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Optogenetic intervention of seizures improves spatial memory in a mouse model of chronic temporal lobe epilepsy

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Summary

Objective—To determine if closed-loop optogenetic seizure intervention, previously shown to reduce seizure duration in a well-established mouse model chronic temporal lobe epilepsy (TLE), also improves the associated comorbidity of impaired spatial memory.

Methods—Mice with chronic, spontaneous seizures in the unilateral intrahippocampal kainic acid model of TLE, expressing channelrhodopsin in parvalbumin-expressing interneurons were implanted with optical fibers and electrodes, and tested for response to closed-loop light intervention of seizures. Animals that responded to closed-loop optogenetic curtailment of seizures were tested in the object location memory test and then given closed-loop optogenetic intervention on all detected seizures for two weeks. Following this, they were tested with a second object location memory test, with different objects and contexts than used previously, to assess if seizure suppression can improve deficits in spatial memory.

Results—Animals that received closed-loop optogenetic intervention performed significantly better in the second object location memory test compared to the first test. Epileptic controls with no intervention showed stable frequency and duration of seizures, as well as stable spatial memory deficits, for several months after the precipitating insult.

Significance—Many currently available treatments for epilepsy target seizures but not the associated comorbidities, which therefore are often not improved through these treatments. There is a need to investigate new potential therapies that may be able to improve both seizure burden

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Ethical Publication Statement

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

Conflicts of Interest

We declare no conflicts of interest.

and associated comorbidities of epilepsy. In this study, we showed that optogenetic intervention may be able to both shorten seizure duration and improve cognitive outcomes of spatial memory.

Keywords

behavior; closed-loop; cognitive impairment; comorbidity; temporal lobe epilepsy

Introduction

Temporal lobe epilepsy (TLE) is the most common form of epilepsy found in adults and tends to be medically refractory to currently available treatments. The seizures that occur in epilepsy are usually difficult to predict and can be greatly disruptive to the lives of patients. However, in addition to seizures, epilepsy is often accompanied by a host of comorbidities that can include a range of cognitive problems.[1] In the case of TLE, it is known that there are problems such as deficits in memory retention, remote memory, and spatial memory.[2–4] Moreover, many current anti-epileptic drugs (AEDs) have a broad course of action and can potentially cause significant side effects as well, including cognitive problems.[5] New treatment strategies for TLE are needed that can both target seizures with high accuracy and precision, and also alleviate aberrant network activity which may arise as a comorbidity of epilepsy or inadvertently as a side effect of AEDs.

Several tools are already available to study such potential new treatment strategies in animal models of TLE, including activation or inhibition of specific neuronal populations by optogenetics or chemogenetics.[6–8] In different rodent models of TLE, animals show both spontaneous seizures[9] and comorbidities such as cognitive deficits in spatial memory[10,11], and thus provide a suitable model to test the effect of specific network manipulations on lowering the seizure burden and cognitive comorbidities. Optogenetics is a particularly promising experimental seizure therapy in animal models of epilepsy due to its ability to target specific cell types at high temporal specificity via light pulses.[12–14] Previous studies showed that optogenetic tools can be effectively deployed to stop chronic seizures in animal models of TLE in an on-demand manner to minimize potential off-target effects.[15–17] However, it remains unknown if closed-loop seizure intervention, in addition to temporarily decreasing seizure duration, also ameliorates other comorbidities associated with TLE such as cognitive deficits in learning and memory.

As interference with normal neural functions due to epileptic activity can disrupt learning and memory[2], we hypothesized that optogenetic suppression of seizures in an on-demand manner, due to its temporal and spatial specificity, may not only alleviate seizure burden but potentially also improve memory performance in TLE. To this end, we tested if closed-loop optogenetic intervention of seizures, in individuals in which it is verifiably effective in shortening seizure duration, can improve spatial memory in the well-established intrahippocampal kainic acid model of TLE.

Methods

Animals

All procedures were approved and performed in accordance to the Administrative Panel of Laboratory Animal Care of Stanford University (Protocol 30183), the Institutional Animal Care and Use Committee of the University of California, Irvine (Protocol 1999–1719), and with the animal care guidelines of the National Institute of Health. Male and female mice used in this study were generated from crossing lines expressing cre in parvalbumin (PV)-expressing interneurons (PV-Cre;B6;129P2-Pvalb$^{tm1(Cre)Arbr}$/J; Jackson Labs stock #008069) to cre-dependent floxed-Stop ChR2 animals (Ai32; Rosa-CAG-LSLChR2H134R-EYFP-deltaNeo, obtained from the Allen Institute; also available as Ai32D, Jackson labs stock #012569)[18]. From this cross, animals expressing channelrhodopsin-2 in PV-expressing cells (PV-ChR2) (Figure S1) were used for all experiments. In addition, siblings negative for the opsin were used as nonepileptic behavioral controls. Animals were given food and water *ad libitum* and housed in a 12-hour light/12-hour dark cycle. All animals were group housed, except after going through electrode and optical fiber implant surgery, after which they were singly housed.

Stereotaxic surgeries

Intrahippocampal kainic acid (IHKA) injections were performed as previously described.[16] Briefly, mice were placed under isofluorane (4%) anesthesia and given local anesthetic, 0.5% bupivacaine, at the site of incision. Kainic acid (80–100 nl, 20 mM in saline, Tocris Bioscience) was injected into the dorsal hippocampus (from bregma: 2.0mm posterior, 1.25mm left, 1.6 mm ventral). Animals were at least six weeks of age or greater. After injection, animals were allowed to recover and then were returned to the vivarium for at least two weeks to allow for emergence of chronic spontaneous seizures.

For optrode implants, animals that had undergone intrahippocampal kainic acid injections were given implants as previously described.[16] A bipolar depth electrode (PlasticsOne) attached to an optical fiber (0.37NA, Low OH, 200 μm diameter optical fiber (Thorlabs) terminating in a zirconia ferrule (Kientec Systems) was implanted into the left dorsal hippocampus ipsilateral to the kainic acid injection (from bregma: 2.5mm posterior, 1.75mm left, 1.25m ventral). Another optical fiber was implanted at the contralateral side (from bregma: 2.5mm posterior, 1.75mm right, and 1.25mm ventral). The electrode and optical fibers were cemented to the skull using micro screws (McMaster-Carr and U.S. Micro Screw) and dental cement (Prime Dent Light Curable and Teets Cold Curing). Animals were given buprenorphine SR (0.5 mg/kg) as an analgesic. They were returned to the vivarium after the surgery to recover for at least three days before beginning videoEEG monitoring and closed-loop optogenetic intervention of seizures. Placement of optical fibers were verified *post hoc* (Figure S2).

VideoEEG monitoring and closed-loop optogenetic intervention

Animals with optrode implants were put through 24/7 video EEG monitoring within a dedicated animal housing and recording room. Each animal was connected to an electrical commutator (PlasticsOne) routed to an amplifier (BrownLee 410), and in turn connected to a

digitizer (National Instruments USB-6221) and a computer running custom MATLAB recorder software.[19] Animals were continuously monitored to establish the presence of spontaneous electrographic and behavioral seizures through the seizure detection algorithm programmed in the MATLAB recorder.

For closed-loop optogenetic intervention, epileptic animals were connected from the implanted optrode to an optical rotary joint (Doric Lenses) using an optical fiber patch cord (Doric Lenses) and ceramic split sleeve (Precision Fibre Products Inc). The optical rotary joint was then connected through an optical fiber patch cord to a blue laser (473 nm, Shanghai Laser & Optics Century Co., Ltd). Lasers were controlled by the MATLAB recording software that was individually tuned to each mouse by gradual adjustments to light intensity and seizure detection criteria settings as previously described.[16,19] Briefly, the custom MATLAB program is able to detect seizures using detection criteria provided by the experimenter for LFP spikes (filtering, amplitude threshold, width, template matching), LFP spike clusters (inter-spike interval, inter-cluster interval, minimal duration) and artefact rejection (filters, signal features), while combing them using Boolean logic. Processed files are verified and if necessary corrected by the experimenter on their detection accuracy of seizure starts and ends. This program was set to detect seizures with the criteria of all spike clusters that lasted at least five seconds in duration and were separated by at least three seconds from another cluster of spikes. The laser was also set to respond to either 50% or 100% of detected seizures, depending on the experiment. When analyzing the effectiveness of laser stimulation by setting response rate to 50% of detected seizures, experimenters were blinded to light status of each seizure during analysis. Laser stimulation consisted of 50 ms light on, 100 ms light off, for 15 seconds total. After light stimulation, another light stimulation was prevented for the next 30 seconds to avoid overstimulation.

Behavior testing

The object location memory (OLM) test was performed as previously described.[20] To test for hippocampus-based place learning and memory, animals were first handled for four days for two minutes per day. Following this, animals were habituated to the empty testing arena, which was an acrylic box (30×23×23 cm) lined with bedding, for five minutes per day for six days, with handling days overlapping with habituation days. After this phase, two identical objects were placed into the arena, and the mice were allowed to freely explore the objects for 10 minutes and then returned to their homecage. After 24 hours, one object was moved to a new location and mice were returned to the arena and allowed to explore for five minutes. All tests were performed approximately between 2pm and 6pm. Videos were taken of each session using a Panasonic Video Camera or Logitech Webcam and manually scored for the time duration in which animals explored the objects (oriented towards the objects and within 1 cm from the object), using previously described criteria for exploration.[20] Experimenters were blinded to the intervention type of the animals. Animals that showed side preference during training (DI>20) [20] or explored less than 2.8 seconds in testing, or had a behavioral seizure during testing, were excluded. In cases where a follow-up OLM test was performed, all aspects of the test were the same except the arena was changed to a novel colored box, new bedding and different objects. A discrimination index was calculated using the time duration spent exploring each object: (time exploring moved object – time

exploring unmoved object)/total exploration time*100. Other analyses such as quantification of running speed and distance traveled was calculated using Any-Maze (Stoelting Co).

Statistics

In all experiments, normality was tested using the Kolmogorov-Smirnov test to determine the use of parametric and nonparametric tests. Comparison of discrimination index between control and IHKA animals was made using the two-tailed Mann-Whitney Test. Correlation between discrimination index and time from IHKA treatment was made with linear regression analysis and calculation of Pearson's R. Running speed comparison between IHKA and control animals utilized the student's t-test. Comparison of seizure duration and seizure frequency between different months from IHKA was evaluated using the Kruskal-Wallis ANOVA followed by Dunn's Test for individual comparisons. Seizure duration in no light and light conditions was compared using the two-tailed Mann-Whitney test. Comparisons of discrimination index between two sequential behavior tests utilized the Wilcoxon signed rank test. For OLM tests, it was estimated that 8 animals per group would allow a detection of discrimination index change of 20 with a standard deviation of 14, based on a sample size calculation for a 0.05 probability of type-1 error and a 20% false negative rate. All values are presented as mean ± s.e.m., and a p-value < 0.05 was considered significant. Statistical analyses were performed using OriginPro 2019 and Microsoft Excel 365.

Results

Spatial memory deficits occur early and remain consistent in a rodent model of TLE

Along with chronic seizures, rodent models of TLE showed deficits in learning and memory, especially in tasks requiring spatial memory[10,21,22], similar to that observed in patients with TLE.[3] Previous experiments with IHKA rats and mice established that after an initial status epilepticus, the seizure probability successively increases in a sigmoid fashion until eventually reaching a plateau during the so-called chronic period.[23–27] To determine if learning and memory deficits are dependent on the time from the initial insult, we tested animals using the OLM test as described previously.[20] This task has been shown to be hippocampus dependent[28] and relies on the animals' ability to recall the location of items that they encountered previously and preferentially explore the object that has been moved (Figure 1A). We tested three separate groups of animals, each at a different time point: 1) 10 days post IHKA, during the so-called latent period[23], 2) 3–4 months post IHKA, during the chronic phase, and 3) 6–7 months post IHKA, which is well into the chronic period.

The three IHKA groups displayed deficits in the OLM task compared to non-epileptic controls at 10 days post IHKA (control: DI=29.5±2.2, n=6; IHKA: DI=5.9±4.6, n=10, $p<0.001$), 3–4 months post IHKA (control: DI=35.6±4.0, n=5; IHKA: DI=11.0±2.8, n=9, $p=0.002$), and 6–7 months post IHKA (control: DI=30.6±3.7, n=14; IHKA: DI=7.9±2.0, n=9, $p<0.001$)(Figure 1B, Table S1). As we showed in a previous study(29), IHKA mice displayed deficits compared with non-epileptic controls and did not discriminate effectively between the moved and unmoved object (DI<20). Quantification of the total exploration time of the objects remained the same between IHKA-treated and control animals at 10 days

(control: 4.0±0.4s; IHKA: 4.5±0.3s), 3–4 months after IHKA (control: 3.9±0.4s; IHKA: 4.1±0.2s), and 6–7 months after IHKA (control: 5.0±0.2s; IHKA: 4.5±0.4s), indicating that the deficit in OLM performance in IHKA animals was not due to lack of exploration (Figure 1C).

More specifically, when comparing each animal's OLM performance to the time of IHKA injection, there was no significant correlation between time the animals' performance and the time since they received IHKA (Fig 1D, Pearson's R=0.06). In addition, we found no significant differences between the discrimination indexes of male and female animals in either IHKA and controls (control female: DI=30.3±3.5, n=16; control male: DI=27.1±4.0, n=12; IHKA female: DI=9.5±3.4, n=23; IHKA male: DI=6.0±1.8, n=27)(Figure 1E), therefore all behavior data were combined and presented as a combination of both sexes. While the velocity of animals in the OLM tests was significantly increased in IHKA compared to control animals during the five minutes of exploring the empty box over six days of habituation (control: 0.028±0.002 m/s, n=34; IHKA: 0.048±0.002 m/s, n=50, $p<0.001$)(Figure 1F), overall exploration time in OLM tests between IHKA and controls remained similar despite this difference in overall speed of movement (Figure 1C). Note that all animals were detached from the closed-loop stimulation equipment during all phases of OLM testing.

Seizure frequency and duration in the intrahippocampal kainic acid model of TLE are stable in the chronic period

As our goals are to possibly alter the seizure burden and deficit in spatial learning after epileptogenesis during the chronic phase in IHKA animals, we tested if the seizure burden continues changing over time or if the animals eventually reached a relatively constant seizure phenotype with a steady state in seizure frequency as previously suggested in IHKA rats and mice.[23–27] While there have been several studies that describe the early development of epilepsy in the IHKA mouse model[23,24,26,27,30,31], few if any have investigated the stability of seizure burden across several months into the chronic period.

To test this, we recorded IHKA treated animals that were implanted after reaching the chronic phase of temporal lobe epilepsy utilizing continuous videoEEG. The recorded videoEEG data were analyzed *post* hoc to identify and quantify seizures in a semi-automated manner. While animals were observed to have both seizures without behavioral manifestation, i.e. electrographic seizures (Figure 2A), and seizures with behavioral manifestation (Figure 2B), electrographic seizures were much more frequent in the murine IHKA model.[32] We first recorded animals over the course of 24 hours to observe seizure duration and frequency over time (n=4)(Figure 2C, D). Though seizure frequency fluctuated over the course of the day, there was no significant difference in the number of seizures over different hours (Figure 2D), but there was a significant difference in seizure duration over the course of the day ($p<0.001$) (Figure 2C). Due to this, we chose to analyze only a specific time period of the day, i.e. the first five hours after the light-on phase, for long term videoEEG analysis.

VideoEEG data was taken from this time span from epileptic animals on one day per month over the course of six months and was analyzed for seizure frequency and duration (n=4)

(Figure 2E, F). Overall, there was no difference in seizure frequency or duration (p=0.76 and p=0.24 respectively), suggesting relative stability of the seizure phenotype this far into the chronic phase of TLE.

Optogenetic intervention of seizures utilizing PV interneuron activation improves spatial memory

The results of the OLM tests at different time points after IHKA administration suggest that the deficit in OLM performance is consistent from the time of epileptogenesis and into the chronic phase of epilepsy. The results also indicate that the seizure parameters are relatively stable well into the chronic period of the IHKA model and do not continue to increase in seizure frequency as has been observed during earlier stages.[23–27] Previous work showed that optogenetic activation of PV interneurons in animals expressing channelrhodopsin-2 specifically in PV cells was able to significantly decrease the duration of electrographic seizures.[16] To test if using this method of seizure suppression has effects on spatial learning ability, we designed an experimental paradigm (Figure 3A) in which IHKA animals were first tested for effective closed-loop optogenetic seizure suppression and then those animals where the intervention was efficacious in curtailing seizures (see Discussion) were given an initial OLM test along with control IHKA and non-epileptic controls, to measure baseline performance. Following the initial OLM test, the experimental IHKA group received continuous closed-loop optogenetic seizure intervention for one week, after which time the second OLM test was performed. This group received continuous optogenetic intervention for 15 days until the final OLM testing day with the exception of a 3-hour span daily for the last eight days of the intervention in which the second OLM test took place. This experimental paradigm required multiple successful sequential steps lasting over a minimum of nine weeks, and therefore acquiring epileptic animals that reached the endpoint was extremely challenging (Table S2).

We first confirmed that PV-ChR2 animals that received a modified closed-loop optogenetic intervention paradigm[16] showed a significant decrease in seizure duration when blue light was provided compared to when no light was provided during a seizure (No light: 16.5±1.5s; Light: 8.0±0.8s, n=8, $p<0.001$)(Figure 3B, C). The individual seizure detection settings determined during this time were utilized for all later closed-loop optogenetic seizure suppression experiments.

We found that the epileptic group receiving optogenetic intervention performed significantly better in the second OLM test compared to the first test (OLM 1: DI=8.5±4.1; OLM 2: DI=21.3±4.1, n=8, p=0.04)(Figure 3D, left panel). In comparison, the epileptic control group with no intervention did not significantly change their performance (OLM 1: DI=9.5±2.7; OLM 2: DI=8.5±3.9, n=18, p=0.58)(Figure 3D, center panel), while non-epileptic controls showed a significant decrease in performance between the two tests (OLM 1: DI=27.5±2.6; OLM2: DI=21.7±2.4, n=34, p=0.01)(Figure 3D, right panel). Opsin negative siblings were used as nonepileptic controls as their performance in OLM remained the same as opsin positive animals (Figure S3). The nonepileptic controls scored an average DI of greater than 20 in both OLM tests (in spite of the decreased performance in OLM, most likely attributable to retesting effects), signifying that they were still able to clearly

discriminate between the moved and unmoved object in both tests.[20] No differences were found in the exploration times between the first and second OLM tests within each group (Epileptic with laser, OLM 1: 4.4±0.3s, OLM 2: 4.6±0.5s; Epileptic w/o laser, OLM 1: 4.6±0.2s, OLM 2: 4.5±0.2s, Nonepileptic, OLM 1: 4.8± 0.2s, OLM 2: 4.4±0.2s)(Figure 3E, Table S1).

Discussion

Epilepsy treatments that reduce the burden of seizures and improve comorbidities associated with epilepsy represent an unmet need in the development of new therapies. In this study, we confirmed that optogenetic activation of PV interneurons in a closed-loop manner is able to significantly decrease the duration of electrographic seizures in the IHKA rodent model of TLE.[16] The key finding of the current study is that in addition to reducing the seizure burden, this treatment also significantly improved performance in a spatial memory task compared to performance prior to the optogenetic seizure suppression. This suggests that an on-demand seizure treatment is able to improve memory even without treatment during the performance of the actual task, as animals are not given light stimulation during the OLM task. In addition, the stability in seizure duration and frequency (Figure 2) and consistent deficits in OLM performance over time after IHKA (Figure 1) are significant because they suggest the therapeutic window for closed-loop optogenetic seizure treatment may potentially remain open throughout the chronic phase.

PV interneuron stimulation and improved cognition

Our findings are further supported by studies that found that targeting and activation of parvalbumin interneurons at specific stimulation frequencies was able to ameliorate spatial learning deficits. For example, rhythmic PV interneuron activation in the hippocampal CA1 in sleep-deprived animals during non-REM sleep enhanced performance in memory tasks.[33] As seizures in IHKA mice can occur during all brain states, with a majority (~90%) arising from a non-theta state[34], animals in our present study likely received some light activation of PV interneurons during non-REM sleep at a theta frequency (~7 Hz) similar to that used in the described study[33] Rhythmic activation of PV interneurons has been shown to have therapeutic effects in the gamma frequency as well. This stimulation paradigm has been shown to reduce the pathology in a model of Alzheimer's disease, by activating glial responses.[35] Glia are also important in in TLE[30] thus providing another possible means by which our intervention may have affected learning outcomes in this study. Other results also have shown that PV activation using chemogenetic methods is able to decrease seizure burden but does not improve learning performance.[36] However, one caveat in the latter study is that the chemogenetic intervention occurred during the learning phase of the OLM task, unlike this current study where optogenetic intervention was instead provided outside of the behavioral task. This suggests that intervention may need to be provided at specific timepoints outside of the behavioral task, or in an on-demand manner which could reduce interference with normal PV function.

Limitations of the current results and their implications for future studies

This study purposely tested the effects of seizure suppression on memory performance only in those chronically epileptic animals where the closed-loop optogenetic intervention was successful in significantly curtailing the seizures. This experimental design was implemented in order to minimize variability arising from the fact that not all mice in which closed-loop optogenetic intervention of seizures is attempted actually show successful seizure suppression (e.g., due to a malfunctioning or incorrectly placed optrode and other technical confounds[16,19]). Given the latter caveat, a number of interesting questions arise from the findings from this study. First, whether varying the timing or targeting of the optogenetic seizure intervention can affect the improvement in memory. For example, it is possible that intervention is only required during the week the animal spends in behavior testing, or even only the period of memory consolidation between training and testing. Second, the mechanism by which the optogenetic intervention of seizures is able to ameliorate spatial memory deficits remains unknown. It is possible that the shortening of the seizure durations and thus lowering the overall seizure burden in epileptic animals in itself is able to improve cognition by reducing overall disruption of network activity. Third, it would have to be established in future studies if PV interneuron activation is actually necessary for the improvement in location discrimination or if the same effect can be achieved using other neuronal targets. For example, seizure suppression was achieved not only by PV interneuron activation, but also by inhibition of principle neurons in the dorsal hippocampus.[16] Fourth, it is unknown if targeting seizures is required for the beneficial effects in learning and memory or if random or periodic PV activation could also work, especially considering experimental results indicating that PV interneuron activation itself can improve different pathologies.[33,36] Fifth, the long-term effects of closed-loop intervention and optogenetic stimulation will need to be determined, i.e., whether the beneficial effects of the closed-loop seizure suppression end immediately after the end of the treatment, or if there are any lasting effects on seizures or cognitive ability. Sixth, it should be noted that it is not yet entirely clear whether unilateral optogenetic intervention in this paradigm achieves curtailment of seizures primarily by exciting the somata, dendrites or axon terminals of those ipsilateral PV cells that survived the kainate administration (Figure S1), despite being greatly reduced in number[37,38], and/or by activating the ipsilateral axon terminals of contralaterally located PV cells that have been recently shown to undergo a prominent sprouting response after IHKA treatment.[39] Due to the difficulty of acquiring enough animals that reach the endpoint of our prolonged multi-step experimental paradigm (Figure 3A), these interesting and important questions will need to be pursued in carefully designed future studies.

Conclusions

We show here for the first time that closed-loop optogenetic intervention of seizures in a model of temporal lobe epilepsy is able to improve spatial memory. By taking advantage of the benefits of the spatial, temporal, and cell specificity of optogenetics, this type of intervention avoids nonspecific targeting of other neurons and other cells. Our findings demonstrate that the closed-loop optogenetic intervention improves the learning ability of epileptic animals and decreases seizure duration, providing strong support for further work in this area as a therapeutic for epilepsy and its comorbidities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points

- After the initial insult and the eventual development of spontaneous seizures in a rodent model of temporal lobe epilepsy, seizure frequency and duration in the absence of intervention remain relatively constant for several months
- Similarly, spatial memory deficits are also stable over several months in epileptic mice without intervention
- Closed-loop optogenetic seizure intervention in a model of temporal lobe epilepsy is able to improve spatial memory
- Together, these findings indicate that even well into the chronic phase of epilepsy, closed-loop seizure intervention in individuals in which it is able to shorten seizure duration may also improve spatial memory

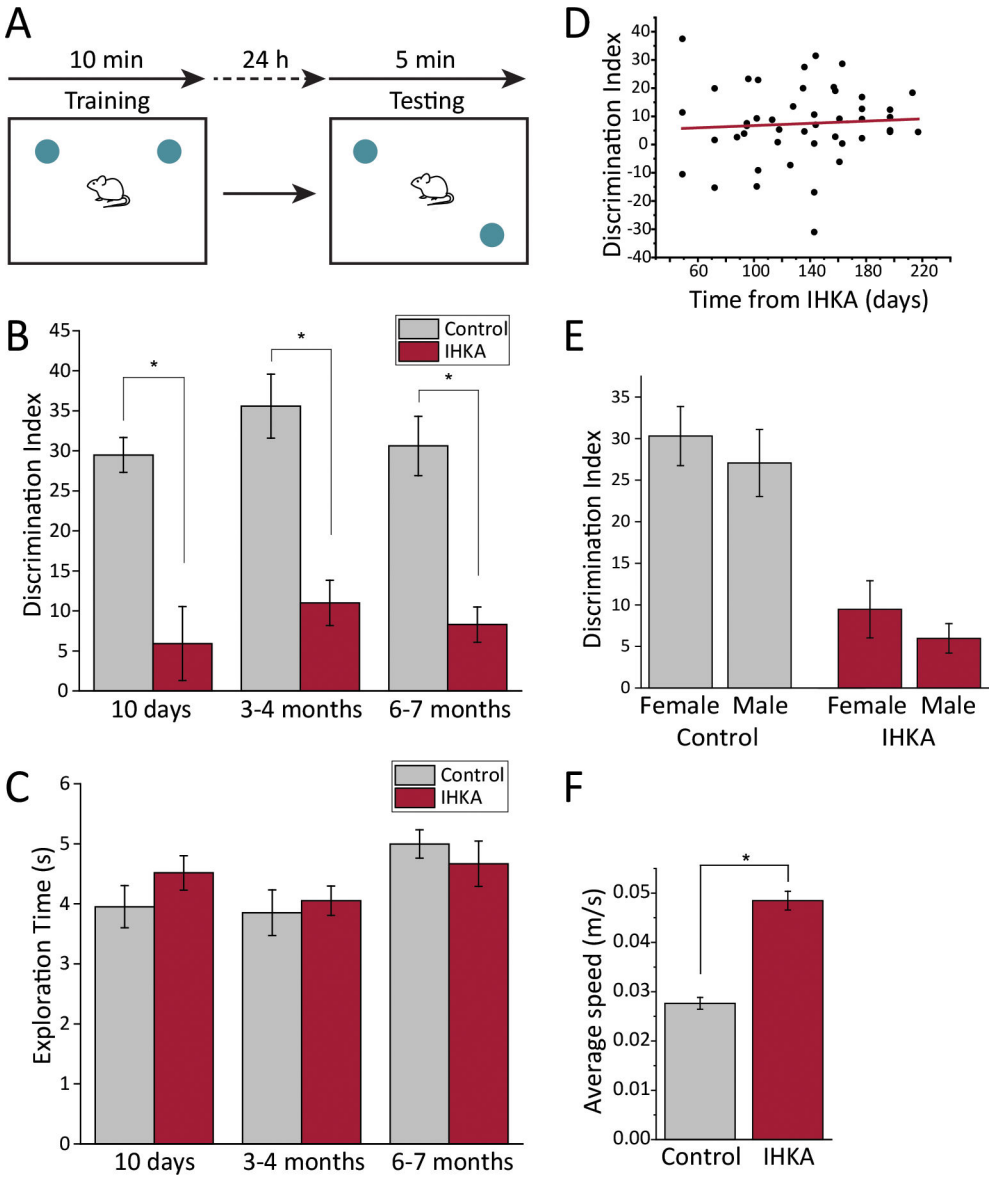

Figure 1.

Spatial learning and memory deficits in the intrahippocampal kainic acid model. (A) In the object location memory test animals are exposed to identical objects for 10 minutes, then brought back to the testing chamber 24 hours later, where one object is moved to a new location and the animals are allowed to investigate for 5 minutes. Animals are timed for how long they explore each object and a discrimination index is calculated. (B) Comparison of OLM performance between IHKA animals and non-IHKA injected animals in three different cohorts at three timepoints after IHKA treatment. Animals given IHKA treatment had significantly lower discrimination indexes than controls in all three tests, whether they were tested 10 days post IHKA (control: n=6; IHKA: n=10; $p<0.001$, $Z=-3.09$, Mann-Whitney Test), 3–4 months post IHKA (control: n=5; IHKA: n=9; $p=0.002$, $Z=2.8$), or 6–7 months post IHKA (control: n=14; IHKA: n=9; $p<0.001$, $Z=3.24$). (C) Comparison of the total time that animals spent exploring both objects in each test between IHKA and non-

IHKA animals. There were no significant differences between exploration times in control and IHKA animals within each time point (p=0.26, p=0.70, p=0.37 respectively, Mann-Whitney Test). (D) Correlation of the time from IHKA injection compared to OLM performance in animals. Linear regression analysis showed no correlation between time from IHKA and OLM performance (Pearson's R = 0.09). (E) Comparison of OLM performance between female and male animals in both control and IHKA categories (control female: n=16, control male: n=12; IHKA female: n=23, IHKA male: n=28) showed no significant differences (control: p=0.24; IHKA: p=0.56, Mann-Whitney Test), and therefore both sexes were combined in all cohorts for analysis. (F) Video tracking analysis revealed that IHKA animals ran significantly faster than controls in OLM tests (IHKA: n=50; control: n=34; $p<0.001$, Student's t-test).

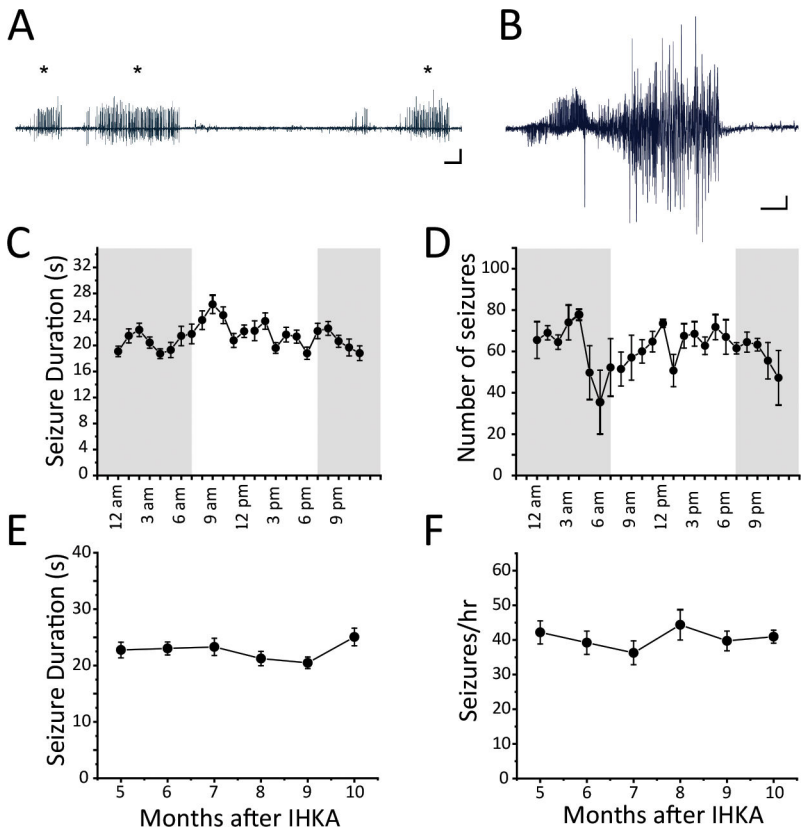

Figure 2.

Quantification of electrographic seizures in the IHKA model over time. Animals given IHKA showed both (A) electrographic seizures with no behavioral manifestation and (B) and behavioral seizures. Scale bars are (A) 1 mV, 5 sec, and (B) 2 mV, 5 sec. Asterisks in (A) indicate events classified as electrographic seizures. (C, D) Seizure duration and seizure frequency were quantified in a semi-automated manner in IHKA treated animals (n=4) over the course of 24 hours and no significant differences were found in (C) seizure frequency ($p=0.34$, Degrees of freedom (DF)=23, Kruskal-Wallis ANOVA followed by Dunn's Test) but a significant difference was found within the (D) seizure duration ($p<0.001$, DF=23, 9 am seizure duration significantly different compared to 4 am, 5 am, 7 am, $p<0.05$, and 9 am seizure duration significantly different compared to 6 pm, 10 pm, 11 pm, $p<0.01$). Shaded areas indicate light-off phase of light cycle. Following this, seizure duration and seizure frequency were analyzed for one day a month over the course of 6 months (E, F) which was limited to the same 5–6 hour period of time each day, approximately 7 am until 12 pm, due to the fluctuations seen in (C, D). No significant changes were seen in seizure frequency ($p=0.76$, DF=5, Kruskal-Wallis ANOVA) and seizure duration ($p=0.24$, DF=5) in these animals (n=4) during this time.

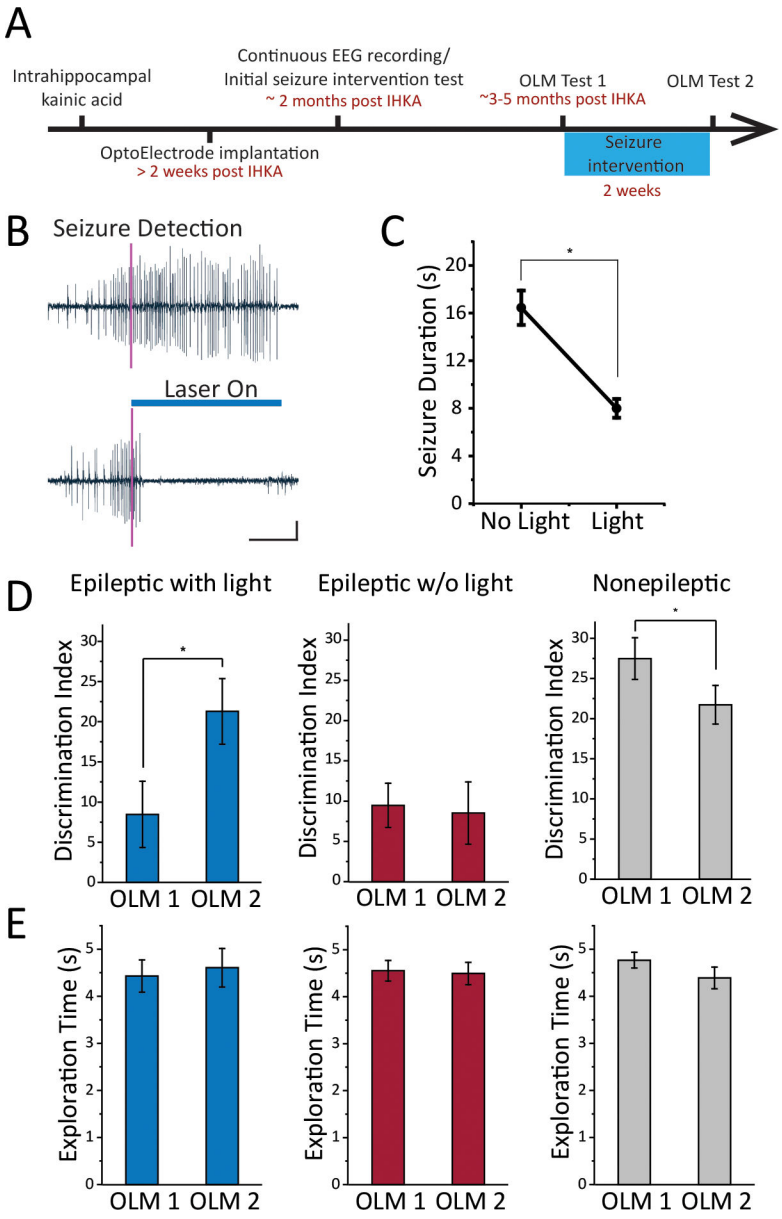

Figure 3.

Effects of optogenetic intervention of seizures on learning and memory. (A) Behavioral paradigm for performing optogenetic intervention in animals using two OLM tests with closed-loop optogenetic seizure intervention provided in between. (B) Example of seizure suppression when closed-loop optogenetic intervention system responds with laser light directed to the dorsal hippocampus after seizure detection (pink line, lower trace) to activate PV interneurons in PV-ChR2, compared to when no laser light is provided. (C) By programming the closed-loop system to randomly respond to 50% of detected seizures with laser light, seizures with light treatment were significantly shorter than seizures without light intervention (n=8 animals, at least 50 seizure events analyzed for each animal, $p<0.001$, $Z=3.1$, Mann-Whitney Test). (D) When animals were given closed-loop optogenetic intervention for two weeks prior to and leading up to the testing day of a second OLM test,

animals that received closed-loop intervention performed significantly better during the 2nd test compared to performance in the first test (left panel: n=8, p=0.04, Z=−2.03, W=3, Wilcoxon Signed Rank Test). Implanted epileptic animals that did not receive closed-loop stimulation did not improve performance in the second test compared to the first (center panel: n=18, p=0.58, Z=0.55, W=144) while naïve control animals showed normal learning in both tests (DI>20), though they performed significantly lower in the second test compared to the first test (right panel: n=34, p=0.008, Z=2.65, W=362). (E) No significant differences were observed between the exploration time of animals between the first and second OLM tests in all three groups (p=0.94, Z=−0.07, W=17; p=0.87, Z=0.16, W=121; p=0.1, Z=1.63, W=312.5, respectively, Wilcoxon Signed Rank Test).