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ORIGINAL PAPER

The Barnes Maze Task Reveals Specific Impairment of Spatial Learning Strategy in the Intrahippocampal Kainic Acid Model for Temporal Lobe Epilepsy

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Abstract

Temporal lobe epilepsy (TLE) is an acquired form of focal epilepsy, in which patients not only suffer from unprovoked, devastating seizures, but also from severe comorbidities, such as cognitive dysfunction. Correspondingly, several animal models of TLE exhibit memory dysfunction, especially spatial memory. The Morris water maze test is the most commonly used test for assessing spatial learning and memory in rodents. However, high stress and poor swimming abilities are common confounders and may contribute to misinterpretation. Particularly epileptic mice show altered behaviour during the test as they fail to understand the paradigm context. In the Barnes maze test, a dry-land maze test for spatial learning and memory that uses milder aversive stimuli, these drawbacks have not yet been reported. In the present study, we use this task to evaluate spatial learning and memory in the intrahippocampal kainic acid mouse model of TLE. We demonstrate that the epileptic mice understand the Barnes maze paradigm context, as they learn the location of the escape-chamber by using a serial search strategy but fail to develop the more efficient spatial search strategy. Our data indicate that the Barnes maze may be a better alternative to the Morris water maze for assessing search strategies and impairment of learning and memory in epileptic mice.

Keywords The Barnes maze · Temporal lobe epilepsy · Intrahippocampal post-status epilepticus Kainic Acid model · Spatial learning and memory · Search strategy

Introduction

Temporal lobe epilepsy (TLE) is the most prevalent form of focal epilepsy, accounting for 50% of all epilepsy cases. The aetiology is variable but can be associated with earlier head trauma, viral infections, brain tumours or febrile seizures. TLE patients suffer from unpredictable and disabling focal seizures, mostly in the hippocampus, but also commonly encounter severe comorbidities [1]. Given that hippocampal structures play a key role in episodic learning and memory, it is not surprising that cognitive deficits account for one of the major comorbidities in TLE [2]. Although neurodegeneration [3], changes in plasticity [4] and massive gliosis [5] are suggested to be associated with these cognitive deficits, the exact pathological mechanism underlying learning and memory deficits in TLE is not yet fully resolved [3]. Animal models of TLE can serve as a valuable tool to evaluate new treatment strategies for treatment of seizures and comorbid cognitive dysfunction in TLE [6].

Several mice and rat models of TLE show disturbances in learning and memory [6–10]. In rodents, the most commonly used behavioural test to evaluate spatial memory is the Morris water maze [11]. However, despite extensive use of this test, the results can be prone to misinterpretation.

Ilse Smolders and Dimitri De Bundel have contributed equally to this work.

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For instance, the high amount of stress caused by the Morris water maze task and poor swimming abilities of mice [12] are known to strongly affect task performance [12, 13]. Several studies observed abnormal behaviour in epileptic mice during the Morris water maze task. This could also be ascribed to the lack in understanding the stressful task context rather than specific deficits in spatial learning and memory [8, 10]. In the intrahippocampal kainic acid (KA) mouse model for TLE, clear impairments in the Morris water maze task have been observed in male C57BL/6J [3] and to a lesser extent in female NMRI mice [8]. However, it should be noted that impaired task performance was more apparent in larger mazes [3], suggesting that epileptic mice performed well in the smaller mazes by using a non-spatial scanning strategy. Therefore, a more in-depth analysis of search strategy would give more insights into spatial learning and memory abilities of the epileptic mice. In the present study, we show that the Barnes maze, a dryland maze that uses mild aversive stimuli to assess spatial learning and memory in rodents [14], is highly suitable for detecting spatial learning deficits in the intrahippocampal KA mouse model, since specific search strategies can be readily assessed.

Methods

Animals

All experiments were performed on male C57BL/6J mice (Janvier Laboratories, France), 12 weeks old at the time the Barnes maze experiment was initiated. In total 26 mice were used for the present investigations: ten mice were subjected to status epilepticus (the KA group), eight mice served as the sham-injected control group and eight mice were used as the age-matched naive control group. All mice were housed in a temperature (19–23 °C) and humidity (30–70% relative humidity) regulated environment with a 14/10 h light/dark cycle and received food pellets and water ad libitum. Mice were habituated one week to the animal house upon arrival and were subsequently subjected to surgery. All mice were single housed for four weeks before starting the behavioural experiment (Fig. 1). Mice were daily acclimatized to the behavioural testing room for a minimum of 1 h prior to commencing experiments. All procedures were carried out in accordance with the National Rules on Animal Experimentation and were approved by the Ethical Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel, Brussels, Belgium. To the best of our abilities, results were reported in accordance with the ARRIVE guidelines [15].

Intrahippocampal Post-Status Epilepticus KA Mouse Model

The intrahippocampal post-status epilepticus KA model is obtained by unilateral injection of KA in the CA1 region of the dorsal hippocampus [16, 17]. To this end, mice were anesthetized with isoflurane (Vetflurane®, 1000 mg/g, Virbac) and KA (200 ng in 50 nL 0.9% NaCl, Sigma) was slowly injected over 1 min using a 10-µL microsyringe (Hamilton) at the following stereotaxic coordinates: AP, − 2 mm; ML, − 1.5 mm; and DV, − 2.1 mm, respectively [16–18]. The syringe was maintained in place for an additional 2 min to limit backflow along the injection track. Sham-injected animals were prepared identically and were injected with the same volume of NaCl.

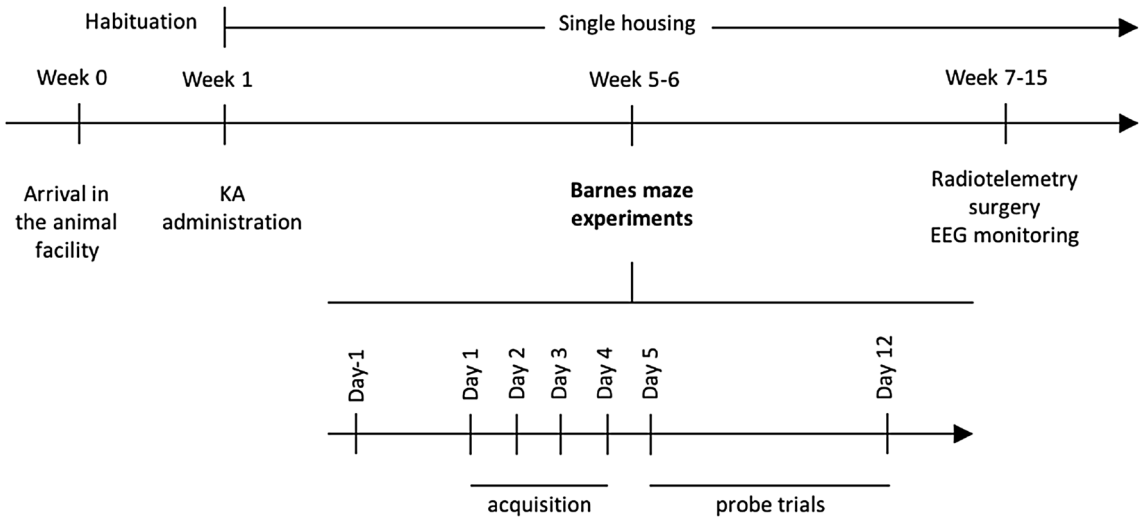

Fig. 1 Time schedule of the experimental procedures

Barnes Maze Test

Behavioural testing in the Barnes maze was performed four weeks after KA injection. The intrahippocampal KA mouse model is characterized by a KA-induced status-epilepticus, followed by a latent period of two weeks, after which mice develop spontaneous hippocampal paroxysmal discharges [16, 17]. The Barnes maze is a dry-land maze used as a test for visual-spatial learning and memory. The maze is an elevated, circular, light-grey platform with a diameter of 100 cm (Ugo Basile, Varese, Italy). A hidden, black chamber was located under one of the 20 holes around the perimeter of the platform. Bright lights were placed around the maze, which served both as mild aversive stimuli (± 600 lx in the centre of the platform) and as visual cues. First, the animals were familiarized to the escape chamber, by placing them into the chamber for 120 s, with a familiar chocolate flavoured treat. The same day they were habituated to the maze by placing them in the middle of the platform and after 60 s gently guiding them towards the escape chamber, in which they stayed for 120 s receiving the treat. Two days afterwards (acquisition day 1), the animals were trained for four consecutive days to escape from the exposed platform surface to the dark recessed chamber, as previously described [19]. The location of the escape chamber was randomly assigned for each mouse, but remained the same throughout the acquisition phase. Every day, the animals were placed in a closed, red holding tube in the centre of the platform. After 10 s, the tube was lifted, and the animals were allowed to explore the maze. Mice that did not enter the escape chamber within 180 s, were gently guided there by the experimenter. All mice were kept in the escape chamber for 120 s. After each training session, the platform was cleaned with 70% ethanol to avoid any odour cues. Four trials per day were conducted, with inter-trial periods of 15–20 min, and for each trial, the amount of errors (primary and total), the escape-latency (primary and total) and search strategy were measured using an automated video tracking system (Ethovision, Noldus). Primary errors are defined as the amount of head-deflections or pokes into incorrect holes before the first encounter with the escape chamber. Total amount of errors is the amount of errors made before the animal enters the escape hole (i.e. when all four paws are inside the escape chamber). Similarly, escape-latency is defined as the time required for the mice to make initial contact with the escape chamber. The data of the four trials were averaged per day. On day 5, 24 h after the last training, a 90 s-probe trial without escape chamber was conducted and the amount of errors, escape-latency and search strategy were analysed until the first encounter with the target hole. Three types of search strategies were evaluated, spatial (directly visiting the target location or visiting one adjacent hole before the target location), serial (visiting at least two adjacent holes in a serial manner before visiting target location) or random search strategies (random crossings of the platform before visiting target location) [20, 21]. A final trial was conducted one week after the last training. If a generalized spontaneous seizure occurred during a trial, the mouse in question was returned to its home cage and the trial was repeated after 30 min. After termination of the Barnes maze test, all KA-treated mice were implanted with a radiofrequency transmitter and electrodes for electroencephalography (EEG) monitoring for unequivocal evaluation of spontaneous seizures. Age-matched naive controls were used for comparison.

EEG Radio Telemetry in Freely Moving Animals

For EEG recordings, a radiofrequency transmitter (ETA-F10, Data Sciences International, DSI) was implanted intraperitoneally in isoflurane-anesthetized mice. The electrodes were tunnelled subcutaneously towards the skull and the peritoneum and the skin were closed using, nonabsorbable suturing material (Ethilon II, 4-0, M-2, Ethicon) and tissue adhesive (3M, Vetbond), respectively. The stainless steel coated, bipolar recording electrode (E363/3/SPC Invivo1) was implanted at the site of KA injection, using the same coordinates as for KA injection. A second electrode was placed over the cerebellum as the ground and an additional screw above the contralateral hippocampus served as anchor. Electrodes were fixed to the skull with dental acrylic cement (Integrity®, Denstply). After surgery, mice were injected i.p. with 0.5 mL of 0.9% NaCl (Baxter) and placed on a heating pad to recover. DietGel® Recovery (Clear H_2O®) was provided in the cage to facilitate post-operative food intake. The telemetry system (DSI) consists of a receiver plate (RPC-1 receiver, DSI), which receives data from the telemetric implant (ETA-F10) and transmits it to the data exchange matrix (MX2, Matrix 2.0, DSI) and a core unit, responsible for acquiring and processing the data. Ponemah software (DSI) was used to acquire real-time EEG data at a sampling rate of 500 Hz. A 60-Hz low-pass filter and a 50-Hz notch filter were applied to the data after acquisition with the Neuroscore software (DSI). The EEG recordings were visually analysed for detection of the spontaneous, focal seizures. Hippocampal paroxysmal discharges are defined as polymorphic, high-amplitude (> 2 times background amplitude) sharp waves with a duration ≥ 5 s and inter-event interval of ≥ 1 s, as previously reported [17]. After behavioural testing in the Barnes maze, all mice were 24/7 EEG monitored in their home cages for at least 1 week. For each mouse the same 3 h of EEG-recording (11–14 h) were analysed for 5 days. Only mice that developed seizures were included in the final analysis.

Statistics

First, the obtained data was tested for normality with the D'Agostino-Pearson omnibus test. Multiple groups of data were analysed by ANOVA for parametric data with the Bonferroni test for posthoc comparisons using Prism v7.0. Discontinue data were analysed using the Pearson's Chi-square test. All tests were performed with $\alpha = 0.05$. P values < 0.05 were considered significant. All results are expressed as mean ± SEM.

Results

Barnes Maze Task Performance After Unilateral Intrahippocampal KA Injection

During the acquisition phase of the Barnes maze task, all three experimental groups showed significant learning, as revealed by a decrease of the primary escape-latency (Fig. 2a). For primary escape latency, there was no significant interaction (two-way repeated measures (RM) ANOVA, F (6, 60) = 1.07, P = 0.39, n = 7–8) but a main effect of acquisition day (two-way RM ANOVA, F (3, 60) = 38.40, P < 0.0001, n = 7–8) and treatment (two-way RM ANOVA, F (2, 60) = 5.05, P = 0.017, n = 7–8), with KA-injected mice performing significantly worse than both sham mice and naive mice on day 1 (Bonferroni post-hoc analysis, P < 0.05, Fig. 2a). For primary errors, there was no interaction (two-way RM ANOVA, F (6, 60) = 0.44, P = 0.87, n = 7–8) but a main effect of acquisition day (two-way RM ANOVA, F (3, 60) = 13.44, P < 0.0001, n = 7–8) and treatment (two-way RM ANOVA, F (2, 60) = 26.42, P < 0.0001, n = 7–8), with KA-injected mice performing significantly worse than either sham or naive control mice on day 1, day 2, day 3 and day 4 (Bonferroni post-hoc analysis, P < 0.05, Fig. 2b).

Mice were tested for long-term memory performance during probe trials carried out on day 5 (one day after the final acquisition day) and on day 12 (1 week after the final acquisition day) (Fig. 2c, d). For primary escape latency, there was no significant interaction (two-way RM ANOVA, F (2, 20) = 0.019, P = 0.98, n = 7–8) or main effect of probe trial day (two-way RM ANOVA, F (1, 20) = 2.62, P = 0.15, n = 7–8) but a significant effect of treatment (two-way RM ANOVA, F (2, 20) = 9.59, P = 0.0012, n = 7–8), with KA-injected mice performing significantly worse than naive mice on probe trial day 12 (Bonferroni post-hoc analysis, P < 0.05, Fig. 2c). For primary errors, there was no significant interaction (two-way RM ANOVA, F (2, 20) = 0.59, P = 0.56, n = 7–8) or main effect of probe trial day (two-way RM ANOVA, F (1, 20) = 2.41, P = 0.14, n = 7–8) but a significant effect of treatment (two-way RM ANOVA, F (2, 20) = 23.55, P < 0.0001, n = 7–8), with KA-injected

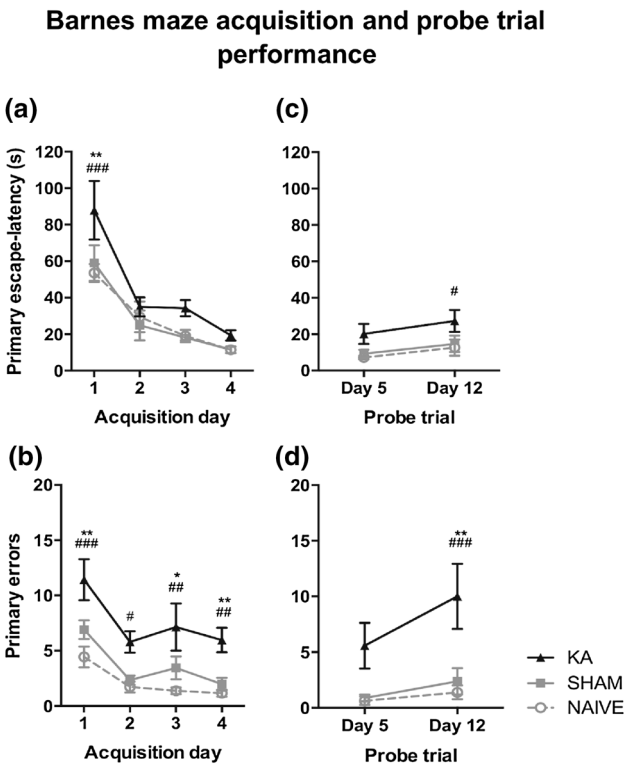

Fig. 2 Barnes maze performance. The graph shows **a,c** the primary escape-latencies (time needed to find the escape hole for the first time) and **b, d** the amount of primary errors (number of holes visited prior to finding the escape hole for the first time), averaged per day during **a, b** the acquisition phase and **c, d** during the probe trials. The results of two-way RM ANOVA and the Bonferroni multiple comparisons post-hoc test are illustrated on the graph by asterisk (comparison KA vs. sham controls) or rhomb (KA vs. naive controls). * or $^{\#}$P < 0.05; ** or $^{\#\#}$P < 0.01; *** or $^{\#\#\#}$P < 0.001. Data are represented as mean ± SEM, n = 7–8 per group

mice performing significantly worse than either sham mice or naive control mice on probe trial day 12 (Bonferroni post-hoc analysis, P < 0.001, Fig. 2d). Together, these data indicate that KA mice are less capable of finding the escape hole, especially after a delay of one week, but that overall, mice maintain a memory trace for finding the escape platform for a period of up to one week after initial acquisition.

Barnes Maze Task Search Strategies After Unilateral Intrahippocampal KA Injection

Three search strategies were identified: random, serial and spatial (see "Methods" section). For all acquisition trials, primary escape latency and primary errors were pooled per search strategy (Fig. 3). For primary escape latencies, there was a significant main effect for search strategy (One-way ANOVA, F (2, 345) = 87.25, P < 0.0001). The random search strategy was significantly slower than the serial search strategy, which in turn was significantly slower than

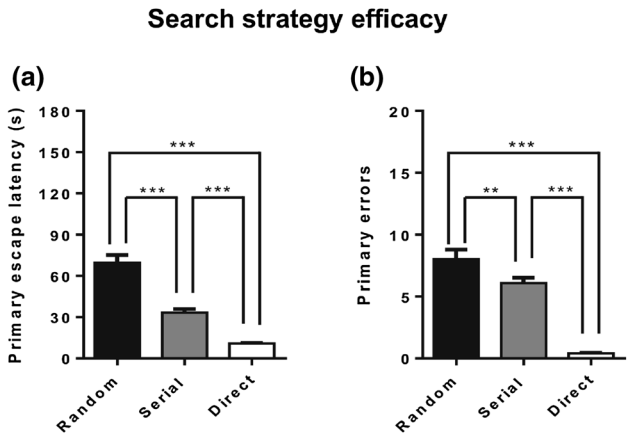

Fig. 3 Search strategy efficacy. Three types of search strategies were identified: random, serial and spatial (see "Methods" section). Illustration of **a** the primary escape latency and **b** the amount of primary errors for all acquisition trials, pooled per search strategy. Data were analysed by One-way ANOVA followed by Bonferroni multiple comparisons post-hoc test. Significance is illustrated on the graph by asterisk, *P < 0,05; **P < 0,01; ***P < 0,001. Data are represented as mean ± SEM, n = 79–143 per group

the spatial search strategy (Bonferroni post-hoc analysis, P < 0.0001, Fig. 3a). For primary errors, there was a significant main effect for search strategy (One-way ANOVA, F (2, 345) = 77.92, P < 0.0001). The random search strategy led to significantly more errors than the serial search strategy, which in turn led to significantly more errors than the spatial search strategy (Bonferroni post-hoc analysis, P < 0.001, Fig. 3b). These differences in search strategy efficacy may thus readily explain differences in Barnes maze performance.

We next evaluated whether KA mice employed different search strategies during the acquisition phase of the Barnes maze (Fig. 4). For the random search strategy, there was a significant effect of acquisition day (two-way RM ANOVA, F = 22.59, DF = 3, P < 0.0001) but no significant effect of treatment (two-way RM ANOVA, F (2, 60) = 1.47, P = 0.254) or interaction (two-way RM ANOVA, F (6, 60) = 22.59, P = 0.50, Fig. 4a). More specifically, 82% of all mice used the random search strategy during the first trial compared to a remaining 13% during the last trial of acquisition phase (Supplementary Figure S1), indicating that mice in all treatment groups learned to use more efficient escape strategies. For the serial search strategy, there was a significant effect of treatment (two-way RM ANOVA, F (3, 60) = 8.4, P = 0.0022), no significant effect of acquisition day (two-way RM ANOVA, F (3, 60) = 1.32, P = 0.28) but a significant interaction (two-way RM ANOVA, F (6, 60) = 4.94, P = 0.0004). Further analysis revealed that KA mice used the serial strategy significantly more later in acquisition compared to sham and naive control mice that learned to use this strategy less (Bonferroni post-hoc analysis, P < 0.05, Fig. 4b). During the last trial of the fourth acquisition day, 71% of the KA mice preferred the serial strategy, while only 25% sham mice and 12% of the naive mice chose this strategy (Supplementary Figure S1). For the spatial search strategy, there was a significant effect of treatment (two-way RM ANOVA, F (2, 60) = 19.23, P < 0.0001) and acquisition day (two-way RM ANOVA, F (3, 60) = 12.77, P < 0.0001) without significant interaction (two-way RM ANOVA, F (6, 60) = 2.18, P = 0.057). Overall, mice favoured a spatial strategy in later trials, but KA mice used this strategy significantly less compared to sham and naive control mice (Bonferroni post-hoc analysis, P < 0.05, Fig. 4c).

The search strategy was also assessed during the probe trials on day 5 (one day after the acquisition phase) and day 12 (one week after the acquisition phase) (Supplementary

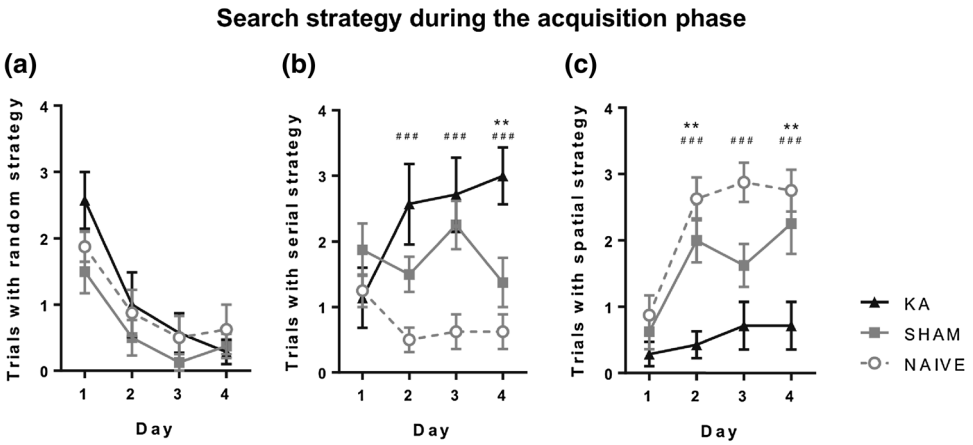

Fig. 4 Acquisition phase search strategies. The graph shows the number of trials a mouse uses **a** random **b** serial or **c** spatial strategies to find the escape hole, averaged per mouse per day. The results of two-way RM ANOVA and the Bonferroni multiple comparisons post-hoc test are illustrated on the graph by asterisk (comparison KA vs. sham controls) or rhomb (KA vs. naive controls). *or $^{\#}$P < 0.05; **or $^{\#\#}$P < 0.01; ***or $^{\#\#\#}$P < 0.001. Data are represented as mean ± SEM, n = 7–8 per group

Figure S1). For both probe trials, we performed the Chi-square test, which showed a significant difference in search strategies between the three groups (df = 2, P = 0.04). During the first probe trial, 43% of KA mice, 75% of sham mice and 88% of naive control mice used a spatial strategy. Moreover, 57% of KA mice, 26% of sham mice and 12% of naive control mice used a serial strategy. During the second probe trial, 14% of KA mice, 63% of sham mice and 50% of naive control mice used a spatial strategy. Here, 72% of KA mice, 25% of sham mice and 50% of naive control mice favoured a serial strategy. These observations suggest that once familiar with the task, mice remember how to find the escape hole, but progressively forget where to find it using a spatial strategy. Moreover, epileptic mice were capable of using the hippocampus-independent serial strategy [22], but failed to consistently use the hippocampus-dependent spatial strategy, resulting in poorer task performance (i.e. longer escape-latencies and higher amounts of errors).

Spontaneous Focal Seizures After Unilateral Intrahippocampal KA Injection

The intrahippocampal KA mouse model is characterized by an immediate status epilepticus [16, 17] and followed by a latent period of two weeks after which mice develop spontaneous limbic seizures that reach a stable amount of seizures per day four weeks after the injection. Some mild behavioural changes could be observed during the status epilepticus, such as mild clonic movements of the forelimbs, rotations and immobilization, as described previously [16, 23]. During the chronic phase of this model, two types of epileptiform discharges were observed, focal paroxysmal discharges and generalized seizures. To evaluate the presence of spontaneous paroxysmal discharges that are not associated with observable behavioural convulsions it is necessary to carry out intracerebral EEG monitoring (Fig. 5). On average 36 ± 5643 (n = 7) focal seizures per hour occur, comparable to other studies found in literature [16, 17, 23]. Generalized seizures only occurred once every day to once every three days and can be identified by behavioural convulsions and typical discharges on the intracerebral EEG trace (Fig. 5). Between 7 and 15 weeks after KA injection, mice were checked for electrographic seizures. Only mice with proven seizures were used in the final behavioural analysis.

Three mice from the KA-treated group were as such excluded from analysis, as one mouse was killed for reaching its humane endpoint (> 20% body weight loss) two days after the status epilepticus, one mouse died from a spontaneous seizure four weeks after KA injection and in one mouse spontaneous seizures were not confirmed due to technical reasons. The final group sizes were 7 KA-treated mice, 8 sham control mice and 8 age-matched naive controls.

Discussion

The intrahippocampal post-status epilepticus KA mouse model is a widely-used mouse model of TLE, showing similarities to human TLE in terms of the disease course, structural hippocampal changes, drug resistance [17], and the presence of comorbidities [6, 24].

The Morris water maze has long been the test of choice for assessing spatial learning and memory in rodents. The Barnes maze is slowly increasing in popularity. Its major advantage is the use of a less aversive context, bright light instead of water, causing the test to be less stressful for the

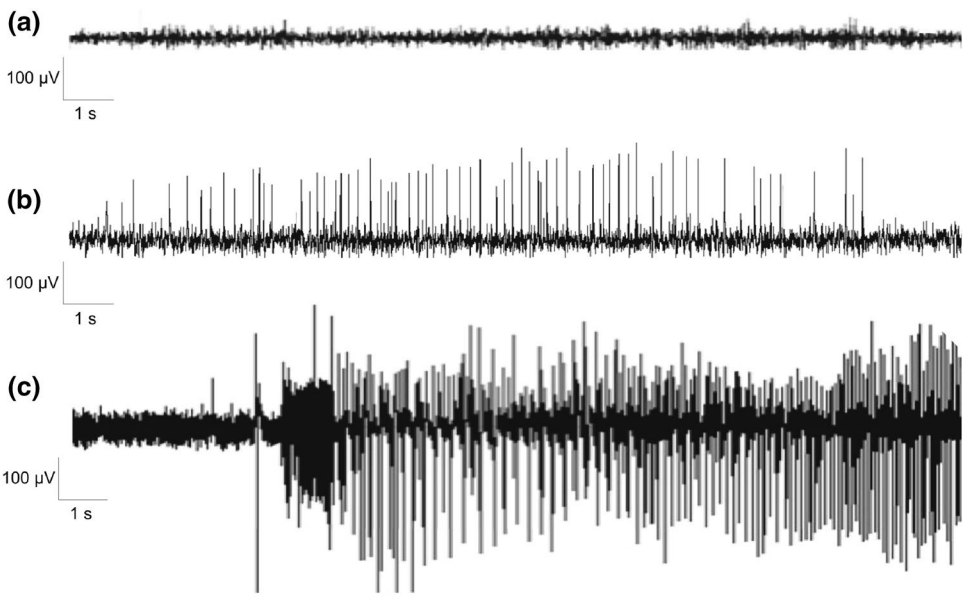

Fig. 5 Representative EEG traces of **a** normal activity in a sham-injected mouse; **b** a typical paroxysmal discharge during the chronic phase in the intrahippocampal KA mouse model and **c** a generalized seizure, sporadically occurring during the chronic phase in the intrahippocampal KA mouse model

experimental animals. Higher corticosterone levels have been observed in mice performing the water maze task compared to those subjected to the Barnes maze paradigm, which are inversely correlated to task performance in the Morris water maze, leading to misinterpretation of data [12]. Moreover, as stress is an important seizure precipitant in epilepsy patients, stressful behavioural tasks could elicit seizures in epileptic animals [25, 26]. Other drawbacks of the Morris water maze are the longer training periods necessary for mice to learn the location of the hidden platform and the labour intensity for the experimenter. Another test for evaluating spatial memory is the radial arm maze, a dry-land maze using food restriction as a motivator for mice to forage the maze. The radial arm maze has similar drawbacks to the Morris water maze, such as the use of a strong aversive stimulus (food deprivation) and intensive training periods which may induce a high amount of stress [25]. However, because the Barnes maze test is less stressful, mice may not be motivated sufficiently to enter the dark escape chamber. In our experiments, 16 out of 23 mice refused at least once to enter the escape chamber without guidance by the experimenter. Fortunately, evaluation of primary parameters, essentially the measurement until the first encounter of the mice with the correct target hole, can circumvent this issue, as mice are often motivated enough to first assure their way out of the open platform prior to further exploration of the platform [19, 20]. A fundamental difference between the three cognitive tests lies in the type of memory being assessed. The radial arm maze is superior for evaluating working memory, as mice need less recuperation time in between trials. Assessing working memory is less straightforward in the Barnes maze and Morris water maze, which are more suited to evaluate spatial learning and reference memory. Furthermore, mice trained in the radial arm maze learn both via spatial and associative cues, while in the Barnes maze and Morris water maze mainly spatial learning is used. Moreover, similar to the Barnes maze, the radial arm maze allows search strategies to be assessed [27]. For these reasons, the radial arm maze and Barnes maze could be used as tests standing side-by-side in a cognitive testing battery.

In the present study, KA-injected epileptic mice performed significantly worse compared to sham and naive control mice. Significant learning was observed for all experimental groups and all mice showed a shift in search strategy, from random to serial or spatial. While epileptic mice continued using a serial search strategy, sham and naive controls increasingly adopted a spatial search strategy, resulting in a lower amount of primary errors. These data suggest that epileptic mice learn how to locate the escape-chamber by using a systematic, serial search strategy, but that they do not learn the spatial location of the escape-chamber. In other words, KA treated mice are clearly impaired in spatial learning and memory. This suggests that in previous studies using the Morris water maze to assess spatial learning and memory [3, 6], KA treated mice may have used a non-spatial strategy, such as swimming at a specific distance from the borders of the maze. Such a strategy may also explain why KA treated mice are less effective in a larger maze [3, 6], since correct distance from the border of the maze would be more difficult to estimate. A study using a virtual navigation task similar to the Barnes maze task showed significantly more errors during the task in refractory unilateral TLE-patients, who readily reached an asymptotic level between two to five training trials [28]. This is in line with the data gathered in our study, in which after four training trials, KA treated mice did not further improve in error making and likewise reached a plateau. The authors in the previous study attributed poor task performance to a specific spatial impairment rather than general learning and memory impairment, and argued that additional training would not be beneficial [28]. Similarly, another study showed that TLE-patients performed significantly worse over all trials in a real-world route learning task [29]. The patients made more errors, although small improvements were observed over the trials [29]. Interestingly, our data show that KA treated mice fail to consistently develop a spatial strategy, but are nevertheless capable of developing and remembering a non-spatial strategy to successfully carry out the Barnes maze task. Moreover, several mice that had used a spatial strategy during the first probe trial regressed to a non-spatial strategy during the second probe trial 1 week later.

Particularly in experiments regarding epilepsy, the use of the Barnes maze shows a lot of potential. Several papers reported that epileptic mice show altered swimming patterns during the water maze task, mice stayed only in one part of the maze and did not seem to look for the platform [8, 10]. The authors considered this as a problem in understanding the context of the paradigm. However, in the Barnes maze, mice clearly searched for the escape chamber [30]. Also in our present study, the epileptic mice adopted a serial search strategy rather than the random strategy, pointing out that they learned the context of the test and were capable of learning a systematic strategy [31]. Another potential of the Barnes maze paradigm lies in its compatibility with in vivo" devices. Continuous EEG monitoring is often an inevitable part of epilepsy research, and therefore mice are implanted with a radio telemetric transmitter and intra-cerebral or cortical electrodes. This technology cannot be used in combination with the Morris water maze, as the weight of the transmitter and electrodes hinders the ability to swim. Moreover, it would be possible to install EEG-receiver plates underneath the Barnes platform and perform EEG recordings during the acquisition of the trials, allowing to correlate the amount of seizures with their simultaneous performance in the Barnes maze. Additionally, as anti-seizure drugs often contribute to cognitive impairment in TLE patients, this

test could be used to further analyse the effect of the current available anti-seizure drugs on cognition in this mouse model of TLE. This would not only provide in-depth pharmacological information to the cognitive dysfunction in this TLE mouse model, but will also allow this model to be used as a screening test for novel anti-seizure drugs for their possible cognitive side-effects at a preclinical stage.

In this pilot study, we showed by using the Barnes maze, that epileptic mice were less accurate, needed more time to find the location of the escape chamber and used a different strategy on the maze compared to both control groups. Moreover, we showed that learning and memory impairment was specific to a spatial strategy. However, a relatively small group size of 7–8 mice was used, taking into account inter-individual variability in memory tests, it is favourable to confirm this data using a second batch of mice. Moreover, it would be interesting to repeat this experiment in other laboratory environments, to account for inter-laboratory variability. The Barnes maze is thus a valuable test to study spatial learning and memory, when performing a search strategy analysis, and specifically, to study memory dysfunctions in mouse models of epilepsy.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures were carried out in accordance with the National Rules on Animal Experimentation and were approved by the Ethical Committee for Animal Experiments of the Faculty of Medicine and Pharmacy of the Vrije Universiteit Brussel, Brussels, Belgium.

Research Involving Human Participants This article does not contain any studies with human participants.

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