiment 2 (30 min locomotor activity, immediately after completion of treatment, with a 15-min pretreatment habituation in the locomotor chamber) or Experiment 3 (30-min locomotor activity, immediately after completion of treatment, with no pretreatment habituation in the locomotor chamber). In Experiment 4 (30-min locomotor activity, 45 min after treatment, with no pretreatment habituation in the locomotor chamber), however, there was a significant reduction in locomotor activity in the ECS-treated group compared to sham controls. Data are presented as means ± SE ** $p < 0.01$.

The NOR behavioral paradigm was chosen because it is a minimally stressful test that relies on rodents' innate curiosity as opposed to their reaction to stressful stimuli [12]. In addition, NOR assesses nonassociative, nonspatial memory [30], which has received limited attention in prior studies examining acute effects of seizures outside the context of epilepsy. One study using functional magnetic resonance imaging (fMRI) volumetric analysis found that a subset of the patients with temporal lobe epilepsy studied had reduced perirhinal cortex volumes compared with controls [31]. While there has been limited research examining the ties between the perirhinal cortex and epilepsy, several studies suggest that this region may play a role in the progression and morbidity of temporal lobe epilepsy (see [32] for review). Given that the perirhinal cortex also plays a significant role in recognition memory [33], this form of memory deserves further attention, particularly in the context of studying the immediate cognitive and behavioral effects of acute seizures.

4.1. ECS and short-term recognition memory

Our first observation, consistent with prior experience and the literature, is that mice showed decreasing open-field locomotor activity with each successive exposure to the testing arena. This is important to note as each of the described experiments used a different pattern of exposure to the open field in order to explore the effects of ECS on behavior. In Experiment 1, mice showed successively decreasing locomotor activity before ECS or sham treatment, demonstrating that they were capable of nonassociative memory acquisition and retention prior to acute seizure induction. The concept underlying this conclusion is that mice have less incentive to actively explore an open field when it is more familiar, and are thus less active [17]. Additionally, it suggests that since mice in the Experiment 2 posttreatment open field (15-min open-field test conducted immediately prior to seizure treatment) displayed less locomotor activity than mice in the Experiment 3 posttreatment open field (no pretreatment open-field test), ECS treatments did not induce marked retrograde amnesia. This is an unexpected finding given that ECT administered to humans with psychiatric conditions has been shown to lead to temporary anterograde and retrograde amnesia [34]. Alterations were likely influenced by parameters of the treatment regimen such as seizure intensity, seizure frequency,

duration of treatment, and the time of memory testing relative to ECT.

The primary results from Experiment 1 indicate that three acute ECS treatments did not impair memory acquisition in the NOR test, a finding in line with a study that induced a single 20- to 30-second flurothyl seizure [18], yet contrasting another study that utilized PTZ to induce a 60- to 70-second seizure [19]. Memory acquisition may have been impaired in the latter study compared to the former study because the PTZ-induced seizures were of longer duration. The fact that memory acquisition was not impaired by ECS in the present study, in contrast to prior work with PTZ [19], is noteworthy. Whereas a singular ECS was likely less intense than seizures induced by PTZ injection, the present study induced three ECS within a 30-min time frame, likely making the procedure more analogous to a single PTZ injection. Previous studies have indicated that a single PTZ injection in mice will first cause focal seizures that progress to generalized seizures, which generally appear in clusters [35]. It is worth noting, however, that the latter study involved mice and that the PTZ dose was 80 mg/kg [35], whereas the former study involved rats and the dose was 50 mg/kg [19]. Differences in other test parameters may also contribute to observed differences between studies: 60-min separated training and test phases in earlier studies [18,19] compared to 15 min in the present paradigm. We set the interval at 15 min because preliminary NOR testing data showed that a 15-min interval provided the most reliable control condition with robust memory acquisition.

Seizures result in a variety of acute physiological and biochemical changes in the brain, and the time course of these changes likely impacts the time course of related behavioral alterations. Postictal changes following ECS include increased brain levels of GABA [36], adenosine [37,38], and opioid peptides [37,39]. These neurotransmitters can alter synaptic transmission and may mediate ECS-induced locomotor deficits. Endogenous opioid peptides may play a particularly important role as prior studies administering ECS in rats found that resulting catalepsy and behavioral suppression was reversed by treating animals with naloxone, an opioid receptor-specific antagonist [40,41]. Electroconvulsive seizures have also been shown to induce expression of immediate early onset (IEO) genes, and thus, it is possible that the delay in locomotor effect, as well as the lack of effect of ECS on memory, is related to the time course of IEO gene activation. Perhaps the changes in gene expression necessary to exert noticeable effects on memory did not have time to develop. Interestingly, acute ECS stimulation has been associated with the activation of a wide variety of IEO genes and a significant increase in the concentrations of their respective proteins, which include various neurotrophic factors and immune system-related proteins [28,42–44]. However, chronic ECS treatments have had the opposite effect, eventually lowering respective protein expression over time [42,45]. This idea has been hypothesized to explain the antidepressant properties of ECT in humans [19].

Given that ECS has been associated with transient cognitive impairment and that immune system activation has been implicated in inducing short-term cognitive deficits [44-46], it is possible that deficits in short-term memory after an acute ECS could be related to a short-lived increased expression of proinflammatory cytokines such as TNF-alpha, IL-1beta, and IL-6 [42]. One study showed that 10 ECS treatments over 10 days in B6 mice produced deficits in both anterograde fear learning and memory and also increased microglial activation [28]. The authors reasoned that increased immune system activity in the hippocampus, particularly due to the increased presence of ionized calcium-binding adaptor molecule, was a likely explanation of observed memory impairments. Other markers of glial activity, such as S100B, have been found to increase in serum 1 to 3 h after ECS [44]. It is possible that the increased expression of specific biomolecules minutes or hours later than the training/testing time frame of the current study (45- to 75-min posttreatment) would have had a demonstrable effect on short-term memory acquisition under these conditions.

Future studies might benefit from measuring memory and behavioral performance in parallel with immune expression in mice after having received one or multiple ECS treatments. It is possible that neuroinflammation is a common denominator in epilepsy and its comorbidities, being observed in the context of epileptogenesis [47], autism spectrum disorder [45], anxiety, and depression [47]. Many studies have documented changes in the expression of various genes following ECS [44,48,49]. Thus, with ample literature to guide future studies, ECS appears to be means of examining the detrimental effects of seizure-mediated immune expression on epilepsy and its comorbidities.

Future research should also further explore the factors (such as seizure duration, regions of the brain activated, and gene expression changes) that dictate whether individual seizures disrupt memory acquisition or alter behavior. This can be done by comparing and contrasting the effects of different methods of seizure induction. For ECS specifically, groups could be administered various levels of current to elicit either focal, generalized tonic–clonic or maximal seizures (these currents are characterized in [20]). An earlier study demonstrated that manipulating ECS intensity can affect the degree of retrograde amnesia experienced by rodents [29]. The ECT studies in humans have resulted in similar findings, wherein higher doses of electrical current and higher frequency of treatments lead to increased cognitive impairment [34]. Once these factors are better elucidated, greater insight will be gained into mechanisms by which seizures themselves contribute to comorbidities associated with epilepsy.

There are several limitations of these experiments that should be addressed. One is that they only assessed nonassociative nonspatial memory. Prior studies have reported a significant impact of seizures on associative and spatial memory [18,19], and thus, the possibility that acute seizures differentially affect these two forms of memory in the short term cannot be discounted. One variant of the NOR paradigm is the novel placement test (NPT), which assesses spatial recognition memory in the test phase by changing the location of one of the two identical objects from the training phase to determine whether the object in the new location is explored more [15,23]. Combined with ECS, the NPT could provide information that would complement and extend the results presented here. Another limitation of the present study is related to the number and timing of seizures relative to behavioral testing. Previous ECS studies demonstrating memory impairment administered more ECS treatments but on a less frequent basis (7 to 10 ECS treatments, once daily) compared with the present study, and reported memory impairment at more distant time points, typically 24 h after the last ECS treatment (e.g., [27]). The same premise holds true for the paradigm of ECT studies on humans, as previously discussed. It is not possible to determine whether the lack of ECS-induced memory impairment in the present study was related to fewer ECS treatments or the fact that the time interval separating the last ECS treatment from the first training phase was shorter than in other studies (e.g., [23]).

4.2. The effect of ECS on other behaviors

Results of the present study document that in addition to effects described above, ECS significantly altered other behaviors in mice. One of the key findings was that ECS-mediated decreases in locomotor activity developed after a brief latency. Across Experiments 1, 2, and 3, ECS and sham-treated mice demonstrated similar locomotion in periods prior to and shortly after (0–30 min) treatments. However, in Experiments 1 and 4, ECS-treated mice traveled significantly less distance than sham-treated mice in periods more distant from treatments (45 min or more). When tested 45 min posttreatment in a 30-min open-field trial with two objects (Experiment 4), decreased locomotor activity of ECS-treated mice relative to sham-treated mice was observed, a difference that was more pronounced in the second 15-min interval. Prior evidence points strongly to the possibility that ECS had a delayed effect (~45 min) on mouse locomotor activity. The finding is consistent with

a previous study [18], which reported that flurothyl-induced seizures decreased mouse locomotion 2 h after treatment. However, other studies, which examined locomotor activity in rodents 24 h after 7 or more daily ECS treatments, found that ECS either significantly increased or had no effect on locomotor activity relative to sham [27,50–52]. The effect of ECS on locomotor activity could potentially be dopamine-mediated. Whereas it is difficult to fully discern factors responsible for the discrepancies between results or the cause of the delayed locomotor effect in the present study, one possibility is that the series of ECS treatments led to dopamine depletion within the studied time frame, a common postictal occurrence [38].

Another notable finding of the present study was that although the administration of both ECS and sham treatments elevated behaviors thought to be related to anxiety, ECS-treated mice were more likely to demonstrate anxiety-like behavior than sham-treated mice. The ECS-treated mice also showed less interest in exploring objects than sham-treated mice. Given the relationship between postictal depression and endogenous opioids [53,54], it is possible that decreases in apparent object interest may have been influenced by opioid-mediated decreases in motivation. It is also noteworthy that increased levels of anxiety-like behavior seemed to have no effect on memory, given that high levels of stress (including corticotropin releasing factor (CRF)) are known to affect learning and memory processes [17]. Previous work found that B6 mice demonstrated increased anxiety-like behavior in the open-field test and impaired spatial recognition memory after experiencing several acute, virally-induced seizures [23]. However, the seizures were accompanied by significant neural damage and increased anxiety-like behavior after the epileptic events. Conversely, the changes in anxiety-indicative behavior in the present study, although statistically significant, were more modest in magnitude. On the other hand, studies that used ECS found seizures to either have no effect on or decrease anxiety-like behaviors. This behavioral finding is consistent with the well-documented ECS-mediated increase in GABAergic transmission [36]. Unlike the present study, however, most of these reports did not use naïve animals, but rather used mice or rats modeling depression or other psychiatric conditions (e.g., [55]). It is also noteworthy that less interest in objects did not reduce memory in ECS-treated mice, since it would be expected that increased object interest would increase object encoding and lead to a stronger memory trace. Another limitation is that measures of anxiety and object interest were potentially skewed by differences in locomotion, as discussed in Section 2.7.

4.3. The benefits of ECS-NOR paradigm

The paradigm utilized in this study isolates the short-term contributions of seizure activity on cognition and behavior related to epilepsy comorbidities by eliminating confounding variables inherent in chronic epilepsy and status epilepticus-dependent models. It also avoids problems associated with behavioral paradigms that are susceptible to locomotor and stress-related effects. Compared to other behavioral tests, the NOR paradigm specifically limits animal stress and requires little training. It can be used to also study other behaviors (e.g., anxiety, interest). Electroshock is a reliable and reproducible means of seizure induction and is also a valuable therapeutic modality in depression. It facilitates integrating the biology of seizures and behavior, providing a means to better understand their functional relationship. Thus, the present work provides a foundation for future studies that can determine the corresponding transcriptional and signaling events induced by ECS and their correlations to cognitive outcomes. Overall, results document that electroshock seizures have an acute impact on motor and cognitive function in mice, and a represent a starting point for future research aiming to better understand the etiology of epilepsy and behavioral comorbidities.

Sources of funding

This research was supported by funds from Cooper Medical School of Rowan University.

Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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