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Cognitive deficits in a rat model of temporal lobe epilepsy using touchscreen-based translational tools.

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Abstract

Objective: Cognitive deficits are commonly observed in people with epilepsy, but the biological causation of these is challenging to identify. Animal models of epilepsy can be used to explore pathophysiological mechanisms leading to cognitive problems, as well as to test novel therapeutics. We utilised a well-validated animal model of epilepsy to explore cognitive deficits using novel translational assessment tools – automated rodent touchscreen assays.

Methods: To induce epilepsy, adult Wistar rats were subjected to Kainic Acid-induced Status Epilepticus or sham control (n=12/group). Two months following induction, animals underwent the Pairwise Discrimination and Reversal learning touchscreen tasks, novel object recognition and the Y maze test of spatial memory.

Results: In the Pairwise Discrimination paradigm, only 40% of epilepsy animals acquired the discrimination learning criterion, compared to 100% of sham animals ($p=0.003$). Epilepsy and sham animals that successfully acquired the discrimination progressed onto the reversal phase which measures cognitive flexibility. Interestingly, there were no differences in the rate of reversal learning; however, on the first reversal session, Epilepsy rats committed more perseverative errors than shams (6.3 ± 0.9 vs 1.8 ± 0.5 , $p<0.0001$). Additional behavioural analysis revealed Epilepsy rats were significantly impaired in novel object recognition and short-term spatial learning and memory.

Significance: Using translationally relevant behavioural tools in combination with traditional assays to measure cognition in animal models, here we identify impairments in learning and memory, and enhanced perseverative behaviours in rats with epilepsy. These tools can be used in future research to explore biological mechanisms and treatments for cognitive deficits associated with epilepsy.

Keywords: Cognitive behaviour, translational touchscreens, seizures, temporal lobe epilepsy, animal model.

Highlights:

- Cognitive deficits are commonly observed in patients with epilepsy, but their biological causation is mysterious, and no treatments exist
- Here we examine cognitive deficits in an animal model of epilepsy utilising translational touchscreen technology

- Animals with epilepsy exhibit deficits in visual discrimination learning, and enhanced perseverative errors
- Touchscreen technology provides an opportunity to study the biological basis of cognitive disorders in epilepsy.

Introduction

Epilepsy is a chronic debilitating neurological condition, which is characterised by the occurrence of spontaneous seizures. Current medications act to suppress neuronal excitability and reduce the likelihood of seizures. But the disease of epilepsy is far greater than just seizures: patients frequently experience psychiatric and cognitive comorbidities which have major impacts on patient's quality of life ¹⁻³. Although cognitive deficits can exist prior to the onset of epilepsy ^{4, 5}, cognitive decline occurs over time and is related to the frequency of seizures in patients ⁶, intimating that the epileptic state itself contributes to cognitive impairment. Chronic exposure to anti-epileptic medications can also cause cognitive disturbance in epilepsy patients ⁷, and many other epilepsy variables can also impact cognition, such as seizure type, age of onset, seizure frequency, and associated neuropathology ⁸. The cognitive domains impacted in patients are very broad, particularly patients with temporal lobe epilepsy, and can include impairment in learning ^{9; 10}, memory ^{11; 12} and executive function ^{13-15; 5; 9}. Despite these well-recognised and common features of epilepsy, little is known about the causes of cognitive and psychiatric disorders in epilepsy, and to date there are no targeted therapies which can improve these comorbidities.

Animal models of epilepsy can be utilised to begin to address some of these knowledge gaps ¹⁶. With respect to cognitive function, many rodent epilepsy models exhibit impairments in a range of cognitive domains, suggesting that these have a biological causation ¹⁷. **For example, models of temporal lobe epilepsy, such as the post-Status Epilepticus models, exhibit deficits in a variety of cognitive domains. These include, but are not limited to, tasks of spatial learning and memory ¹⁸, non-spatial learning and association tasks such as novel object recognition ^{19; 20}, social memory ²¹, and fear-associated learning ²², as well as executive functions including attention ²³ and working memory ²⁴. However, many previous animal studies of epilepsy have limited their assessment to spatial learning and memory using the Morris water maze. While especially popular for understanding mechanisms of spatial learning and memory, it is possible that rodents with epilepsy may appear to be impaired in such tasks, for example due to**

excessive stress responses²⁵ caused by the environment, or motor impairments, thereby giving false positives. In addition, we have anecdotal observations from our laboratory of ‘barrel-rolling’ and diving of epileptic rats in this task, which could also confound outcomes. Therefore, there is a pressing need to develop advanced behavioural tests which avoid some of these common issues and assess specific higher order cognitive function in animal models of epilepsy. Ideally, these would encompass several cognitive domains using tasks analogous to human cognitive testing, bridging the translational divide between humans and rodent models.

To make the first attempts at this in a rat model of epilepsy, here we used the rodent touchscreen technology with automated cognitive tasks²⁶, which have strong face validity to human cognitive test settings. Indeed, several of the tasks which can be used are analogous to the human equivalent²⁷. We first were interested to observe whether epileptic rats could perform the operant-based touchscreen task, and then examined a test of visual discrimination learning followed by reversal learning to assess cognitive flexibility.

Materials and methods

Animals

Adult male Wistar rats (9-10 weeks old) were obtained from the Australian Resource Centre (Perth, Australia). Upon arrival to our laboratories in the Melbourne Brain Centre, University of Melbourne, rats were individually housed and given one week to habituate. Rats were gradually shifted to a reverse phase light cycle (lights on from 9 p.m - 9 a.m) over 2 weeks. Throughout the study, rats were given free access to food and water, except during touch screen testing when food availability was restricted. All experimental procedures received approval from the Florey Animal Ethics Committee (#17-015).

Induction of Status Epilepticus

Rats were gently handled for two days prior to induction of Status Epilepticus (SE), which was achieved via systemic administration of Kainic Acid **using the Hellier protocol**²⁸ (KA – Tocris Biosciences), as previously described^{29; 30}. **We chose to use this protocol in rats since the epilepsy (diagnosed by recurrent spontaneous seizures) develops reliably in our hands, and repeated low dose limits (although does not prevent entirely) overdose of KA, which is a concern if large single doses are delivered. Also, we preferred to use this model over amygdala kindling, since even after many stimulations and seizures, animals rarely develop spontaneous seizures, and true epilepsy.** Rats (n=17) were repeatedly

injected with low dose KA, starting at 5mg/kg ip with subsequent doses of 2.5 – 5mg/kg every 30-45 minutes if the animal had yet to enter SE. Animals were closely monitored after each injection for the initial signs of SE, including staring, head nodding, hyperactivity, searching, wet-dog shakes (i.e.: Racine scale I to II seizures³¹). If any of these behaviours were observed, the time interval between injections was extended to 45 mins, and KA dosage also reduced. Seizure behaviour progresses to include repeated wet-dog shakes - the hallmark of the developing SE in rats. These become almost continuous, and are followed by limbic motor seizures: forelimb clonus (Racine III), rearing (Racine IV), rearing and falling (Racine V), and ‘popcorn’ seizures (Racine VI). Eventually SE develops, where seizures become continuous incorporating the different Racine classes for more than 30 mins. Status Epilepticus was terminated 4 hours after onset (defined as the time of the first Class IV/V seizure) with diazepam (5mg/kg i.p.). Additional doses of diazepam were delivered if necessary when animals re-entered SE. **Table 1 indicates the number of KA and diazepam injections, as well as the severity of the SE.** One rat did not exhibit signs of SE after four doses of KA, and was excluded from the study. Animals were individually housed after SE, and received saline injections and pain relief (Rimadyl, 5mg/kg) if warranted. Dietary supplements aided recovery, including mashed food, honey and sugar water for the following 3-5 days. Mortality rate was 24% over the first week, due to either unexplained death (n=1), or unsatisfactory improvement in health, leading to euthanasia (n=3). Animals which survived SE were termed Epilepsy rats (n=12). Sham control rats (n=12) were injected with equivalent dose of vehicle (saline) and one dose of diazepam. These rats were enrolled in the touchscreen study, but subsequent to this, one other Epilepsy rat was euthanised, leaving 11 Epilepsy rats to be enrolled in the other behavioural tests.

Touchscreen test for Pairwise Visual Discrimination and Reversal Learning

Apparatus

Behavioural experiments were conducted in automated touchscreen-based operant systems for rats (Camden Instruments Ltd., UK) as previously described^{32; 33}. Briefly, the operant chamber is trapezoidal (30cm in height × 33cm in length) in length, comprising of black plastic walls that connect to a touchscreen (38cm wide, screen resolution 1024 × 768) at one end and a reward magazine attached to a pellet dispenser at the other. Rewards were 45mg dustless sugar pellets. The entire unit is housed within a sound-proofed and ventilated fibreboard box (51cm × 60cm × 72cm) equipped with a fan, tone generator and house light. A mask partially covered the touchscreen to confine access to only two response windows

where stimuli are displayed for visual discrimination. Each of the response windows is fitted with infrared beams, which are broken and detected when the rat nose-pokes that window. The operant chamber and touchscreen task related inputs/outputs were controlled by Whisker³⁴ and ABET II Touch Software (Campden Instruments Ltd., Loughborough, UK).

Pretraining for Operant Conditioning

Four weeks after SE, a restricted food diet was introduced to maintain rats (n=12/group) at 85-90% of their free feeding weight (water available *ad libitum*). **This restricted diet was maintained for the duration of the touchscreen study, and rats were returned to an *ad libitum* diet on conclusion of this.** Pretraining for the pairwise discrimination (PD) task allowed rats to accustom to the operant chambers, and involved various stages that facilitated appropriate screen touching behaviour. Pretraining for the PD task has been described previously in detail³². In brief, rats were incrementally taught to: 1) associate stimuli presented on the touchscreen with reward delivery, 2) nose-poke stimuli to receive reward, 3) initiate stimulus presentation by breaking an infrared beam at rear of chamber and finally, 4) to avoid touches to non-illuminated response windows. Rats had to complete 50 trials in 60min at 80% correct percentage accuracy on two consecutive days of training to advance to the learning task. Two Epilepsy rats failed to achieve this criterion during pretraining (consistently insufficient trials completed) and were excluded from further touch screen testing.

Pairwise Discrimination (PD) task - discrimination and reversal paradigm

The PD task is a two-choice visual discrimination task comprising of an acquisition and reversal learning paradigm. The protocol used in this study was a modified version of previous studies^{32, 35}. During the discrimination phase, a pair of stimuli (an aeroplane and a spider) was presented together on two response windows of the screen, one being the correct (rewarded) stimulus (S+) and the other the incorrect (S-). A correct response via nose poke resulted in illumination of the reward magazine light, delivery of a tone and reward pellet. Incorrect responses were punished with a time out with house light on and an ITI of 20s followed by a correction trial. Correction trials ensued until a correct response was made. Stimuli were presented randomly in the two response windows. Animals had to complete 60 trials in a 60min session (excluding correction trials)³⁵. Once they reached criterion (80% percentage correct accuracy on two consecutive days), animals were advanced to the reversal paradigm. Animals were given a maximum of **30** sessions to achieve criterion.

To assess reversal learning, the stimuli were reversed such that the stimulus previously identified as the unrewarded stimulus now became the rewarded response, and

vice versa for the rewarded stimulus. All rats that advanced to reversal were given 15 sessions on consecutive days (60 trials per 60 min session) each. Latency to response, percentage correct response accuracy and perseverative index (incorrect responses/number of correction trials) were calculated to determine performance for both acquisition and reversal phases.

Y Maze test of short term spatial memory

This task was commenced 2 weeks after completion of the touchscreen studies, when all animals had returned to their baseline weights. The Y maze was made of acrylic and beige in colour comprising of three arms in the shape of a Y. Each arm of the maze was identical in size: 4 inches (w) × 22 inches (l) × 5 inches (h) (San Diego Instruments). Spatial cues were positioned at the end of each arm within the maze. During the training trial, each rat was placed at the end of one arm (home) and allowed to explore two arms (termed the home and familiar arms) of the Y maze for 10 mins, with the third arm (novel arm) blocked. Following an ITI of 1h, the test trial began. Rats were placed in the home arm, and allowed to explore all three arms. The number of entries and time spent in each arm were calculated using the software TopScan (CleverSys Inc). Odour cues were minimized by spraying and wiping down the arms with 80% ethanol between trials and animals. Two Epilepsy rats repeatedly jumped out of the maze and were excluded from the analysis.

Novel Object Recognition test

The NORT testing protocol consisted of three phases: habituation, familiarisation and testing³⁶. During habituation, rats were allowed to explore an empty square arena (60 x 60 cm, grey acrylic) for 10 mins. Following habituation, the familiarisation phase was initiated, where rats were placed in the arena with two identical objects and allowed to explore for 5 mins. Objects used were a plastic water bottle and a transparent reagent bottle with a blue cap. Objects were placed on their sides, equidistant from the side walls. Rats were returned to their home cage for 1h, and then the test phase began. For this, one of the two familiar objects was removed and replaced with a novel object. Rats were allowed to explore the familiar and novel object for 5 mins during the testing phase. Exploration time was defined by the snout being within 1-2 cms of the object or touching the object. The number of times an object was visited and time spent exploring the novel object (T_N) and familiar object (T_F) was calculated using the TopScan (CleverSys Inc) software. The arena and objects were wiped down with 80% ethanol between trials and animals to ensure odour cues were minimized. The Discrimination

Index (DI) was calculated as $[DI = (T_N - T_F) / (T_N + T_F) \times 100]$ to assess preference for novel or familiar object, wherein a positive score was indicative of a preference for the novel object, a negative score indicative of preference for the familiar object and zero score for a null preference. Three Epilepsy rats had seizures during the testing trial and were excluded from the analysis.

EEG Electrode Implantation Surgery and video EEG monitoring

Following behaviour testing, and 4 months following SE, Epilepsy and sham rats were implanted with electrodes for video EEG monitoring. Electrodes were surgically implanted as previously described in detail ³⁷, with slight modifications. Briefly, animals were anaesthetised with 2% isoflurane and aligned in a stereotaxic frame. Following a midline scalp incision, six small burr holes were made in the skull ensuring that the dura was not breached, through which the electrodes (Plastics One, Bioscientific, Australia) were implanted and held steadily in place with acrylic dental cement (Henry Schein Halas, Australia). Animals (n=12 sham and n=11 Epilepsy) were implanted with 4 epidural electrodes - 2 to serve as reference and ground and 2 as active electrodes implanted bilaterally over the somatosensory cortex (± 2 mm anterior/posterior, ± 2 mm lateral to bregma, and 2 mm anterior, ± 2 mm lateral of lambda). Additionally, two 200 μ m twisted bipolar stainless steel depth electrodes (Plastics One) were positioned into the dorsal hippocampi (-4.6mm posterior from Bregma; 2.6mm lateral from midline; and -3.1mm from the skull surface). One Epilepsy animal did not complete surgery due to difficulties intolerance of anaesthesia. Animals were housed individually and allowed to recover for one week before video-EEG recordings commenced using Compumedics™ EEG acquisition software ³⁸. Animals were recorded continuously for 7d to quantify seizure frequency. Electrographic seizures were then analysed using Profusion EEG 4 software by an observer blinded to group.

Data Analysis and statistics

All group data was first tested for normal distribution – subsequent tests employed either parametric or non-parametric testing. The proportion of animals reaching criterion in the PD task was assessed using Fisher's exact test. **Sessions to criterion was assessed using a Log-Rank survival curve (Mantel-Cox test)**, and latencies were analysed using student's t-tests. **Percentage accuracy within each session and perseveration indices were assessed using mixed models ANOVA for the acquisition phase of the pairwise discrimination task** and

two-way repeated measured for the reversal learning phase. Time spent in the novel arm of the Y maze was compared using Mann-Whitney U test. Distance travelled in the Y maze and discrimination index in the novel object recognition test were compared with student's t-tests. Correlations between seizure frequency and behavioural outcomes were assessed using Pearson's correlation coefficient in Epilepsy animals. All analyses were done using Graph Pad Prism and results were considered statistically significant at $p < 0.05$. Data represented as mean $\pm$ SEM except non-parametric data, which is median $\pm$ range). Where practical, individual data points are included.

Results

Table 2 summarises the number of animals which were entered, and that completed, each procedure in the study.

Epilepsy rats display deficits in discrimination learning

Most Epilepsy rats progressed through the pretraining phase of the touchscreens equally as well as sham animals, indicating that they were able to learn the simple operant conditioning procedures (Figure 1). The primary outcome in pairwise discrimination task (Figure 2) is the rate of learning to reach the criterion. We assessed this in two ways: first, the proportion of rats which achieved criterion, and second, the number of sessions required to achieve this. All sham animals (12/12) successfully reached the discrimination learning criterion, whereas significantly fewer Epilepsy rats did (4/10; $p = 0.0028$, Figure 2A). **Epilepsy rats also acquired criterion at a faster rate than the sham group ($\chi^2 = 7.72$, $p = 0.0055$, Figure 2B).** When comparing the session accuracy over time, the Epilepsy group were significantly delayed in their rate of learning, compared to shams ($p = 0.0011$; Figure 2C). We also measured the perseverative index during discrimination learning, with the Epilepsy rats showing significantly more perseverative responses than shams ($p = 0.043$; Figure 2D). No differences were observed in task latencies, including correct touch, incorrect touch, and reward collection (all $p > 0.05$; Figure 2E), ruling out these factors as confounds. **Also, all animals ate all sugar pellets provided to them during habituation, so coupled with the reward latencies, it appears as though all subjects were sufficiently motivated to perform the task.**

Four Epilepsy rats and 12 shams successfully acquired visual discrimination learning, and progressed to the reversal phase (Figure 3). One of the Epilepsy rats repeatedly failed to complete sufficient trials in these subsequent sessions, so was removed from the analysis.

Reversal learning— a measure of cognitive flexibility – was not significantly different between Sham and Epilepsy rats ($p=0.21$; Figure 3A). Assessing the perseveration index across all sessions of reversal learning revealed no difference between groups ($p=0.96$; Figure 3B). However, on the first day of reversal, Epilepsy rats showed significantly greater perseverative behaviour than shams ($p<0.0001$). No differences were observed in task latencies (all $p>0.05$; Figure 3C).

Epilepsy rats display impairments in short term memory and object recognition

The Y maze task assesses short term spatial memory. Epilepsy rats explored the novel arm of the Y maze significantly less than sham controls in this task ($p=0.0013$; Figure 4A), indicative of spatial memory impairment. There was no difference in distance travelled in the entire maze ($p=0.6$; Figure 4B). Epilepsy rats also showed significant impairment in object recognition memory, exhibiting a significantly lower discrimination index compared with sham animals ($p=0.038$; Figure 4C).

Effects of epilepsy severity on behaviour

All Epilepsy rats exhibited spontaneous seizures during the monitoring period at the conclusion of the behavioural exams (see Figure 5A for an example EEG recording of one seizure), whereas no seizures were identified in shams. Five months after SE, the median number of seizures experienced by each animal per week was 22 (range: 1-119) recorded during the one week monitoring period (Figure 5B). Average duration per animal of these seizures was 44 ± 6 sec (Figure 5C). Seizures were almost exclusively Class IV-V on the Racine scale. Using only Epilepsy animals, we performed Pearson's correlations between the total number of seizures and behavioural performance. A significant inverse relationship was observed between seizure frequency and the novel object recognition task ($r=-0.76$; **$p=0.046$**), such that the greater the epilepsy severity, the worse the performance. There were no other significant relationships observed with other behaviours (all $p>0.05$). However, the ability to complete the acquisition of the visual discrimination task was only achieved by animals with low seizure counts (less than 1/day), but not all animals with low seizure counts were able to complete the acquisition.

Discussion

Animal models are valuable assets which can be used to investigate the biological mechanisms that underlie cognitive impairment in epilepsy. Previous rodent studies on

cognitive dysfunction using the post-SE models have predominantly focussed on long-term spatial learning and memory deficits using conventional behavioural paradigms such as the Morris water maze that are modestly translatable to clinical evaluations of cognitive deficits in epileptic patients. However, tests such as this in which the animal is motivated by aversion to perform the task introduces potential confounds, since elevated stress and anxiety levels could impair performance. In comparison to these thoroughly validated tasks for rodents, translational touchscreen testing offers a less stressful, fully automated environment with minimal experimenter intervention. Here we demonstrate, to the best of our knowledge, for the first time the use of an automated touchscreen paradigm to assess cognition in a rat model of temporal lobe epilepsy.

The first question we asked was whether rats with epilepsy could perform the fundamental operant conditioning (pretraining) in the touchscreens. Two rats could not (18%), but the majority could, and so these continued to the pairwise visual discrimination and reversal learning touchscreen tasks. These allow probing and examination of particular components of higher order cognitive functions ^{26; 35; 39; 40} including visual discrimination, cognitive flexibility, learning and memory ³². **The acquisition phase of the task in particular assesses visual perceptual and associative learning ⁴¹, and relies on the integrity of brain regions such as anterior cingulate and rhinal cortices ^{42; 43}. We found Epilepsy rats as a group displayed a robust discrimination learning deficit and required significantly greater number of sessions during the task acquisition phase. Interestingly, we found a strong inter-individual variability between the Epilepsy rats during acquisition, with the majority of the animals not able to appropriately complete this learning task. The epilepsy model used here exhibits extensive pathology in hippocampus, including atrophy and mossy fibre sprouting ^{44; 45}, which is likely the cause of spatial deficits commonly observed ⁴⁶. But the model also exhibits marked pathology in other areas, including anterior cingulate and rhinal cortex ⁴⁵ which are required for the acquisition of the task. While we did not conduct detailed histological or functional assessment of these regions on completion of behaviour, it is possible that variation in the level of pathology in anterior cingulate and rhinal cortex could account for the variability in competency in this task.**

The reversal learning component of the visual discrimination task is a measure of cognitive flexibility and is used to assess the rodent's ability to inhibit and dissociate from previously rewarded responses. Following the acquisition phase, sham and Epilepsy rats that achieved criterion were assessed for reversal learning. We found no differences in learning

rates between sham and Epilepsy rats in this assessment – indeed, if anything, the Epilepsy rats that were able to be tested (a small sample size) showed enhanced reversal learning. However, in the first session of the reversal task, Epilepsy rats found it difficult to suppress prepotent responses and displayed significantly increased perseveration. This finding agrees with a related study finding increased perseverative errors during extinction of a food-procuring task using intrahippocampal kainic acid treatment in rats ⁴⁷, **although some caution should be exercised with this conclusion, due to the low sample size caused by few animals eligible for this part of the study.** The prefrontal cortex and associated striatal structures are known to play an important roles in reversal learning, and cognitive inflexibility and perseveration has been associated with dysfunction in the prefrontal cortex ⁴⁸. Based on this, and on evidence that prefrontal cortex can experience pathology and functional disturbance in post-SE models ^{49; 50}, this could explain the excessive perseveration observed in our study.

To complement touch screen testing, we also performed conventional behavioural paradigms such as the Y maze and NORT to encompass other neurocognitive domains. Epilepsy rats displayed short term spatial memory impairment in the Y maze, and deficits in object recognition in the novel object task, compared to shams. These findings agree with previous studies in Epilepsy rats (eg: ^{19; 20; 51}), but is in contrast to others ^{24; 52}, which could be due to differences in experimental protocol. It is interesting to consider that these two tasks involve different brain regions – spatial memory involves hippocampus, whereas rhinal cortex has been implicated in object recognition ⁵³. Therefore, we have identified a broad cognitive deficit in Epilepsy rats, spanning different domains and brain regions, which aligns with the reported cognitive profile in patients with epilepsy ¹. **Some consideration should be given to potential motor deficits in these tests, in particular the Y maze (where deficits in locomotion might provide a false readout of exploration).** Indeed, examining individual data points in Figure 4B would suggest that some animals do not explore much at all. However, at a group level, no differences in exploration were noted, and further, the two Epilepsy animals which explored least recorded a % time exploring the novel arm at 123% - right on the group average. Motor impairment may also impact touchscreen performance, although we did not observe any group differences in correct/incorrect response times, or reward latency. In addition, the post-SE model is not renowned to be accompanied by marked motor impairment, so we do not have any reason to suspect that this played a role in cognitive testing.

One important consideration we were unable to address in the current study is the role of recurrent seizures occurring during the period of the touchscreen task (2-3 months) as we did not have the ability to monitor the animals for seizures during this period after the daily test sessions were complete. Undoubtedly, some animals had seizures during this period (although none experienced seizures within the touchscreen chambers themselves), so we are unable to conclude whether the seizures, or epilepsy per se, were the cause of the behavioural disturbance observed in this aspect. Seizures themselves would be expected to impair performance and interfere with the learning process⁵⁴, but the extent of this is not known, and very difficult to ascertain. It is interesting to note, however, that only animals with few seizures (as measured after completion of behavioural studies) were able to successfully complete the pairwise discrimination task (and reversal), suggesting that those with greater disease burden is associated with worse cognitive outcome. When attempting to directly assess this through correlative analyses, we only identified a significant inverse correlation between novel object exploration and seizure frequency, but not other behaviours. **It should also be noted that the n=3 animals which progressed to the reversal phase of the pairwise discrimination task were impaired in the other cognitive tasks we conducted (data not shown), suggesting differential vulnerability to different tasks.**

Taken together, in the present study we demonstrate cognitive deficits using a range of different behavioural exams, including those utilising advanced touchscreen technology. Most animals with Epilepsy were able to be tested in the chambers, although only a few, with low disease severity, were able to successfully complete the pairwise discrimination task – a relatively simple test of non-spatial learning. In addition to deficits in novel object recognition, short-term spatial learning and memory, Epilepsy rats which were able to complete the pairwise discrimination showed early perseverative behaviours in the absence of any reversal learning deficits. The findings of this study demonstrate for the first time the use of translational touchscreen tasks to characterise cognitive impairment in an animal model of Epilepsy. Future studies can be designed to explore biological mechanisms underpinning these deficits, **explore such biological mechanisms with directed studies in clinical populations**, as well as development of therapeutic strategies.

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position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

Table legend:

Table 1: Variables of methodology of the Status Epilepticus procedure, including the resulting severity of epilepsy. The three rats with shaded background refer to the three animals that performed the touchscreen task very well. There are no patterns of protocol which would suggest that these variables contribute to the relative sparing of cognitive function, or epilepsy severity.

Table 2: Number of subjects entering, and completing, each procedure in the study.

Figure legends:

Figure 1: Rats in both Epilepsy (n=10) and Sham (n=12) groups progressed through the pretraining phases of the touchscreens equally well, with no differences ($p>0.05$) at any stage. Data does not include the Epilepsy rats (n=2) which were unable to complete training.

Figure 2: Epilepsy rats are impaired on the Pairwise discrimination task. A) Significantly fewer ($p<0.01$) Epilepsy rats achieved criterion on the PD task, compared to Sham rats. B) Survival analysis indicates when each animal achieved criterion, and Epilepsy rats were significantly slower to achieve this ($p<0.05$). C) The percentage accuracy in responding also indicates that Epilepsy rats are impaired in the PD task, with a slower improvement in accuracy scores over the course of the study ($p<0.001$). D) Perseverative responses during the PD task were significantly higher in the Epilepsy rats ($p<0.05$), but no differences were observed with the latency to respond, or to collect rewards between the groups (E).

Figure 3: Epilepsy rats have normal reversal accuracy, but display increased perseveration in the reversal task. A) Rats which successfully completed the PD were tested for cognitive flexibility. In this, Epilepsy rats showed normal learning in the reversal phase, compared to Sham rats. B) However, Epilepsy rats showed significantly higher perseverative behaviours in the first reversal trial, compared to the Sham rats ($p<0.0001$). C) No changes in response latency were observed in this phase.

Figure 4: Epilepsy rats show impairments in other cognitive paradigms and behaviours. A) Epilepsy rats exhibit impaired short-term spatial memory in the Y maze, with significantly lower time spent in the novel arm ($p<0.05$), compared to Sham rats. B) No difference in the total distance travelled was observed in this test. B) In keeping with the PD results, Epilepsy rats also showed impaired discrimination in the novel object recognition task, compared to Sham rats ($p<0.05$).

Figure 5: All animals exposed to Kainic Acid-induced Status Epilepticus develop epilepsy. A) Example electrographic recording of a spontaneous (convulsive) seizure, as recorded bilaterally in both cortical (Ctx) and hippocampal (HPC) electrodes. B) The number of seizures each rat experienced over one week varied between 1-119. C) The average seizure length also varied considerably between animals (range 10 – 61.8 sec).

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% time spent in seizure class

weight (g)	No. KA injections	KA dose (mg/kg)	IV	V	VI	SE severity	Diazepam dose (mg/kg)	No. diazepam injections	No. seizures recorded in chronic phase
413	3	15		80	20	extreme	15	3	55
426	2	12.5		50	50	extreme	15	3	49
418	2	12.5	50	50		very severe	10	2	4
429	3	17.5	50	50		very severe	15	3	1
433	2	12.5	50	50		very severe	10	2	95
459	4	22.5		50	50	extreme	20	4	4
455	4	20	50	50		very severe	20	4	1
438	4	20	50	50		very severe	15	3	5
431	3	15		50	50	extreme	15	3	not recorded

405	3	15	50	50	very severe	10	2	39
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	Entered		Completed		
Procedure	Epilepsy	Sham	Epilepsy	Sham	Explanation for subject dropout
SE/sham	17	12	12	12	one rat did not enter SE, four rats did not survive
Touchscreen					
Pretraining	12	12	10	12	two rats did not complete sufficient trials
PD	10	12	10	12	
Reversal	3	12	3	12	only those completing PD progressed to reversal
Y maze	11	12	9	12	two rats repeatedly jumped out of maze
NORT	11	12	8	12	three rats had seizures during the task
Electrode implantation	11	12	10	12	one rat suffered complications with anaesthesia
Video-EEG	10	12	10	12

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