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Specific alterations in the performance of learning and memory tasks in models of chemoconvulsant-induced status epilepticus

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Abstract

Cognitive impairment is a common comorbidity in patients with Temporal Lobe Epilepsy (TLE). Learning and memory impairments can be recapitulated in animal models, particularly those using chemoconvulsants. Here, we used two relatively low-stress behavioral paradigms, the novel object recognition task (NOR) and a spatial variation, the novel placement recognition task (NPR) to reveal deficits in short and long term memory, in both kainic acid (KA) and pilocarpine (Pilo) treated animals. We found that both KA- and Pilo-induced significant deficits in long term recognition memory but not short term recognition memory. Additionally, KA significantly impaired spatial memory as detected by both NPR and Morris water maze. These deficits were present one week after SE. The characterization of memory performance of two chemoconvulsant-models, one of which is considered a surrogate organophosphate, provides an avenue for which targeted cognitive therapeutics can be tested.

1. Introduction

Cognitive impairment is a common co-morbidity of the epilepsies and is especially pronounced in patients with medically refractory seizures and patients with Temporal Lobe Epilepsy (TLE). These impairments, including deficits in declarative and spatial memory (Abrahams et al, 1999, Guerreiro et al, 2001, Ploner et al, 2000, Viskontas et al, 2000), contribute significantly to the disability experienced by people with epilepsy. While many clinicians associate cognitive dysfunction specifically with TLE, there is increasing awareness that these deficits are also common in people with other epilepsy syndromes, including extratemporal (Adams et al, 2008) and genetic generalized epilepsies (Adams et

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al, 2008, Akanuma et al, 2008, Christensen et al, 2007, Filho et al, 2011, and Herman et al, 2008). Whether cognitive deficits are merely the side effect of medication, arise from the seizures themselves, or share a common underlying etiology is the topic of much speculation. Additionally, the threat of progressive cognitive impairment resulting from frequent uncontrolled seizures (Pitkanen & Sutula, 2002) underscores the need for further research on mechanisms of cognitive dysfunction associated with epilepsy. Such research is complicated in the human due to a multitude of factors including genetic background, medication status and history, and other comorbid conditions such as anxiety and depression, any of which can negatively impact cognitive function. Animal models may afford the greatest opportunity to dissect out the roles each of these play in contributing to cognitive impairment associated with epilepsy.

A wide variety of animal models have been developed for the study of specific types of epilepsy (reviewed in Sarkisan et al, 2001) and although no single animal model can precisely mimic the human condition, several recapitulate important facets of the disease. Perhaps the most commonly studied experimental models of TLE are the chemoconvulsants, kainic acid (KA) and pilocarpine (Pilo). Systemic administration of either KA (an analog of glutamate) or Pilo (a cholinergic agonist) results in a characteristic pattern of intense limbic seizures typically culminating in status epilepticus (SE) or a period of continuous seizure activity. Animals that experience SE-induced by either KA or Pilo are likely to go on to develop spontaneous seizures and become epileptic (Hellier et al, 1998). Chemoconvulsant-induced neuropathology is very similar to TLE-related neuropathology and includes neuronal loss, gliosis, mossy fiber sprouting and synaptic reorganization.

Chemoconvulsant TLE models offer advantages for studying cognitive dysfunction associated with epilepsy. First, use of animal models allows for the identification of potential mechanisms of seizure-induced cognitive damage. Secondly, the chemoconvulsant models allow preclinical testing of therapeutic candidates for cognitive dysfunction occurring in acquired epilepsy (Brooks-Kayal et al., 2013). Additionally, chemoconvulsants such as Pilo, mimic long-term neurochemical and behavioral changes occurring following exposure to chemicals such as nerve agent and/or metabolic poisoning (Jett, 2012). In fact, Pilo has been used as a surrogate of nerve agent neurotoxicity and seizure activity plays a mediating role in its long term toxicity. Therefore the use of chemoconvulsant models to study seizure-induced cognitive dysfunction may yield therapeutic targets beyond TLE.

Behavioral testing in seizure-prone animals represents a series of unique challenges to both selection of paradigms and timing of task performance. Some of the most commonly used paradigms to evaluate learning and memory include the Morris Water Maze (MWM), the Radial Arm Maze (RAM), Contextual Fear Conditioning (CFC), and Delayed Non-Matching to Sample (DNMS). These paradigms have been used to reveal learning and memory deficits in various models of epilepsy including chemoconvulsant-induced SE models, kindling models and some genetic models. However, the use of these tasks to screen for targeted therapies against cognitive deficits associated with the epilepsies may be problematic. First, the use of aversive techniques (water, shocks, and food restriction) to spur performance in these paradigms introduces a possible confound of stress. Furthermore, any drug that affects stress responses or anxiety levels may improve performance and be

interpreted as improving learning and memory, when it simply functions as an anxiolytic. It is therefore ideal to test cognitive outcome on multiple tasks to get an accurate idea of learning and memory performance. Secondly, stress is among the most frequently self-reported precipitants of seizures in patients with epilepsy (Frucht et al., 2000; Spector et al., 2000; Nakken et al., 2005; Haut et al., 2007; Sperling et al., 2008), so it stands to reason that behavioral tasks that elicit stress may induce seizures in animals with a lowered seizure threshold. It is therefore ideal to include in the cognitive testing battery, tasks that are low-stress to get an accurate idea of learning and memory performance.

The current study tested the effects of two chemoconvulsants, KA and Pilo, on learning and memory performance using two minimally stressful variants of the novel object recognition paradigm, the novel object recognition task (NOR) and the novel placement recognition task (NPR). We also measured locomotion and anxiety-related behavior in the open field in order to assess these parameters during the latent period and also to determine if these factors could account for differences in learning and memory. Finally, we employed the Morris Water Maze (MWM) to allow for comparisons between memory paradigms.

2. Materials & Methods

2.1. Animals

Male Sprague-Dawley rats (250–300g) were purchased from Harlan Laboratories (Indianapolis, Indiana). Upon arrival, animals were housed two per cage in static clear polycarbonate cages with wire bar lids and microisolator air filtration covers. Animals had *ad libitum* access to both food and filtered water. Room conditions were maintained at 21° C with a 14:10 light/dark cycle. Animals were treated in accordance with NIH guidelines and all protocols were approved by the IACUC of the University of Colorado Denver.

2.2. Chemoconvulsant Injections

All animals were handled for approximately 2 minutes per day starting a week before treatment both to accustom the animals to the investigator and to potentially reduce any stress associated with handling on subsequent testing days. On the day of treatment, animals were randomly assigned to either the control or experimental (KA or Pilo) group. The experimental groups were administered injections of the chemoconvulsants kainic acid (KA, 11 mg/kg, subcutaneously; s.c. Sigma Aldrich) or Pilocarpine Hydrochloride (340 mg/kg, s.c. Sigma Aldrich) in buffered PBS. Animals treated with Pilo were injected with scopolamine (1mg/kg, IP) 30 minutes prior to Pilo to limit peripheral cholinergic effects and diazepam (10mg/kg, IP) 90 minutes after Pilo to terminate SE (as is the standard of procedure for a cholinergic agonist to reduce mortality). Control animals for the Pilo group were injected subcutaneously with scopolamine, and equal volumes of saline in place of Pilo and diazepam. Control animals for the KA group received a single injection of saline in place of KA. After treatment, the experimental group was observed for the characteristic progression into SE. Chemoconvulsant administration typically elicits seizures of increasing severity starting with staring and wet dog shakes and then progressing into unilateral forelimb clonus, bilateral forelimb clonus before reaching the most severe seizures which result in rearing and loss of balance (Tremblay et al, 1984; Sperk, 1994). Ultimately,

administration of chemoconvulsants results in a period of continuous seizure activity for at least 30 minutes known as SE. Only animals that progressed into SE as defined by 5 seizures resulting in bilateral forelimb clonus and loss of balance within an hour (Racine, 1972) followed by a period of continuous seizure activity were included in subsequent studies. The day following treatment, all animals received a 1ml subcutaneous injection of saline to help prevent any dehydration the animals treated with chemoconvulsants may have experienced. Treatment occurred at 77 days of age and is considered day zero for the behavioral testing timeline. All animals were observed, yet left undisturbed for a recovery period of 3 days.

2.3. Behavioral Testing

2.3.1 Apparatus and stimuli—Behavioral testing apparatus consisted of two identical behavioral arenas constructed of mat black expanded PVC (70cm × 70cm; wall height = 47.6 cm). A false floor made up of four removable PVC pieces were used to facilitate multiple spatial arrangements of the objects. The stimulus objects consisted of vinyl dog toys (Lil' Buddies) that varied in color, shape, and texture but were of similar size and constructed of the same material. Ten unique toy types were used across testing days allowing for one toy type (or two in the case of the novel object recognition task) to be used on a particular test day and not be used again throughout testing for that particular animal. A pilot study of the objects, performed with a separate cohort of animals, showed that no specific object elicited more investigation than any other. To prevent objects from being dislodged by the animal, the objects were zip-tied to inverted jars that screwed firmly into place on individual segments of the false floor. Stimulus objects were cleaned between all tasks with a disinfectant (70% isopropyl alcohol) and an odor remover (Nature's Miracle). The behavioral arena was cleaned between all tasks with a non-toxic deodorizing solution (Simple Green).

2.3.2 Experimental Design—In order to assess the learning and memory profile of animals treated with chemoconvulsants compared to untreated controls, we utilized a battery of behavioral paradigms. Testing was performed on consecutive days across a time span of about one week. After recovery from chemconvulsant-induced SE, animals were acclimated to testing parameters through conditions approaching the experimental paradigms that were to be used as highlighted in Figure 1. Specifically, animals were first exposed to the dedicated behavioral suite where the entirety of the study was performed (room acclimation). On the next day, animals were tested in an open field for a period of ten minutes in order to gage locomotor activity and indices of anxiety, this also served as the initial acclimation to the behavioral arenas. The following day, stimulus objects were placed into the arenas and the animals were allowed to acclimate to the objects. Specifically during this “object investigation”, animals investigated two identical objects for a period of ten minutes before being placed back into their home cages. Objects used during the toy acclimation were not used again in any later testing. The four days following the toy acclimation, animals were subjected to learning and memory testing. The behavioral testing consisted of one task per day alternating between novel placement recognition (NPR) and novel object recognition tasks (NOR) and two delay lengths, 5 minutes and one hour. A separate cohort of animals was used for the NOR task at the 24 hour delay (NOR24) and

Morris Water Maze tasks both to reduce the likelihood of carryover testing effects and to ensure that all tasks were performed within the latent period of epileptogenesis.

2.3.3. Procedure—All behavior testing occurred at about the same time each day (i.e. 9:00am- 3:00pm) and after a period of at least one hour to allow the animals to acclimate to the room. Test order was randomized for each day of testing using a random number generator (random.org) and the arena tested in was counterbalanced across days for each animal. Whether the novel stimulus object for the NOR task was presented on the left or right side of the behavioral arena was counterbalanced for each animal. All behavioral testing occurred during the light phase of the light/dark cycle by a single investigator blind to group assignment.

2.3.4. Novel Placement Recognition Task—Briefly, this task consisted of two phases, a learning phase and a memory phase (Figure 2). During the learning phase, animals were placed into the behavioral arena for a period of 5 minutes and allowed to explore two identical stimulus objects before being placed back into the home cage. After a delay, the animals were placed back into the arena where they had the learning phase with the same stimulus objects, except during the memory phase, one of the objects was displaced to a novel spatial location. Cognitively intact animals should notice this change and spend more time investigating the displaced object as opposed to the object in the familiar location. Previous studies have shown that this task is hippocampal-associated (Mumby et al, 2002; Dix & Aggleton, 1999; Mumby, 2002). This task was performed once at a delay length of 5 minutes (NPR5) and again at a delay length of one hour (NPR60).

2.3.5. Novel Object Recognition Task—Similar to the NPR task described above, the novel object recognition task (NOR) also consisted of a learning phase and a memory phase (Figure 2). The task was performed exactly as described above with the exception of during the memory phase, rather than the target object being displaced to a novel spatial location, it was replaced with an entirely new stimulus object. Again, animals should notice this change and spend more time investigating the novel object as opposed to the familiar object. Previous studies have shown that this task is not strictly hippocampal- dependent, but also depends on an intact perirhinal cortex (Mumby et al, 2002; Dix & Aggleton, 1999; Mumby, 2002). As with the NPR trials, NOR was performed first at a delay length of 5 minutes (NOR5) followed by a delay length of one hour (NOR60). A separate cohort of animals was used for the 24 hour delay and the MWM.

2.3.6 Morris Water Maze—The water maze consisted of a tank approximately 6 feet in diameter and 2 feet deep. The water (room temperature) was colored with non-toxic black tempura paint to obscure a plexiglass platform that was located within the top left quadrant of the tank. Animals were placed into the tank from various positions that were randomized across trials for each day. Surrounding the tank were various cues for navigation including markings on each wall and three-dimensional objects hanging above the maze, all within eyesight of the animals. Each animal performed 4 trials per day for 5 days. A trial consisted of an animal being gently placed into the tank facing the inner wall. The animal was allowed to swim freely for 2 minutes or until it reached the platform where it was allowed to rest for

15 seconds. If the animal had not reached the platform in the allotted time its latency was noted as 120 seconds and it was gently guided to the platform and allowed to remain for 15 seconds. Latency to reach platform was measured and quantified using automated behavioral software (TopsScan, Clever Sys, Inc., Reston, VA) over trials and days as an indicator of spatial learning. Twenty-four hours after the last trial on day 5, a probe test was performed to assess spatial memory. Time spent in the target quadrant (that formerly held the platform) was measured over a 30 second period.

2.3.7. Behavioral Analyses—All tasks were video recorded and behaviors of interest were quantified using TopsScan behavior recognition software (Clever Sys. Inc, Reston, VA). Behavior parameters quantified for each task included: locomotor measures such as distance traveled and velocity within the arena (open field, NOR, NPR), and investigatory measures such as time spent sniffing the stimulus objects and latency to sniff either object (NOR, NPR). Investigation of the stimulus objects was recorded as both frequency and duration and was defined as any instance in which the animal's nose was oriented within at least 4 mm of the object. From these measures, the proportion of total visits to the novel object and the proportion of total time spent investigating the novel object was calculated (novel/(novel + familiar investigation)). Previous studies have found that object novelty quickly diminishes during the recognition phase (Dix & Aggleton, 1999; Mumby, 2002), so our analyses were focused on the first 30 seconds of the recognition phase in learning and memory testing. Data presented from the open field task and toy acclimation are the full ten minutes in order to ensure that there were no differences in locomotor activity that could explain deficits in toy investigation during the learning and memory tasks. MWM data was analyzed for latency to platform and swim speed/distance.

2.3.8. Statistical Analysis—Open field data were analyzed using one-factor ANOVA to assess the effect of chemoconvulsants on locomotion and indices of anxiety. NOR and NPR tasks were analyzed using a two-factor repeated measures ANOVA using treatment group as the independent variable and task delay length as the repeating variables. For the MWM, animals performed 4 trials per day for 5 days and performance each day was averaged and analyzed by means of a two-way repeated measures ANOVA with treatment groups and serving as the independent variable and day as the repeating factor. Significant main effects and interactions were probed using Bonferroni post-tests. Differences were considered significant when $p < 0.05$. All statistical analysis were performed using GraphPad Prism 5. All data are expressed as mean $\pm$ SEM.

3. Results

3.1. Open Field

To exclude the possibility that differences in mobility and anxiety could account for differences in exploratory behavior, these parameters were initially examined in an Open field task but monitored throughout testing. Interestingly, total distance traveled within the open field arena was significantly different between groups [Fig. 3a; $F(1,27) = 8.853$, $p = 0.0012$] such that KA-treated rats were slightly hyperactive relative to control [$p < 0.05$], however, velocity within the arena was similar across groups [Fig. 3a; $F(1,27) = 1.879$, $p =$

0.1737]. This difference in total distance traveled did not persist after the open field task, specifically the following day, all animals were acclimated to objects and locomotion during this task, as measured by total distance traveled was not significantly different between groups (data not shown). Additionally, indices of anxiety including frequency and duration of time spent in the center of arena was not significantly different between groups [Fig 3; $F(1,27) = 0.084$, $p = 0.9201$ and $F(1,27) = 1.779$, $p = 0.1894$]. Latency to the center of the arena was also not significantly different between groups [Fig 3; $F(1,27) = 0.2048$, $p = 0.2048$]. Interestingly, both Pilo [$p < 0.001$] and KA groups [$p < 0.05$] had significantly reduced instances of stretch-attend behaviors [Fig. 3; $F(1,27) = 9.271$, $p = 0.001$] which are defined as instances when the animal stretches to sniff the center of the arena while the body is located in the perimeter, these postures are commonly associated with risk assessment (Pinel & Mana 1989). Risk assessment behaviors such as stretch attends are investigatory in nature and allow the animal to gain information about a potential threatening environment (Pinel & Mana 1989). Taken together, these data indicate animals treated with chemoconvulsants do not have any differences in locomotion that may account for differences observed in the learning and memory tasks. Additionally, no differences were observed in measures of anxiety related behavior i.e. frequency and duration in the center of the arena between chemoconvulsant -treated animals and controls, suggesting that differences in the performance of the learning and memory tasks are not due to differences in anxiety. Interesting, the data do suggest that risk assessment behavior may be reduced in animals that experienced SE compared to controls.

3.2. Novel Placement Recognition

To evaluate predominantly hippocampal dependent spatial memory in chemoconvulsant animals, a novel object placement task was performed at two delay lengths: five minutes and one hour. Performance on this task varied depending on treatment group [$F(2, 23) = 10.87$, $p = 0.0005$]. Specifically, KA-treated rats exhibited a lower novel object preference ratio at both delays [$p < 0.05$]. A significant decrease in the proportion of time spent investigating the novel location is suggestive of deficits in spatial memory. Finding deficits at both time points is indicative of significant and persistent deleterious effects of KA-induced TLE on spatial memory. Interestingly, this deficit was not observed in the Pilo treated animals at either delay length.

3.3. Novel Object Recognition

In order to evaluate predominantly perirhinal cortex-associated recognition memory, animals were subjected to a novel object recognition task at three delay lengths; five minutes, one hour and 24 hours. Memory performance on these tasks, again varied as a function of treatment group [Fig. 5; $F(2,50) = 10.53$, $p = 0.0005$]. Post-hoc analysis revealed significant differences between control and KA groups at the one hour delay [$p < 0.05$] and at the 24 hour delay, both KA and Pilo groups were significantly different than control [$p < 0.01$]. Effect of delay length trended towards significance at $p = 0.063$. These data highlight the difference in memory performance between chemoconvulsant models. Specifically, recognition memory appears to be intact after a short retention time in both chemoconvulsant models, however longer delays may be required to reveal deficits in this type of memory.

3.4. Morris Water Maze

To determine the extent to which spatial learning and memory was impaired in chemoconvulsant treated animals compared to controls, animals were tested in the Morris Water Maze (MWM). Latency to reach the submerged platform varied as a function of treatment [Fig. 6A; $F(1, 88) = 69.07$, $p < 0.0001$] and day [Fig. 6A; $F(1, 88) = 25.6$, $p < 0.0001$; Interaction $F(1,88) = 7.771$, $p < 0.0001$]. Post-hoc analysis revealed a significant effect of KA treatment on latency to reach the platform on days 2, 3, 4 and 5 and for a significant effect of Pilo treatment for animals on all days tested. This significantly greater amount of time to reach the platform than controls is indicative of deficits in spatial learning. When tested in a probe trial for spatial memory 24 hours after the last trial on day five, significant differences were detected between groups in the amount of time spent searching the target quadrant [Fig. 6B; $F(1,22) = 3.763$, $p = 0.0393$], suggesting that treatment with chemoconvulsants affects spatial memory in this task. Interestingly, animals injected with KA or Pilo but not experiencing SE showed deficits similar to saline-treated controls (data not shown), suggesting a role for SE as the initiating event in mediating cognitive deficits. These data suggest that chemoconvulsant-induced SE, regardless of the type of chemoconvulsant, significantly and deleteriously affects spatial learning and memory.

4. Discussion

The primary goal of this study was to characterize the learning and memory performance of chemoconvulsant-treated animals. Two mechanistically distinct chemoconvulsants, KA and Pilo, were chosen to initiate TLE and to reveal treatment-induced memory deficits. Two variants of the novel object recognition test were employed, the NOR and NPR tasks, both of which are minimally stressful. By employing multiple delay lengths in both NOR and NPR tasks, we were able to test both short-term and long term memory on each of these tasks. Spatial memory was also evaluated using the MWM, allowing for comparison of performance between two distinct memory paradigms.

Both KA and Pilo animals exhibited significant deficits on the long delays of the NOR task suggesting that exposure to such chemoconvulsants results in impaired long-term recognition memory. Interestingly, short-term recognition memory (i.e. five minute delay) was spared in both chemoconvulsant models on the novel object recognition task (NOR). Additionally, both Pilo and KA groups exhibited spatial learning deficits in the MWM, with Pilo animals being virtually unable to learn the task. Despite the significant impairment in the MWM, Pilo-treated animals exhibited above chance novelty preference ratios for the novel spatial location indicative of intact spatial memory on the NPR task. It is possible that cessation of Pilo-induced SE with diazepam may have protected against deficits on this task but to prevent excessive mortality, the use of diazepam is typically the standard. KA-treated animals on the other hand exhibited significantly reduced novelty preferences relative to controls, indicative of spatial memory impairment. These data suggest that the NPR task may be effective in revealing spatial memory deficits in animals treated with KA but not animals treated with Pilo. These results are not due to differences in seizure susceptibility because all animals went on to develop spontaneous seizures at equivalent observed frequency.

The finding of Pilo animals exhibiting intact spatial memory on the NPR but not the MWM is of interest, particularly given the recently emerging interest in evaluating therapies in models of organophosphate neurotoxicity. It is possible that the stress associated with the MWM, namely the shock of swimming, compromises memory performance for that task. Indeed, a common technique adopted by Pilo treated rats during MWM testing was thigmotaxis (wall hugging) which can indicate increased anxiety and fear (Treit & Fundytus, 1988). This behavior occurred even when the platform was visible, raising concerns about the ability of the animals to participate in the task. Thigmotaxis and other indices of anxiety were not observed in the open field task performed just days before the MWM indicating that it is specific to the swimming task. It may therefore be beneficial to test spatial learning and memory in various tasks, including the NPR task to get an accurate picture of Pilo-induced learning and memory deficits.

Our findings corroborate and extend previous investigations of KA- and Pilo- induced learning and memory deficits, however, to our knowledge, this is the first report of both NOR and NPR testing at multiple delay lengths in KA and Pilo treated animals. Other groups have reported KA-induced spatial memory deficits at delays as short as 2 and 3 minutes on the NPR task performed once the animals are chronically epileptic (Chauviere et al 2009, and Gobbo & O'Mara, 2004). Given that our studies were performed early after SE when seizures are less frequent, our findings extend the literature, showing that these deficits are present before frequent, chronic seizures begin. Identification of a task that reveals memory deficits at a time point when the likelihood of seizure occurrence is low, allows for the study of co-morbid cognitive impairment without the additional confound of persistent seizure activity.

The model- and delay-dependent effects revealed here may help to clarify some of the discrepancies in the literature regarding memory performance of chemoconvulsant-treated animals on the NOR task. For example, Chauviere et al (2010), reported intact recognition memory in Pilo-treated rats on a variation of the NOR task, but recognition memory testing was performed after a 2-minute delay. Our data would suggest a longer delay length may be required to reveal recognition memory deficits in the Pilo model. Another study, Detour et al, (2005) failed to find significant deficits in the NOR task after a 24-hour delay in the Lithium-Pilocarpine model of epilepsy. Given that we found significant deficits on this task at the 24-hour delay in both the KA and Pilo models, recognition memory may be differentially affected depending on the particular SE induction model of epilepsy employed. This is not surprising given that different SE induction models can result in different patterns of neurodegeneration. It is possible that the perirhinal cortex, the brain region that governs recognition memory, may be less affected in the lithium-Pilo model which may account for the observed discrepancies between the models.

Interestingly, other studies testing performance in the open field task have found chemoconvulsant treatment to induce hyperactivity (Gobbo & O'Mara, 2004) and decrease indices of anxiety (Inostroza et al, 2012). The present study found locomotion and anxiety indices of chemoconvulsant treated animals to be similar to those of control animals with no evidence of hyperactivity or changes in anxiety levels. This difference in findings is likely attributable to testing during the early phase of epileptogenesis versus testing once chronic

spontaneous seizures have occurred. The lack of differences in locomotion and indices of anxiety presents a unique opportunity to test for learning and memory deficits without concomitant changes in other indices that might contribute to poor performance on learning and memory tasks confounding interpretation.

In conclusion, while learning and memory deficits have been reported in both KA and Pilo treated animals during the chronic phase of epileptogenesis, fewer reports have tested cognition early after SE. The data presented here demonstrate that deficits in spatial memory are task dependent in the Pilo model and globally impaired in the KA model. Additionally, both KA and Pilo models exhibit impaired long-term recognition memory. Future studies can use this information to study cognitively-targeted therapeutics in these models and should future studies chose to utilize the NOR and NPR tasks, we have shown the delay lengths were deficits are present.

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Highlights

The novel object recognition paradigm can be used to reveal deficits in chemoconvulsant-induced SE models.

KA-treated animals but not Pilo-treated animals are impaired on the Novel Placement task.

Both KA- and Pilo-treated animals exhibit deficits in long-term recognition memory.

Both KA- and Pilo-treated animals are impaired in the Morris Water Maze test of spatial learning and memory.

Figure 1.

Schematic of behavioral testing schedule. NPR- Novel placement recognition; NOR- Novel object recognition; MWM- Morris water maze

Figure 2.
Schematic of novel object testing paradigm

Figure 3.

Open Field Performance of KA and Pilo treated animals compared to controls. Significant differences were detected between groups on measures of locomotion (A) as measured by total distance traveled, KA treated rats were hyperactive but (B) velocity was not different between groups. Indices of anxiety including the duration of time (C) and frequency of visits to the center of the arena (D) were equivalent between groups. A common indices of risk assessment (E), stretch-attend behaviors were decreased in both KA and Pilo treated animals relative to controls however, latency to the center of the arena was not different between groups. Control, $n = 10$; KA $n = 9$; Pilo, $n = 7$. Data expressed as mean $\pm$ SEM. Asterisk (***) denotes a significant interaction ($p < 0.001$) between groups, Pound sign (#) indicates a significant difference ($p < 0.05$) from control.

Figure 4.

Effects of chemoconvulsant induced SE on novelty preference ratios on novel placement recognition trials. At the five minute delay, KA treatment significantly reduced the novelty preference ratios compared to control, while treatment with PILO was not significantly different from control. At the 60 minute delay, a similar pattern was observed, treatment with KA significantly reduced novelty preference ratios compared to controls, while treatment with Pilo resulted in preference ratios not significantly different from control. Control, $n=10$; KA, $n=9$; Pilo, $n=7$. Data expressed as mean $\pm$ SEM. Asterisk (*) denotes a significant difference ($p < 0.05$) between groups.

Figure 5.

Effects of chemoconvulsant induced SE on novelty preferences in the novel object recognition tasks at different delay lengths. Chemoconvulsant treatment did not significantly affect performance at the five minute delay length for either KA or Pilo groups. At the 60 minute time point, treatment with KA significantly reduced novelty preferences however Pilo treated animals were not significantly different from controls. At the 24 hour delay, both KA and Pilo significantly reduced novelty preferences relative to control animals. Control, $n=10$; KA, $n=9$; Pilo, $n=7$. Data expressed as mean $\pm$ SEM. Asterisk (*) or (**) denotes a significant difference ($p < 0.05$ or $p < 0.01$, respectively) between groups.

Figure 6.

Effects of chemoconvulsant induced SE on performance in the Morris Water Maze. (A) Both KA and Pilo significantly affected latency to find a submerged platform using spatial cues. (B) A probe test for spatial memory revealed a significant impairment in Pilo and KA treated rats compared to control. Control, $n=10$; KA, $n=9$; Pilo, $n=7$. Data expressed as mean $\pm$ SEM. Asterisk (***) denotes a significant difference ($p < 0.001$) between groups.