CA1 Network Dynamics During Impaired Working Memory in Temporal Lobe Epileptic Mice

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List of abbreviations

CAT: continuous alternation task

DAT: delay alternation task

EC: entorhinal cortex

EEG: electroencephalographic

FRD: focal rhythmic discharge

GC: granule cell

HFO: high-frequency oscillations

HS: Hippocampal sclerosis

KA: kainic acid

KAR: kainic acid receptor

LEC: lateral entorhinal cortex

LFP: local field potential

MEC: medial entorhinal cortex

MTLE: mesial temporal lobe epilepsy

pHFO: Pathological high frequency oscillations

SWR: Sharp wave ripple

TLE: temporal lobe epilepsy

1. Introduction

Around 50 million people in the world have epilepsy (WHO, 2018). Epileptic patients suffer not only from seizures, which are the clinically defining feature of epilepsy, but also from cognitive deficits. Although cognitive impairments widely impact the patient's quality of life, they have not been the target of common treatments. One main reason for such clinical approaches is that cognitive deficits have been commonly linked to seizure occurrence. In other words, cognitive deficits have been thought to be driven by seizures. However, over the last years, several studies have shown that cognitive deficits and seizures should be considered as two symptoms of epilepsy (Lenck-Santini PP and Sakkaki S., 2020). Studying the neuronal mechanism underlying cognitive deficits is crucial to providing the proper treatment for this comorbidity of epilepsy. In this study, we used in vivo electrophysiology to investigate the hippocampal neuronal network changes underlying memory deficits in temporal lobe epileptic mice that were running a spatial working memory task. We aimed to address whether memory deficits were driven directly by seizure burden, or whether there are other pathological changes in hippocampal networks that lead to memory deficits in epileptic mice.

1.1 Hippocampus structure

The hippocampus has been the subject of 200,681 publications over the last century and has become one of the most well-studied brain regions in both human and animal models (https://app.dimensions.ai/). The hippocampal formation consists of the dentate gyrus, cornu ammonis (CA) fields, and the subiculum. The dentate gyrus routes excitatory inputs to CA3 via mossy fibers. The mossy fiber inputs provide strong excitatory input to CA3 pyramidal neurons from DG granule cells (GCs). Even single GCs are able to trigger action potentials in CA3 pyramidal neurons (Henze, D et al., 2002). CA3 projects Schaffer collaterals to CA1. CA3 pyramidal neurons also project to contralateral CA1 via commissural projections (Kjelstrup et al., 2002; Blackstad 1956; Gottlieb and Cowan

1973), and form recurrent collateral connections with other CA3 cells (Bains et al., 1999). These recurrent excitatory connections are implicated in sharp wave ripple propagation (Buzsáki et al., 1992; Csicsvari et al., 2000). In epilepsy, the stochastic firing of a single pyramidal cell is enough to build an excitatory activity within recurrent collateral of the CA3 leading to an epileptic event (Miles and Wong., 1989; Menendez et al., 2006).

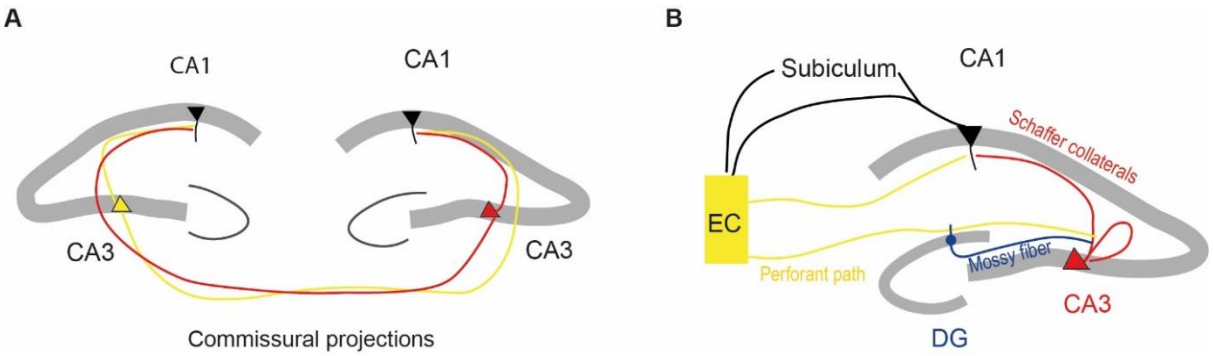

Figure 1.1 - Schematic of rodent hippocampus section.

A, CA3 pyramidal neurons project to CA1 ipsilaterally and contralaterally (via commissural projections). B, Ipsilateral Cortical inputs reach the hippocampus via the perforant path projections from the entorhinal cortex (EC). The perforant path axons terminate on dentate granule cells (GC), and mossy fiber axons of GC terminate on CA3 pyramidal cells. Schaffer collateral axons of CA3 pyramidal cells then project to CA1 pyramidal cells, which project back to the EC (both directly and indirectly via the subiculum). CA3 also provides recurrent collaterals within CA3.

The entorhinal cortex (EC) both provides information to the hippocampal formation and receives information from the hippocampal formation. The EC itself comprises the medial EC (MEC) and the lateral EC (LEC). MEC and LEC receive and compute information from different cortical regions before transmitting that information to the hippocampus. MEC is part of the dorsal pathway, integrating spatial egocentric information from other regions of the cortex. The LEC is part of the ventral pathway and encodes somatosensory information, and projects non-spatial features such as object identity and time (Mishkin et al., 1983; Save and Sargolini., 2017). Hippocampus combines these pieces of information to form memory (Rodo et al., 2017; Sewards and Sewards, 2003;). DG, CA3 and CA1 receive input from EC (O'Keefe and Dostrovsky, 2003; Basu and Siegelbaum, 2015).

Eventually, the hippocampus sends back processed memory output to the deep layer V of the entorhinal cortex via CA1 and subiculum. (Dolorfo and Amaral, 1998).

In this study, we bilaterally recorded in vivo LFP from CA1. CA1 has a unique connection with other parts of the hippocampus that make it able to integrate the direct (non-processed) information from EC and procced information through CA3. CA1 also receives strong excitatory inputs from CA2 (Chevaleyre and Siegelbaum., 2010). CA1 pyramidal neurons send hippocampal output to the subiculum, EC, amygdala, prefrontal cortex as well as cortical areas (van Groen and Wyss., 1990; Fuentealba et al., 2008).

1.2 Epilepsy

According to the World Health Organization, epilepsy is the most prevalent neurological disorder, with a prevalence of over 50 million (WHO, 2018). Epilepsy is a central nervous system disorder characterized by an enduring predisposition to generate epileptic seizures. This definition is practically applied as having two unprovoked seizures >24 h apart (Fisher et al., 2005, 2014). An epileptic seizure refers to transient symptoms caused by abnormal excessive or synchronous neuronal activity in the brain (Fisher et al., 2005). Epilepsy is categorized into three subgroups based on the pathology: acquired, idiopathic, and epilepsy of genetic or developmental origin (Gupta et al., 2010). Epileptic syndromes are classified as generalized and focal seizures (ILAE, 1989). Generalized seizures affect both hemispheres of the brain and are classified into two categories: motor and non-motor seizures (Fisher et al., 2017). Motor seizures include tonic-clonic seizures, clonic, tonic, myoclonic, myoclonic-tonic-clonic, myotonic-atonic, atonic, and epileptic spasms (Fisher et al., 2018, Fisher et al., 2017). Non-motor seizures are also termed absences and include typical, atypical, myoclonic, and eyelid myoclonia (Scheffer et al.,2018, Fisher et al., 2017).

In contrast, focal seizures occur in a small localized regions of the cerebral cortex or a deeper structure of the cerebrum (Fisher et al., 2017). Focal seizures have the following major subcategories: automatisms or hyperkinetic movements and non-motor symptoms like cognitive seizures including impaired language, hallucinations, or perceptual

distortions (Fisher et al., 2018b; Scheffer et al., 2018). The most common form of focal epilepsy is temporal lobe epilepsy.

1.2.1 Temporal lobe epilepsy

Temporal lobe epilepsy (TLE) is the most common form of pharmacoresistant epilepsy in adults. The term TLE is referred to epilepsy conditions due to lesions in the temporal lobe. Among all forms of TLE, mesial temporal lobe epilepsy (MTLE) is the most prevalent one which is recognized by spontaneous recurrent motor seizures, and learning and memory impairments. The main pathological hallmark of TLE is hippocampal sclerosis (Mathern et al., 2008, Wiestler and Blümcke, 2002; Blümcke et al., 2013). Hippocampal sclerosis is characterized by selective neuronal death of pyramidal cells in the CA1, CA3, and CA4 (in humans). Although neuronal death in dentate granule cells is much less, there are two distinct phenomena present in DG: migration of GC cell bodies, called granule cell dispersion, and sprouting of their axons, called mossy fiber sprouting (Thom, 2014).

Although behavior and pathological studies provided a large body of information about epilepsy, studying epilepsy would not be possible without electrophysiological recording, since many seizures are only observed in electroencephalographic (EEG) recordings. These seizures, so called sub-clinical seizures, have no symptoms or clinical signs. Sub-clinical seizures are similar to clinical seizures in EEG signature and the onset region (King-Stephens., 2020) but they do not recruit a sufficient number of neurons to produce behavioral symptoms like clinical seizures (Babb et al., 1987). Interictal spikes, and high-frequency oscillations (HFO), are other epileptiform activities that are present in EEG recording both in TLE patients and animal models (Jefferys et al., 2012). Interictal spikes are high amplitude and fast transients in the EEG emerging from the synchronous discharges of a group of neurons (Engel., 1989). HFOs are high-frequency bands (80-600 Hz) in local field potential (Bragin et al., 1999). HFOs are used to detect the seizure generating zones in TLE patients (Worrell et al., 2004).

1.2.2 Kainic acid model of temporal lobe epilepsy

Heterogeneity is a notable feature of MTLE in humans. Likewise, there is no experimental model that reproduces all the histopathological, electroencephalographic, and behavioral features of TLE. Administration of pilocarpine and kainic acid (KA) has been extensively used to model temporal lobe epilepsy in rodents. The kainic acid-induced status epilepsy, a well-validated model of TLE, was first introduced by Ben-Ari and colleagues (Ben-Ari and Lagowska, 1978; Ben-Ari et al., 1979). For this study, we utilize the supra-hippocampal KA model of TLE. The supra-hippocampal KA model provides localized pathology that mimics human TLE (Ben-Ari and Lagowska, 1978; Ben-Ari et al., 1979a; Loscher W et al., 2010; Bedner et al. 2015). In this model, chronic epilepsy state starts after 4-13 days of the latent period (Bedner et al. 2015).

Kainic acid (KA) is a cyclic analog of L-glutamate and an agonist of ionotropic KA receptors (KARs). KARs belong to a family of ionotropic glutamate receptors, along with AMPA and NMDA receptors. KARs can be found in the entorhinal cortex (Patel et al., 1986), cerebellum (Wisden and Seeburg, 1993), amygdala (Rogawski et al., 2003), basal ganglia (Jin and Smith, 2011), and the hippocampus (Bloss and Hunter, 2010). Of the five types of KAR subunits, GluK2 and GluK5 are found to be related to developing chronic epilepsy (Pinheiro et al., 2013; Artinian et al., 2015). Interestingly GluK5, with a high affinity for KA (dissociation constant of 5–15 nm) GluK2 with a low affinity for KA (KD of 50–100 nm), and GluK1 are all found in the CA3. (Bloss and Hunter, 2010; Bahn et al., 1994). GluK 1 in particular is thought to be related to acute seizures (Fritsch et al., 2014).

In this study we used the supra-hippocampal kainic acid injection to avoid direct hippocampal damage by KA (Bedner et al., 2015). Still, it is unclear whether cell loss in our model following the KA injection is induced by the neurotoxicity of KA's direct actions or because of the seizures it generates. In the intra-amygdaloid KA injection model, another rodent TLE model, the injection of benzodiazepines terminates seizures and prevents hippocampal damage, although local cell loss in the amygdala persists (Ben-Ari Yet al., 1978; Ben-Ari Y et al., 1990). It has been shown that mossy fiber synapse lesions on CA3 neurons can prevent damage in the hippocampus following KA injection (Gaiarsa JL et al., 1994; Nadler JV et al., 1981; Buckmaster PSet al., 1999). These pieces of

evidence suggest that seizures generated by KA can induce secondary damage in vulnerable neurons.

1.3 Working memory

Memory refers to the capacity of the brain to store, retain and recall information. Several types of memory play different roles in cognitive processes. Widely used categorizations of memory include short-term, long-term, and working memories. Long-term memory is defined as memory processing where information is stored indefinitely, potentially for a lifetime (Cowan., 2008). Short-term memory and working memory are two closely related but distinct concepts in cognitive psychology. Short-term memory is a temporary storage system that holds information for a brief period, typically a few seconds to a minute, before it is either forgotten or transferred to long-term memory. Working memory, on the other hand, is not only the ability to maintain but also manipulate information in real-time over a timescale of seconds. Working memory is critical for daily life like problem-solving and decision-making (Baddeley., 2012).

The neuronal mechanisms of memory coding have been extensively studied over the last decades. In vivo electrophysiology and single-neuron imaging techniques led to enormous progress in investigating the neuronal mechanism of memory. Along with all kinds of memories, spatial memory has been widely studied both in human and animal models. Spatial memory is fairly simple to study in animal models and it is translatable to human navigation and spatial memory. One common task utilized to study spatial working memory is the delayed alternation task, which requires maintaining the information from the previous trial over the delay period in order to make the correct decision in the upcoming trial. In this study, we used a figure-8 maze version of the spatial alternation memory task to investigate memory deficits in epileptic mice.

1.4 Role of Hippocampus in memory

Studies about ‘’Patient H.M’’, the most famous patient in neuroscience, provided fundamental information about the role of the hippocampus in memory formation. In 1953,

patient H.M underwent bilateral surgical ablation of his medial temporal lobe structures in order to control his seizures. His seizure frequency was reduced, but he was unable to form new memory after the surgery. The first report on H.M was published a few years later by Penfield.

> ‘It is concluded that the anterior hippocampus and hippocampal gyrus, either separately or together, are critically concerned in the retention of current experience. It is not known whether the amygdala plays any part in this mechanism, since the hippocampal complex has not been removed alone, but always together with uncus and amygdala (Penfield and Milner., 1958).’’

Since then, the hippocampal formation has been extensively studied both in humans and rodents as a crucial region for memory formation and spatial navigation. Hippocampal neuronal activity reflects memory processing across a range of spatial and nonspatial tasks both at the level of fMRI signals in humans and neuronal activity patterns in humans and animals (Eichenbaum 2017). The hippocampus is able to encode memory and maintain or consolidate it over time and has been implicated in a range of working memory tasks in humans, including verbal working memory, visual working memory, and episodic working memory (Jaffe and Constantinidis., 2021). We will briefly discuss the unique properties of the hippocampus, both at single neuron and population scales, which make it capable to process and form memory.

1.4.1 Hippocampal rhythmic oscillations

A major component of the mechanisms of memory is hippocampal network oscillations. These oscillations are observed in local field potential (LFP) recordings. LFP refers to the summation of excitatory and inhibitory dendritic potentials from a population of neurons around the recording site. Those oscillations shape interactions between populations of neurons within and across brain regions (Engel et al., 2001; Fries, 2015). Hippocampus neuronal oscillations including theta, gamma, and sharp wave-ripple (SWR) are thought to be involved in different aspects of memory processing.

Theta

Theta oscillatory (6-12 Hz) activity is present in CA1, CA3, and DG LFP signals while animals run across space. Theta oscillations are considered important for spatial navigation and memory. Early studies showed that lesions of the medial septum (the major theta generator) caused impairments in a spatial memory task which were correlated with the amount of reduction in hippocampal theta rhythm (Winson, 1978, Givens and Olton, 1990; Aggleton et al., 1995; Ennaceur et al.,1996). In CA1, the amplitude and phase of theta waves change as a function of depth, with the largest amplitude theta waves being observed at the hippocampal fissure (Buzsáki., 2002). For this reason, entorhinal afferents are believed to play an important role in theta generation. That said, several other inputs including the CA3 afferents and CA1 recurrent collaterals are also involved in theta generation in CA1 (Zutshi et al., 2022).

Gamma

Gamma oscillations (30-100Hz) are present in the hippocampus during both locomotion and immobility and are known to be important in various behavioral tasks such as memory encoding (Colgin et al., 2009; Carr et al., 2012). The gamma band is usually divided into slow (25-50 Hz) and fast (50-120 Hz) (Colgin et al., 2009) and each frequency band is known to have different origins. Slow gamma (30 – 80 Hz) is thought to be generated from CA3 and spread to CA1. Gamma oscillations are also present in MEC, but in a fast frequency band (~90 Hz) (Chrobak and Buzsaki; 1998). Studies have suggested that fast gamma originates from excitatory postsynaptic currents in MEC and are later propagated to CA1 (Bragin, A. et al.; Yamamoto et al., 2014). Fast gamma is recordable in all parts of the hippocampus. Phase coupling between gamma and theta band in CA1 suggests that fast gamma is a reflection of principal neurons firing (Lasztóczi & Klausberger, 2017). The fact that gamma oscillations emerge in CA1 from different circuits (CA1 and MEC) is thought to be crucial to communication between brain regions (Womelsdorf et al., 2007). Better memory performance correlating with CA3-CA1 gamma synchronization is further proof for gamma oscillations playing a key role in maintaining the temporal organization of hippocampus sub-regions (Carr et al., 2012).

Sharp wave ripple

Sharp wave-ripples (SWRs) are characterized by a high-frequency ripple oscillations (150–250 Hz) and a large negative deflections of the LFP signal. SWRs occur in the hippocampus and EC during awake immobility and slow-wave sleep. The activity of a large population of hippocampus neurons (50,000–100,000) over a very short time (50–150 ms) generates SWR, the most synchronous event in the healthy brain (Sosa et al., 2018). The recurrent collateral system of CA3 pyramidal cells is considered to be essential to generate SWRs. SWRs are later transferred to CA1 via the Schaffer collaterals (Buzsaki et al., 1989; Csicsvari et al., 2000). During SWRs, inhibitory neurons, and pyramidal cells fire phase-locked to the ripple oscillation (Isomura et al., 2006; Ellender et al., 2010). Waking SWR activity is found to be crucial for navigational memory-guided decision-making in rats (Jadhav et al., 2012).

High frequency oscillations in epilepsy

As we mentioned before, SWRs are the most synchronized event in the healthy brain. Such a synchronized network of neurons has the potential to turn into a pathological system like epilepsy. Pathological high frequency oscillations (pHFOs) in epileptic animals has a similar frequency signature to ripples in healthy animals. pHFOs are not only used as biomarkers for the focal onset of seizures but also to study the pathological changes that transitions a healthy network to an epileptic one. In the epileptic animal, the number of SWR events decreases but the frequency of HFO (SWRs and pHFO) is similar to control animals (Marchionni et al., 2019). This data suggests that pHFO and SWRs might emerge from the same neuronal population with different network dynamics, synchronous activities (pHFO) instead of rhythmic activities (SWRs) (Valero et al., 2017). Along these lines, in epileptic animals the same principle neurons contribute to both SWRs and pHFOs but the fraction of pHFO modulated principle neurons during HFO is less than in control animals (Ewell et al., 2019).

1.4.2 Place cells

Place cells are hippocampal pyramidal cells that fire when the animal is at specific positions in the environment. Place cells were discovered by O'Keefe and Dostrovsky using single-unit recording from dorsal hippocampus more than 50 years ago:

> ‘’these 8 units responded solely or maximally when the rat was situated in a particular part of the testing platform facing in a particular direction (O'Keefe and Dostrovsky, 1971).’’

When the animal runs along a trajectory through multiple place fields, place cells fire one after another in a sequence at a time scale of ~100 ms within one cycle of a theta oscillation (Harris et al., 2003). As the animal travels through a place field, the phases of the place cell's spikes occur earlier and earlier relative to each theta cycle, a phenomenon called theta phase precession (O'Keefe and Recce, 1993). CA1 pyramidal cells require CA3 and bilateral MEC input to maintain the developed place field and effective phase precession (Davoudi and Foster, 2019; Schlesiger et al., 2015; Zutshi et al., 2022). Place cell activity coupled with theta is thought to generate an ongoing representation of the location and represent an online upcoming plan during active behavior (Sinnamon., 2005). Most place cells develop a place field immediately after entering the environment (Hill., 1978) but having a stable place field needs more exploration and attention to the environment which often takes several minutes (Frank et el., 2004; Kentros et al., 2004). Place cells change their firing area following sufficient spatial context changes such as the color and shape of the arena and novel objects (Kubie et al., 2020) - a phenomenon called remapping.

1.4.3 Spatial encoding and consolidation

Spatial encoding and consolidation could be described as the process to build an animation (or a movie) with a series of individual picture frames. Each picture is built of small pieces, like pieces in a puzzle. Place cells can be thought of as the puzzle pieces. Yet, putting the puzzle together is not sufficient to build the whole picture that leads to an animation. Pieces need to be arranged in matching frame sizes. Theta oscillations provide

the frame through the mechanism of theta phase precession. It is not surprising that this step, 'encoding', relies on the input arriving from EC. The hippocampus has been delivered the information it needs to form a memory, which can be thought about as rewatching the animation. Later, when the animal has no interaction with the environment, and is resting or sleeping (non-REM), the hippocampus 'replays' the animation in the same sequence but in a compressed time (there is no need to rewatch the animation with the original recording speed!) over and over. The longer it gets replayed the better memory performance is. This step is called 'consolidation'. Memory consolidation is associated with SWRs and it depends on the intrinsic property of the hippocampus itself (as the hippocampus already has the original copy of the animation!). Hippocampus is also able to 'replay' the same sequence in a reversed order. This 'backward reply' mainly occurs when the animal is close to a reward (Diba and Buzsáki, 2007; Karlsson and Frank, 2009; Gupta et al., 2010).

Given the crucial role of theta oscillations and SWRs in the memory process, it is not surprising that disrupting SWRs, both awake and sleep, and theta is sufficient to impair the performance of memory-dependent tasks (Girardeau et al. 2009; Ego-Stengel and Wilson 2010; Jadhav et al. 2012; Nokia et al. 2012).

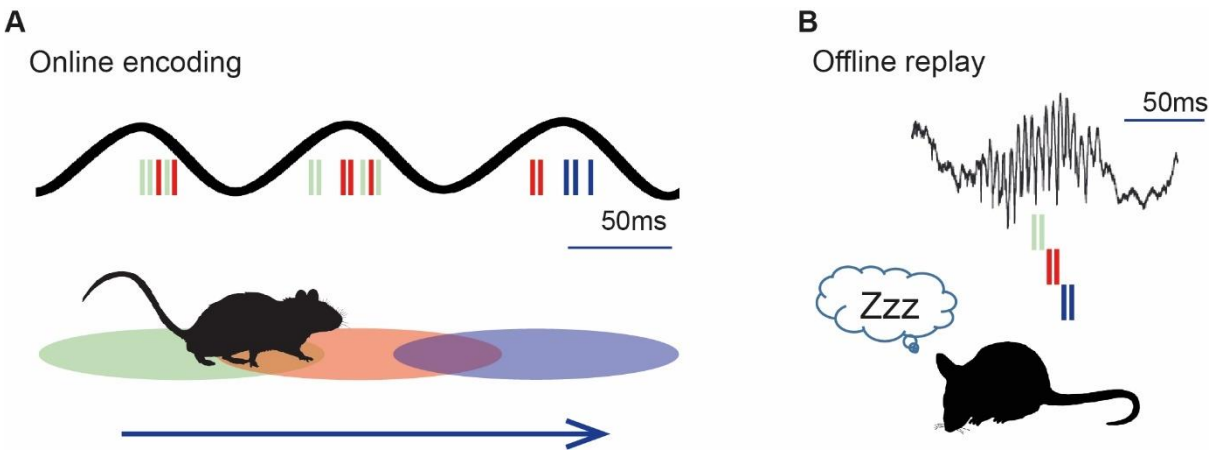

Figure.1.2 - Spatial coding in the mouse hippocampus.
A. Online encoding of spatial trajectories. During exploration, hippocampal networks generate big amplitude theta oscillation (6–12 Hz). CA1 place cells fire sequentially within the theta cycle (theta phase precession). B. Offline reply (memory consolidation). The same place cell sequence gets activated in a compressed time scale during SWR.

1.5 Memory deficits in epilepsy

Cognitive impairments such as psychological and behavioral abnormalities in TLE patients are extremely common and have a major negative influence on their quality of life (Elger et al., 2004; Sen et al., 2018). Among all cognitive deficits, memory impairment are the most common affecting seventy percent of TLE patients (Sillanpää, 2004; Whatley et al., 2010; Kerr, 2012; Fisher et al., 2018; Scheffer et al., 2018). Memory impairment in TLE patients is proposed to be a consequence of the pathological changes in the hippocampus that are associated with TLE. Given that hippocampal structures play a key role in memory, it is not surprising that the extent of the memory deficit could be related to hippocampal sclerosis (Ozkara et al., 2004; Helmstaedter and Kockelmann, 2006; Postma et al., 2020), seizure burden and duration (Aldenkamp and Arends, 2004; Elger et al., 2004; Black et al., 2010), and interictal epileptic activity (Dinkelacker et al., 2016; Ung et al., 2017). However, patients who don't show a lesion with MRI also present memory impairments (Rayner et al., 2019) meaning that cognitive impairments depend on the affected network activity rather than a structural lesion (Gauffin et al., 2021). Spatial memory in TLE patients has been studied using a variety of tasks such as virtual reality version of the hole board (Rosa Cánovas., 2011) and a human equivalent to the Morris water maze (Hort et al., 2007; Amlerova., 2012), which showed spatial navigation impairment in those TLE patients. Consistent with clinical evidence, memory impairments have been reported in animal models of epilepsy (Van Den Herrewegen., 2019). Epileptic mice do not adopt the hippocampus-dependent spatial search strategy while performing memory tasks, but use the less-efficient serial search strategy, resulting in poorer task performance (Leite et al., 1990; Stafstrom et al., 1993; Letty et al., 1995; Nissinen et al., 2000; Yang et al., 2000).

These memory deficits can be explained by degraded place coding in epileptic animals (Masala et al., 2022; Ewell et al.,2019), reduction in theta power (Inostroza et al., 2013; Marcelin et al., 2009; Winson., 1978), and aberrant phase precession patterns (Lenck-Santini and Holmes., 2008) interfering with memory encoding stage. Furthermore, pHFOs are also believed to disturb memory consolidation by activating the neurons in imprecise times and order with respect to sequential neuron activation (Binnie., 2003; Valero et al., 2017; Marchionni et al., 2019).

Altogether these studies suggest that the memory impairment is not caused directly by seizures, but instead by the hippocampal network activity changes in TLE. In this study, we investigate the role of seizure, both clinical and subclinical, as well as the hippocampal oscillations in impaired spatial working memory in epileptic mice.

Key questions

Cognitive deficits are commonly observed in TLE, both in human and animal models. These deficits are thought to be associated with the extent of hippocampus damage underlying TLE. However, not many studies have investigated the hippocampal network's dynamic in online (while performing a memory task) and offline (pre/post memory task) stages.

The goal of this thesis is to investigate hippocampal network dynamics during impaired working memory in TLE mice and answer the following key questions:

- Is the working memory deficit driven by seizures or hippocampal damage?
- Do the epileptic mice experience sub/clinical seizures while running the working memory task?
- Is the physiological network of the hippocampus altered in epileptic mice?
- Is hippocampal network disruption limited to the period of epileptiform activities or it persists during interictal periods?

2. Material and methods

2.1 Animal Ethics and Care

All the experiments were performed on male C57BL/6J mice (Charles River, Sulzfeld). All mice were single housed under a 12 h light/dark cycle, in a temperature (22 ± 2°C) and humidity (55 ± 10%). Food and water were available ad libitum except for the memory task period mice were given enough food to maintain 85% of their initial weight. All efforts were made to minimize pain and reduce the number of animals used. All animal experiments were conducted in accordance with European (2010/63/EU) and federal law (TierSchG, TierSchVersV) on animal care and use and approved by the county of North-Rhine Westphalia (81-02.04.2018.A006/2 - mittel).

2.2 Supra-hippocampal Kainic Acid Injection

Supra-hippocampal kainic acid injection is one of the modified administrations of the intrahippocampal kainic acid injection where KA is injected in the cortical area just above the hippocampus. This technique was introduced by Bedner et al. (2015). Right supra-hippocampal KA injection was used in this project to reproduce the features of the intrahippocampal injection and avoid damaging the CA1 of the hippocampus.

13 weeks old mice were injected with analgesic ketoprofen (Gabrilen, Mibe; 5 mg/kg b.w.; injection volume 0.1 ml/10 g b.w., s.c.) diluted in H2O (Ampuwa, Fresenius Kabi Deutschland). 30 minutes later, mice were anesthetized with ketamine (Medistar; 80mg/kg b.w.) and medetomidinhydrochloride (Domitor, Orion Pharma; 1.2mg/kg b.w.; injection volume 0.1 mL/10 g, i.p.). The Head was fixed in a stereotaxic and body temperature was retained at 37°C using a regulated heating plate (TCAT-2LV, Physitemp). Eyes were covered with eye-ointment (Bepanthen, Bayer) to prevent drying. After removal of the head hair, 10% lidocaine was applied on the head surface to get anesthetized locally. The scalp was removed from the skull with a scraper. Bregma was visible after cleaning the skull with 3% H2O2/NaCl solution and used as the reference to mark the coordinate. A

hole was drilled through the skull with a dental drill. A 10 µl Nanofil syringe (World Precision Instruments) was used to inject KA. the needle was left at the injection point (−1.9 mm AP, 1.5 mm ML, −1.1 mm DV) for two minutes. 70 nL Kainate solution 20mM (Sigma, Lyon, France;) was delivered using a micropump (Micro4 Microsyringe Pump Controller, WPI) for ~3 min (20nL/min). To prevent reflux of the injected fluid, the needle was retained for 2 minutes at the injection site. Control mice were given 70 nL of 0.9% sterile NaCl following the same procedure. After saturating the incision, mice were given atipamezole hydrochloride (Antisedant, Orion Pharma; 2.5mg/kg b.w.; injection volume 0.1 mL/10 g, i.p.) to terminate the anesthesia and glucose monohydrate (Glucosteril, Fresenius Kabi Deutschland; injection volume 0.25 ml, s.c.). Four hours after terminating anesthesia, Diazepam (Ratiopharm, injection volume 0.15 ml/20 g, s.c.) was injected to suppress the convulsion. Mice were kept single housed on a heat-pad and were injected with the analgesic ketoprofen to reduce pain for four days.

2.3 Microdrive Building

Tungsten wire (Tungsten 99.95%, California Fine Wire Company) was cut in approx. 30 cm in length. Two ends of the wire were attached using tape. The loop was twisted in order to have two loops and it was placed on a horizontal holder. Both of the loops were caught using a weighted hook above the magnetic stirrer (HANNA instrument). The weight started turning after increasing the power of the stirrer and formed a tetrode. Both ends of the tetrode were cut after 10 s of heating the tetrode with an air gun (Steinel).

The 8 tetrodes double bundle Microdrives (axona) that were used for this project had 4 tetrodes on each side. The first step in making micordives was to build tetrode carriers consisting of a shuttle and a cannula. Each carrier moves a single tetrode down by turning the drive screw. A cannula was glued to the shuttle after inserting it into the cannula hole. The other side of the cannula was placed in the exit hole at the bottom of the drive. The middle part of the drive was added after inserting all 8 tetrode carriers in the correct position. shuttle screws were put into the screw holes. Reference and ground screws were soldered to 4 pieces of silver wire (Science Products) were inserted from the bottom into the holes of the drive's lid and were fixed with gold pins. The lid was placed on the top of

the half built drive. Prepared tetrodes were loaded into the cannula and the free endings of each wire were inserted in the holes in the lid and were fixed with gold pins. The lid was lowered after inserting all the tetrodes. Tetrodes were cut in 2.5mm lengths and were plated (Platinum Black Plating Solution, Neuralynx) to have a resistance around 260 kΩ.

2.4 Microdrive Implantation

Mice were injected with the analgesic buprenorphine (0.05 mg/kg body weight) and ketoprofen (5mg/kg body weight) to reduce pain. 20 minutes later, mice were anesthetized with 3-4% isoflurane using an oxygen/air mixture (25/75%). Mice were placed on a regulated heating plate (TCAT-2LV, Physitemp) to retain the body temperature at 37°C. After fixing the head in a stereotactic frame, anesthesia was performed via a mask with isoflurane 1-2% at a gas flow of about 0.5 ml/minute. Eyes were covered with eye-ointment (Bepanthen, Bayer) to prevent drying. 10% lidocaine was applied on the head surface to t anesthetize the skin. Skin and other tissues were removed from the skull with a scalpel. Remaining tissue on the skull were cleaned using H2O2 and was then dried using an air spray (Kontakt Chemie DRUCKLUFT 67). A layer of Prime (OptibondTM 3FL, KERR) was applied and air-dried for 5 seconds. A drop of adhesive was applied and dried for 3 seconds. Optibond was light-cured (M+WDental) for 20 seconds to aid the adhesion of dental cement. Bregma was used as the reference to mark coordinates for implanting tetrodes. Two holes were drilled for reference and ground screws anterior to bregma and two holes for tetrode implantation bilaterally (−2 mm AP, ±1.5 mm ML) with a dental drill. Reference and ground screws were placed in the holes such that they made contact with the brain surface and fixed with dental filling material (Tetric EvoFlow). Tetrodes were placed in 70 % ethanol for two minutes and they were implanted in the cortex above the CA1 (≈0.6 mm DV) after removing the dura. Tetrodes were covered with heated gelatinous paraffin before putting dental cement around the drive. Paraffin was made with 40 g of solid wax and 50 mL oil that were mixed at 100 C. Microdrive was fixed on the head using dental cement (Paladur powder and liquid, Kulzer). Mice were injected with glucose monohydrate (Glucosteril, Fresenius Kabi Deutschland; injection volume 0.25 ml, s.c.) and were kept single-housed on a heat-pad, carefully monitored twice daily and were injected with the analgesic ketoprofen to reduce pain on the following four days.

2.5 Spatial Alternation Task

Two weeks after KA/control induction, mice were handled for five minutes daily (4-6 days). Mice were also given sugar pellets (sucrose tablet 200mg, Test Diet) in their home cage. Food restriction was started 17 days after KA/control induction. Animals were given enough food to maintain 85% of their weight. Handling and training phases were started one week earlier for the implanted group.

2.6 Training

The training was started three weeks after KA/control induction. The experiment room was quiet and had dim light conditions using a single light pointing towards the ceiling. We used the figure-8 maze (dimension 80*90 cm) to perform the spatial alternation task. After each session of the experiment, the maze was cleaned using 70 % ethanol. Training consisted of three phases:

Habituation Phase

In this phase, mice were placed in the maze to be habituated to the maze and consume the sugar pallets comfortably. Nine sugar pellets were spread in all the arms of the maze and the animal ran around freely for a maximum of 20 minutes. This phase was repeated until the mouse ate all the sugar present in the maze in less than 5-10 minutes (2-4 days) (Fig.2.1.B).

Forced Alternation Phase

Mice learned the concept of the task which is alternating between the left and right arms of the maze during this phase. They were guided to alternate between right and left using the barriers. They found a sugar pallet at the end of the arm, which is called the reward zone. This session lasted 20 minutes per day (2-4 days) until they made about 30 laps and consumed at least 20 sugar rewards (Fig.2.1.C).

Free Alternation Phase

In this phase, mice had to remember the previous trial and go to the opposite side in order to get the reward without the help of barriers. Mice are required to run 20-30 trials each day. Mice will be passed to the next phase once they performed at least 80% of correct choices in 2 days out of 3 consecutive days. Behavior group proceeded to the next phase a day after reaching the criteria. In the implanted group, food restriction was terminated after this phase. The mice ate freely and rested for 5-7 days before the surgery was performed (Fig. 2.1.D). After Microdrive implantation, this group performed the alternation test while LFP was recorded from CA1 (Fig.2.2.A and B)

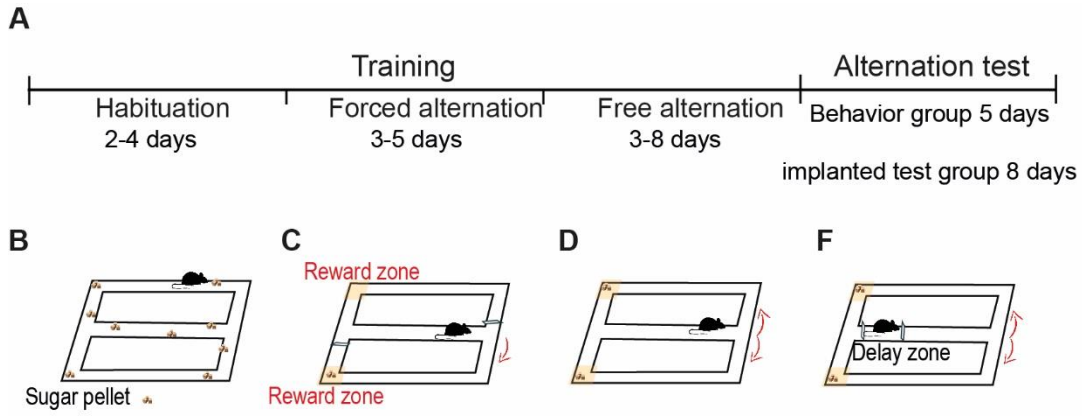

Figure.2.1 - Spatial alternation task.

A**,** Experimental timeline for training mice to perform alternation task on a figure-8 maze. B, Habituation in the maze with sugar pallets. C, In the forced alternation phase, mice were guided to alternate between right and left using the barriers and get a reward. D, Free alternation phase was performed without the help of barriers. F, In the alternation test session, mice performed delay and continuous alternation tasks. In delay alternation, mice were kept in the delay zone for 30 s before alternating.

2.7 Alternation Test

Behavior Group

For the behavior group, this phase was composed of 5 days where mice ran 40-60 laps for two sessions. The delay alternation task (DAT) session comprised trials with 30 second delay and the continuous alternation task (CAT) session had no delay involved. The order of the session type was altered randomly every day. For the delay alternation task, mice were kept in a part of the middle arm of the maze called the delay zone using barriers to make the task Hippocampal dependent (Fig.2.1.F) (Racine and Kimble., 1965). The barrier was removed after 30 seconds and mice were tested on whether they remembered the previous trial side to correctly visit the opposite side to get rewarded.

Implanted Test Group

For the implanted test group, this phase was performed after implanting the Microdrive and was composed of 8 days where mice ran 15-30 trials with delays and were placed in a monitoring chamber (glass bowl) for one hour and ran 15-20 trials for continuous alternation task (Fig.2.2.B).

2.8 LFP recording

One week after implantation, LFP recording was started using a Neuralynx system (Digital Lynx 4SX, Sample Rates 32 kHz, filtering 1-8000 Hz) and Cheetah 6.4.1. along four tetrodes that were placed in the cortex during the surgery, one was left in the cortex to be used as a reference. The rest of them were moved slowly down to the CA1 by turning the screws on the drive. Theta oscillation was used as the marker to navigate the tetrode's locations. Tetrodes with ripples and single-unit activity were selected for further analysis.

2.8.1 LFP recording, video monitoring, and position tracking

Mice were placed in a glass chamber under video monitoring (Conrad Electronic) and LFP recording for at least one hour before and after performing the alternation task on the

maze. The animal's position was tracked on the maze while it was performing the memory alternation task with a camera (PAL Cyamera Kit, Neuralynx, 25 frames/sec) that was placed above the maze. The camera detected the LED (HS LED green 10V, Neuralynx) on the drive with video tracker software (cheetah 5.0, Neuralynx). Position and LFP were synchronized by the cheetah software.

Test Implanted Group

Test implanted mice had been trained and reached the criteria for the free alternation phase before the surgery. One week after implantation, mice were trained to run with a microdrive and recording cable for 5 days by performing 12-20 trials of continuous alternation daily. During this time, tetrodes were moved down to the CA1. The test phase was started two weeks after surgery after mice were accustomed to performing continuous alternation while they were plugged. Mice performed 12-20 trials in delay alternation and 12-23 trials in continuous alternation tasks.

Naive Implanted Group

Naive implanted mice hadn’t been trained to do the task before implanting the microdrive. Spatial alternation task phases (as described before) were performed after tetrodes were placed in the CA1. Each session was flanked by 60 minutes of video monitoring/LFP recording. On the last day of the test phase (day 8), mice were put in the maze after the last monitoring to run around for 15 minutes. This session did not comprise rewards or any task roles. On the following day, mice were trained to perform the task on the same maze but in the opposite orientation and shifted reward/delay locations (Fig.2.2.C). Once animals performed 80% of correct choices in 2 days out of 3 consecutive days they started the test phase. During the test phase, mice performed 12-15 trials in 30 s DAT and 12-20 trials in CAT for 5 days (Fig. 2.2.C).

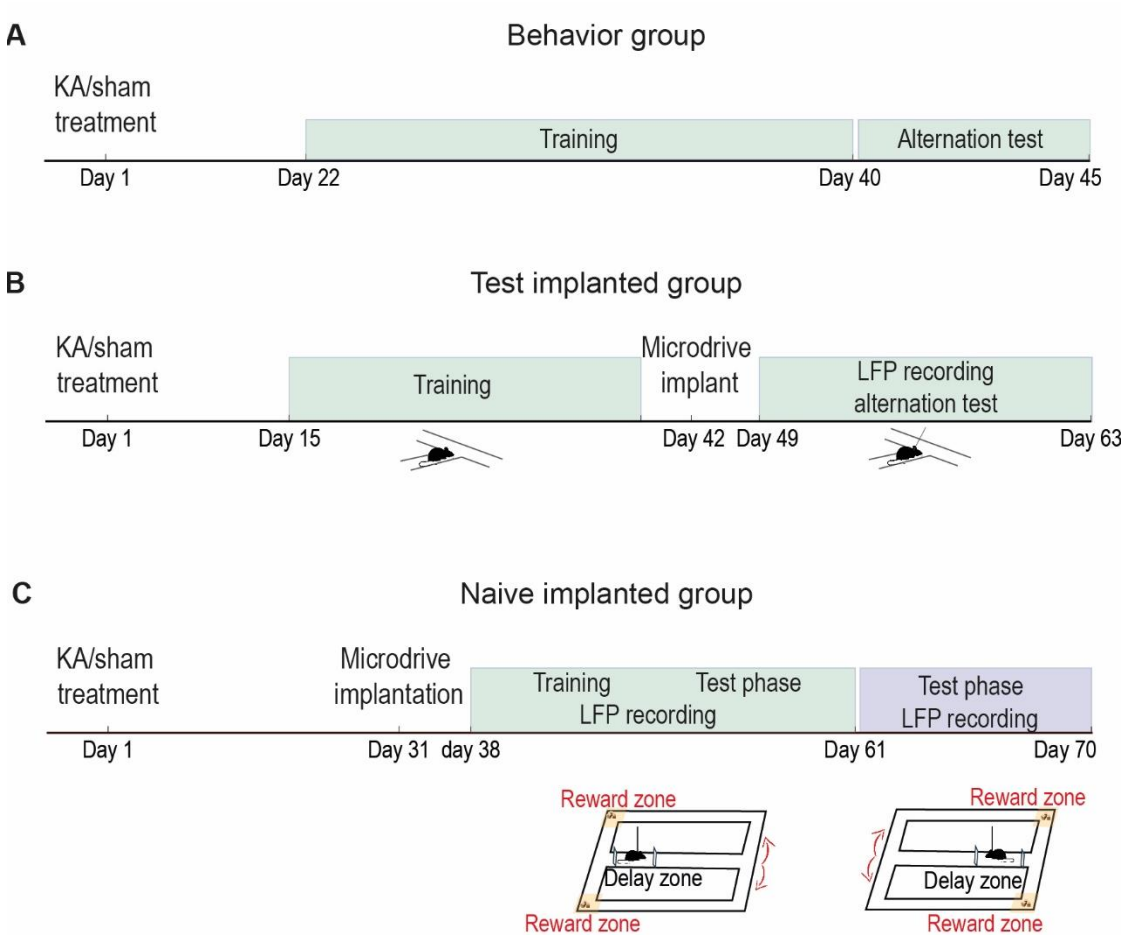

Figure.2.2. Experimental timeline to investigate memory impairment in epileptic mice.

A, Behavior group mice were trained to perform the alternation task and ran the test phase for 5 days. B, Test implanted mice were trained before the implantation and performed the alternation test while LFP was being recorded from the hippocampus. C, Naive implanted mice were trained after implanting the drive and they performed alternation tasks in two various orientations.

2.9 Histochemistry

To verify the tetrode's location and measure the severity of HS, hippocampus slices were stained by TIMM and Cresyl Violet. Mice were deeply anesthetized with ketamine (80 mg/kg body weight) and xylazine (15 mg/kg body weight). Mice were heart perfused with Sulphidesolution 0.37% followed by 4% formaldehyde in PBS. The brain was stored in 4% formaldehyde in PBS. Later, brain was placed in 30% sucrose for 1-2 days before getting cut. The brain was sectioned into 200 µm thick slices with a sliding microtome (Leica SM2010 R). Sections were mounted with 2% gelatin in PBS. 1-2 days after mounting the

sections, slides were stained by Timm. First, they were placed in distilled water for three minutes before incubating in developer solution (120 mL Gum Arabic solution, 60 mL 1.7% Hydroquinone solution, 10 mL Citrate buffer, 1 mL 0.09% Silver nitrate solution). 60 minutes later slides were dipped in warm water (50 C) and fixed in 5% Sodium thiosulfat pentahydrat. slides were hydrated in water for two minutes. After completing TIMM staining slices were dipped 10 times respectively in ethanol 70%, 80%, 90%, 100%, 100%. They were placed in xylene for two minutes before getting dehydrated 10 times in ethanol 100%, 100%, 90%, 80%, 70%, 50%. Slides were stained in Cresyl Violet for 10 s. After dehydration in ethanol 70%, 80%, 90%, 100%, 100% they were placed in Xylene and coverslip with Eukitt. Images of cresyl violet and TIMM staining slices were taken with a widefield microscope (Olympus IX81). Using ImageJ/FIJI software, we measured the thickness of CA1 and granule cell layer.

Immunostaining with a Purkinje cell protein 4 (PCP4) antibody (Santa Cruz, CA) was used to label the CA2 region. The length of CA2 layer was measured by ImageJ/FIJI software.

2.10 Data Analysis

Bilateral CA1 LFP signals recorded by Cheetah 6.4.1. was used for further analysis. Spectrum and coherence analyses were run for all mice including the control and Ka group.

2.10.1 Spectrum analysis

First, raw LFP signals were downsampled to 2 kHz. After breaking the LFP for each part of the maze including delay, stem, choice, outer arm, and reward zones, the spectrogram was calculated for frequency of 1-350 Hz using the wavelet transformation (MATLAB package). Furthermore, spectrograms for each interested frequency band were used to make a comparison between KA and control groups. The frequency bands we used in these studies were theta (6-12 Hz), beta (14-26 Hz), low gamma (26-50 Hz), and high gamma (50-120 Hz).

In order to minimize the variability driven by the exact location of electrodes, we normalized the LFP signal recorded during the memory session to the LFP recorded during the rest period. The rest period was the time with no movement (velocity< 2 cm/s) for at least 2 s while mice were in the maze. The maximum amount of normalized spectrum was detected for each LFP band. The peak of normalized LFP and the frequency of the selected peak were used for further statistical comparisons.

2.10.2 Theta coherence

Bilateral CA1 coherence across theta band (6-12 Hz) was calculated using the chronux time-frequency coherence (in MATLAB). Raw LFP signal was first downsampled to 2 kHz. After breaking the LFP for each part of the maze including delay, stem, choice, outer arm, and reward zones, theta coherence was calculated using windows steps of 2 s and 200 ms resolution.

2.10.3 Seizure Detection

All analyses on seizure detection were conducted in MATLAB using custom written code. Seizures were detected in LFP recording from CA1 using a written MATLAB code. First LFP was band pass filtered (1-200 Hz) and down sampled to sample rates 500 Hz. To detect spikes first, the mean activity was calculated for the whole session, then the threshold was defined based on mean+N*std, where N was manually defined. Peaks that were bigger than the threshold were categorized as spikes. Epileptiform events were defined as a set of spikes that were closer than 2.5 s to the previous spike. Epileptiform events closer than 3 s were merged and counted as one event. Within those epileptiform events those that lasted for at least 2 s were detected as Focal Rhythmic Discharges (FRD), and those that were longer than 10 s were detected as seizures.

2.11 FRD Classification

We used RhythSOM tool to classify FRDs. RhythSOM is a computational tool written in MATLAB by Valero et al., 2017, to classify brain rhythms, in an unsupervised way, by their waveform shapes. FRDs first go through a Principal Components Analysis (PCA). The selected PCs are fed into a Self-Organizing Map (SOM) algorithm from the SOM Toolbox. Units from the map are clusterized by a k-means algorithm. Due to the heterogeneity of epileptiform events along animals, the classifier was run on the FRDs recorded for each mouse. Short FRDs were excluded from this analysis. FRDs duration median was calculated for all the FRDs recorded for each animal and only FRDs longer than the duration median were included to be classified. The FRDs were down sampled to 30-65 Hz before being classified.

3. Results

3.1 Supra-hippocampal KA injected mice develop chronic epilepsy

In order to investigate whether kainic acid (KA) injected mice develop chronic epilepsy, we performed LFP recordings from CA1 starting seven weeks after the KA injection. Previous studies have shown that the latent period in KA supra-HC injected model is 4-13 days (Pitsch et al., 2019). Data from 8 days of recording, 3 h daily, showed that the KA injected mice developed chronic epilepsy (Sub-clinical seizure rate average= 14.11 ± 8.31 per hour, duration average = 18.44 ± 3.79 s, n=6, Fig. 3.1). Furthermore, among 7 epileptic mice at least one stage V seizure was recorded in 3 epileptic mice. One of the mice didn't have any seizures.

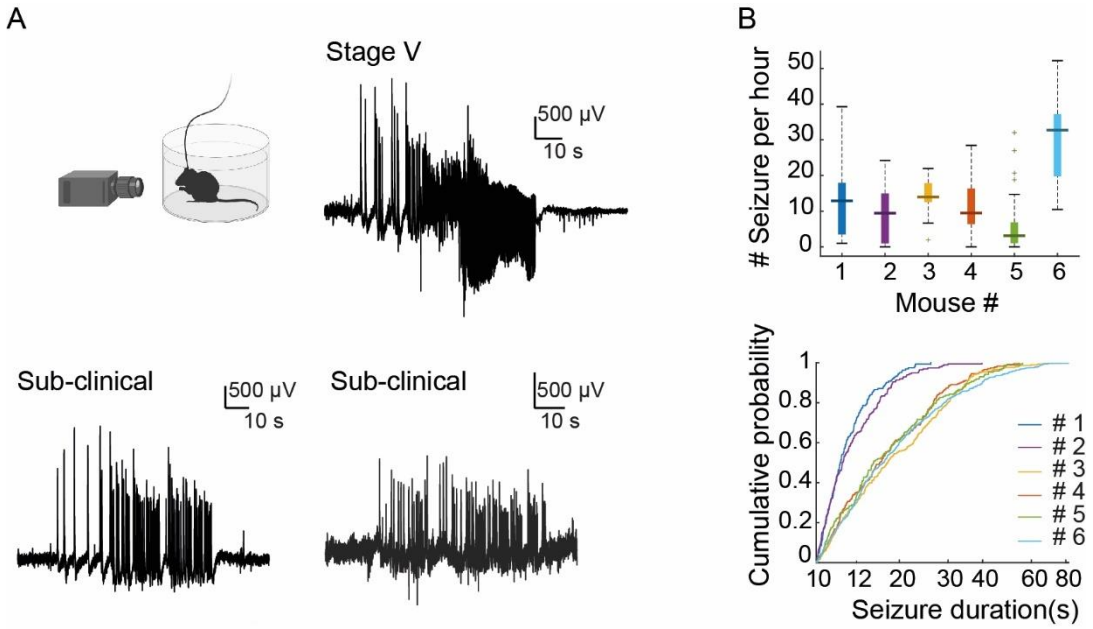

Figure.3.1 – KA supra-HC injected mice develop chronic epilepsy.
A, Examples of stage V and sub-clinical seizures recorded from CA1 seven weeks after KA injection. B, Seizure rate and duration for each KA-induced epilepsy mouse (seizure rate= 14.11 ± 8.31 per hour, duration= 18.44 ± 3.79 s)

3.2 Decrease in CA1 cell layer thickness and granule cell dispersion in KA mice

Neuronal death in CA1 and granule cell dispersion are the two major hallmarks of temporal lobe epilepsy (TLE). Therefore, we measured the CA1 cell layer and granule cell layer thickness three months after the KA injection. Consistent with previous reports, histological analysis demonstrated a decrease in CA1 cell layer thickness as well as granule cell dispersion selectively in the hippocampus ipsilateral to the KA injection (*P< 0.05, **p<0.01, one-way ANOVA, n= 6, fig. 3.2.A). Notably, we did not observe any change in CA2 length in epileptic mice using PCP4 staining (n=6 KA, n=6 control, control= 105.33 ± 9.59 µm, KA contralateral= 110 ± 14.41 µm, KA ipsilateral=119.41 ± 32.88 µm, fig. 3.2.B). This is in line with data from patients with TLE showing survival of CA2 neurons during the epileptogenesis despite the neuronal loss in CA1 and CA3 (Freiman et al., 2021).

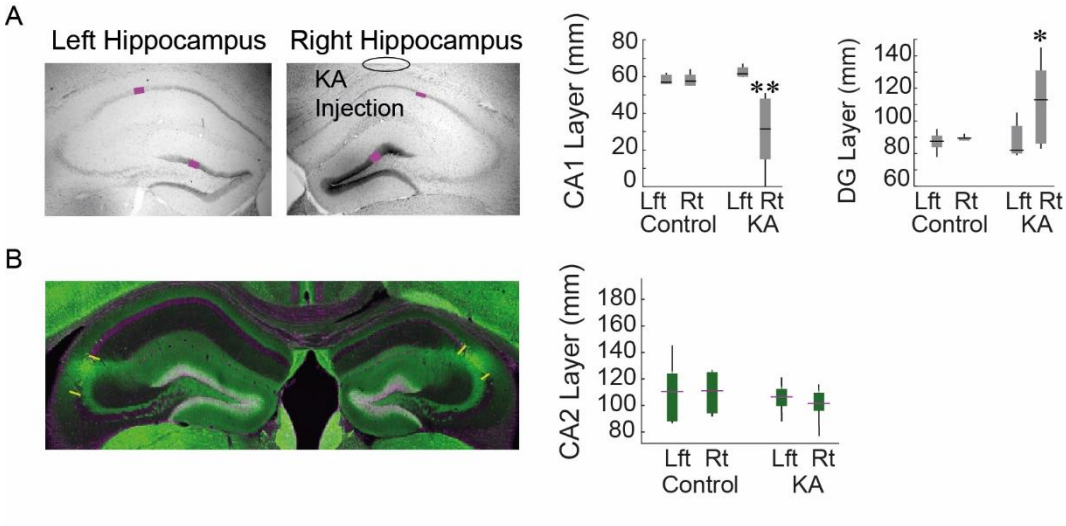

Figure.3.2 – Histopathology 3 months after KA injection.
A, cresyl violet staining shows a significant decrease in CA1 cell layer thickness as well as granule cell dispersion in the injected hippocampus (*P< 0.05, **p<0.01, one-way ANOVA, n= 6) compared to the control. B, PSP4 staining didn't show any change in CA2 length in KA-injected mice.

3.3 Spatial working memory impairment in epileptic mice

It is known that patients with TLE suffer from memory impairment (Fisher et al., 2018; Scheffer et al., 2018). To investigate memory deficits in KA-induced epilepsy mice, we

examined the spatial working memory performance using a figure-8 maze (Fig 3.3.A). To minimize the possible memory deficit caused by microdrive implantation, or behavior variability by the recording cable and recording setup, we first examined the memory performance in 13 KA mice and 9 control mice, all non-implanted (pure behavior experiment), for 5 days. In each session, mice performed the delay alternation task (DAT), which is known to be a hippocampal-dependent spatial working memory task (Racine and Kimble., 1965). The performance was calculated by dividing the number of correct trials by all trials. Epileptic mice had a deficit in performing delayed alternation (KA n=13, control n=9, KA average performance across five days= 62.15±5.34%, control average performance across five days = 73.4±1.89, two-way ANOVA). Moreover, implanted mice (see later data) also had a memory deficit in performing DAT (n=7). To examine whether the impairment is driven by changes to the hippocampus specifically, we tested spatial working memory with a continuous alternation task (CAT) using the same maze. In CAT, mice were not kept in the delay zone and were allowed to run the alternation task continuously. Epileptic mice did not show any deficit in performing the CAT (KA performance= 70.95 ± 4.15%, control performance=76.1± 2.67%, Fig. 3.3). This result suggests that memory impairment in KA mice is most likely caused by pathological changes in the hippocampus underlying epilepsy.

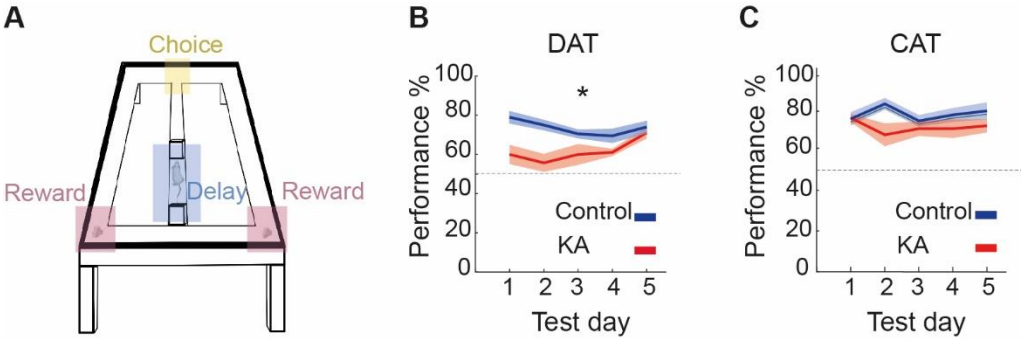

Figure.3.3 – Spatial working memory is impaired in KA-injected mice.
A, a schematic of the figure-8 maze and the zones. Mouse was kept in the delay zone for 30 s at the beginning of each trial. B, KA-injected mice have a deficit in performing the delay alternation task (DAT) ($P < 0.05$, two-way ANOVA) but no deficit in performing the continuous alternation task (CAT).

3.4 Memory impairment and damage of the hippocampus

Pathological and behavioral heterogeneities are the nature of TLE in human and animal models. To address whether the observed memory deficit is related to the severity of damage to the hippocampus, we measured the thickness of the DG and CA1 layer in animals that performed the memory tasks (Tab.3.1). This analysis was done for the microdrive-implanted mice group (KA n=7, control n=4). Epileptic mice with little to no damage in the hippocampus also didn't have a memory deficit (n=3, CA1= fig.3.4.B). However, a decrease in CA1 thickness and granule cell dispersion was observed in the rest of the epileptic mice (n=4). Within the epileptic mice with significant damage in the hippocampus, animals with mild hippocampus damage improved the DAT performance after the 4th day. Finally, mice with severe hippocampal lesions never improved in the DAT performance (fig. 3.4.D).

	CA1 layer (µm)		**DG layer (µm)**	
	Contralateral	**Ipsilateral**	**Contralateral**	**Ipsilateral**
Control	49 ± 4.61	45 ± 7.43	118 ± 23.27	110 ± 23.95
KA- no HC damage	50.91 ± 8.8	43.7 ± 16	106.06 ± 48	138.7 ± 57.19
KA- mild HC damage	49.2 ± 9.7	23.5 ±11.3	177.7± 40.6	433.22±95.08
KA- severe HC damage	24.66 ± 21.3	0	140 ± 21.5	541 ± 43.5

Table.3.1 – CA1 and DG layer thickness for control and KA mice.

3.5 Reduced ipsilateral CA1 theta power in epileptic mice

Extensive cell death in the CA1 layer and granule cell dispersion can result in the reorganization of circuits essential for encoding memory. To address the hippocampal network changes underlying epilepsy, we recorded local field potential (LFP) bilaterally from the CA1 cell layer, while mice were performing the DAT (Fig.3.5.A). In one of the epileptic mice, there was no tetrode in the contralateral CA1 cell layer (KA ipsilateral n=7,

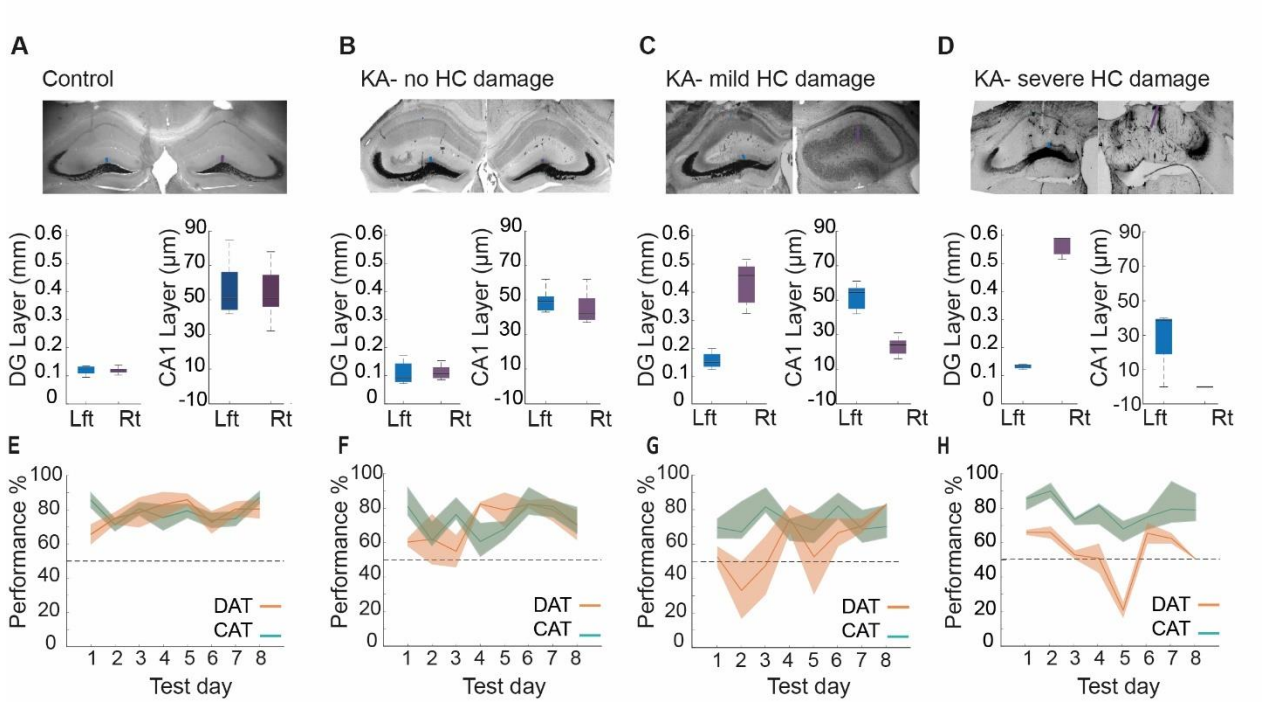

Figure.3.4 – Memory impairment and hippocampus damage in KA-injected epilepsy mice. A, Top, TIMM, and cresyl violet staining in a control hippocampus, bottom, Box plots of CA1 and DG layer thickness of the left and right hippocampus. B, in n=3 KA epileptic mice, there is no difference between the left and right hippocampus. C, some grade of cell loss within CA1 and granule cells dispersion in the right hippocampus in n=2 KA mice. D, severe HC damage including cell loss within CA1 in the left hippocampus and no CA1 cell in the right hippocampus in addition to drastic granule cell dispersion In n=2 KA mice. E, Eight days of working memory testing show no difference between DAT and CAT in control mice. F, There was no dramatic difference between DAT and CAT in the epileptic group with no significant pathology. G, Epileptic mice with Granule cell dispersion and CA1 neuronal cell loss improved their WM impairment after six days. H, WM impairment over the eight days of WMT in the epileptic mice with severe HC damage with no progress in memory over 8 days of performing the memory task.

we linearized the trials and split them based on the mice's location in the maze including delay, stem, choice, outer arm, and reward zone (Fig.3.5.B). We then calculated the average spectrum for the LFP recorded from control mice (ipsilateral CA1), and KA mice (ipsilateral and contralateral CA1) (Fig.3.5.C). LFP from ipsilateral CA1 in epileptic mice consists of big power in the low-frequency band (1-5 Hz) driven by epileptiform activities. The other major difference in epileptic ipsilateral CA1 was the decrease in theta power (6-12 Hz) which was consistent in all parts of the maze (Fig.3.5.D & E). Studies have shown that the speed at which mice move is related to theta rhythm activity (McFarland et al.,

1975). However, theta power reduction in ipsilateral CA1 of the KA-induced epilepsy mice is independent of the animal's speed since there is no difference between the running speed of KA mice compared to control mice (Control mice speed= 7.46 ± 1.14 cm/s, n=5, KA mice speed= 7.47 ± 1.31 cm/s, n=6, Fig. 3.5 F and Tab.3.2).

3.6 Similarity of normalized CA1 LFP spectrum in epileptic and control mice

To address whether the CA1 dynamics during various phases of the memory task in epileptic mice are similar to those in control mice, we normalized the LFP signal, from the CA1 cell layer, in the DAT to the LFP recorded during the rest period. The rest period was defined as the time with no movement (velocity< 2 cm/s) for at least 2 s while mice were in the maze. The maximum amount of normalized spectrum was detected for each LFP band (theta (6-12 Hz), beta (14-26 Hz), low gamma (26-50 Hz), and high gamma (50-120 Hz)), and mice location on the maze (delay, stem, choice, our arm and delay zone). Analyses of oscillation frequencies and power revealed just one significant difference between epileptic and control mice which is an increase in high gamma frequency in ipsilateral CA1 of epileptic mice while running on the outer arm. These findings suggest that although the structural pathological changes in the epileptic hippocampus lead to major alternations like theta power reduction, the hippocampus still maintains a considerable amount of functionality similar to the control group during different phases of the spatial working memory task (Fig.3.6 and Tab.3.2).

	Delay	**Stem**	**Choice**	**Outer arm**	**Reward**
Control (n=5)	1.98 ± 0.21	11.03 ± 1.8	11.22 ± 2.82	10.07 ± 1.48	3.03 ± 0.09
KA (n=7)	2.66 ± 0.52	10.08 ± 2.78	10.65 ± 3.59	10.9 ± 2.35	3.03 ± 0.81

Table.3.2 – Locomotion speed (cm/s) of KA and control mice for each part of the maze.

3.7 Reduced theta coherence between left and right hippocampus in epileptic mice

To address whether the left and right hippocampi in KA-induced epileptic mice are capable of communicating and functioning as a united structure similar to control mice, we calculated the coherence of CA1 theta oscillations. In order to study the coherence before

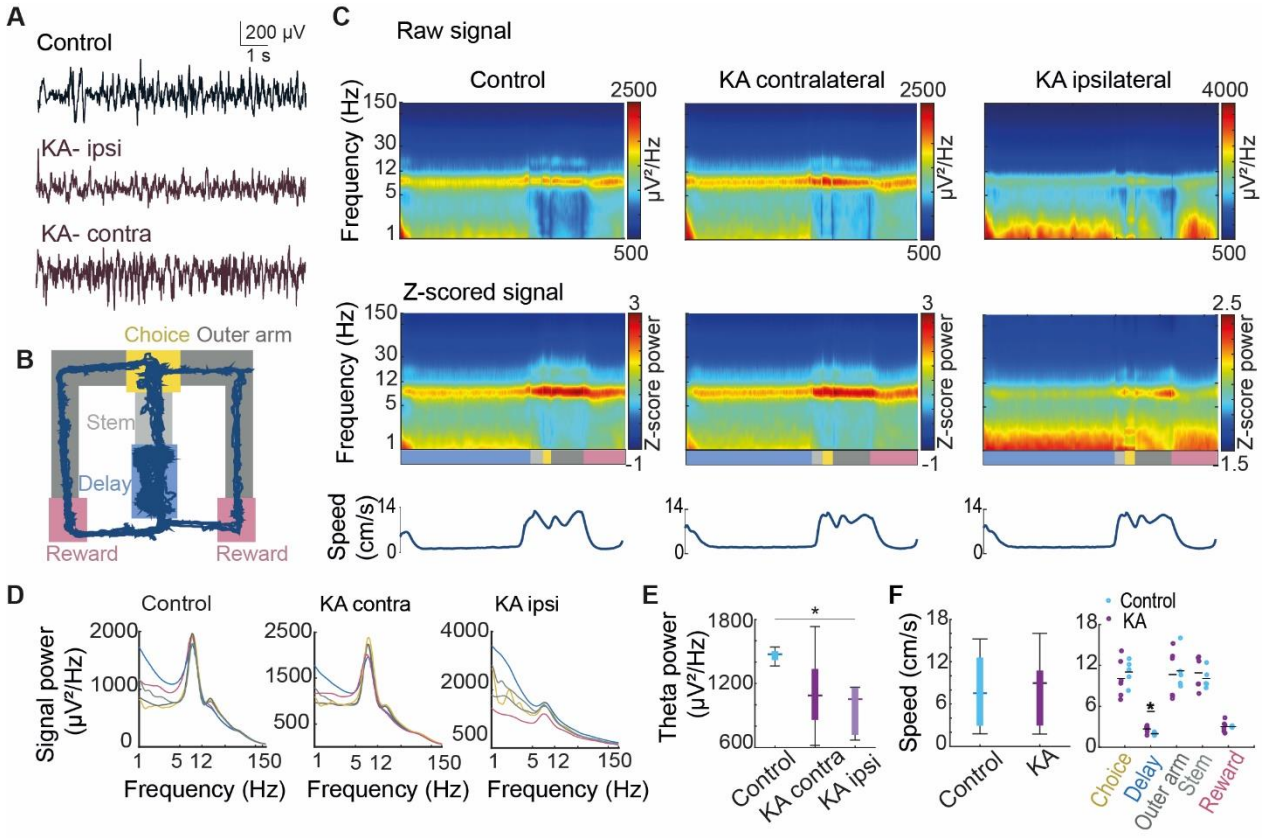

Figure.3.5 – CA1 LFP in control and KA mice while performing delay alternation task (DAT).
A, 10 s of CA1 LFP for control and KA mouse (ipsilateral and contralateral). B, The trajectory of the mouse running the figure-8 maze and different zones of the maze. C, top, the spectrum of raw CA1 LFP recorded from figure-8 behavior sessions, averaged for all mice. Middle, The z-scored spectrum of the raw LFP spectrum. Bottom, the average speed for all trials of figure-8 behavior sessions. D, Power spectrum density (PSD) plot of different zones of the meze including delay, stem, choice, and reward zones for control and KA mice (contralateral and ipsilateral). E, There is a reduction in the theta band in KA-ipsilateral CA1 compared to control and KA-contralateral ($P< 0.05$). F, left, speed of control and KA mice running the figure-8 behavior sessions show no significant difference. Right, the speed plot for each zone of the maze shows that KA mice were more active than control mice during the delay.

and after decision-making, we applied coherence analysis on signals from stem- choice and outer arm zones. We found that epileptic mice showed a theta coherence reduction in both stem-choice and outer arm zones compared to control mice (KA mice n=6, theta coherence outer arm= 0.71 ± 0.73, theta coherence stem-choice= 0.74 ± 0.09, control mice n=4, theta coherence outer arm= 0.78 ± 0.05, theta coherence stem-choice= 0.81 ± 0.05, Fig.3.7.D). Interestingly, in both control and epileptic mice, CA1 LFP signals were more coherent while mice were in the stem-choice zone compared to the outer arm of the maze (Fig.3.7.C). This result suggests that theta coherence is influenced by the different phases of the memory task. These results suggest that mice may require higher theta coherency between two hippocampi when the alternation direction decision is being made.

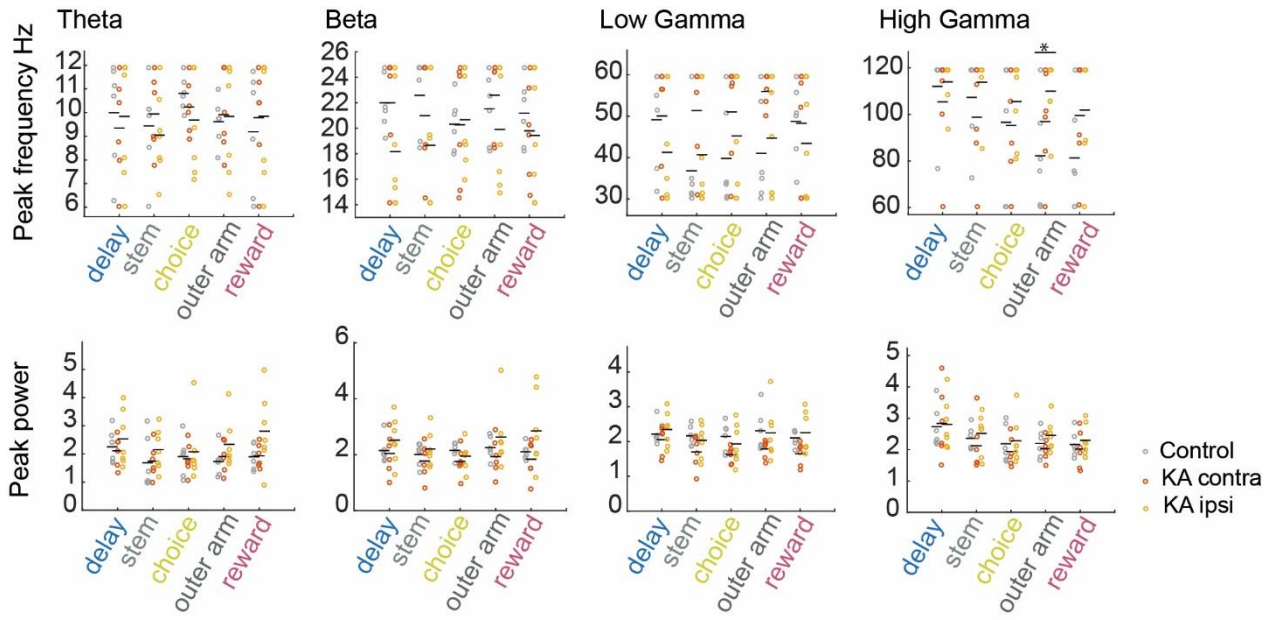

Figure.3.6 – Peak frequency and peak power of the normalized LFP signal LFP to rest period.
Peak of high gamma band in ipsilateral CA1 in KA mice is significantly higher than control mice in outer arm of the maze.

		Theta	Beta	Low-Gamma	High-Gamma
Control (n=6)	**Frequency (Hz)**	9.80 ± 0.55	21.51 ± 0.62	43.11 ± 3.8	95.9 ± 7.79
	Peak	1.89 ± 0.2	2.13 ± 0.17	2.18 ± 0.15	2.32 ± 0.32
KA ipsilateral (n=7)	**Frequency (Hz)**	9.65 ± 1.14	19.36 ± 1.26	43.07 ± 6.38	109.03 ± 10.19
	Peak	2.38 ± 0.39	2.43 ± 0.59	2.16 ± 0.23	2.47 ± 0.26
KA contralateral (n=6)	**Frequency (Hz)**	9.84 ± 0.67	21.12 ± 1.9	51.34 ± 4.7	99.19 ± 6.61
	Peak	1.9 ± 0.07	1.87 ± 0.8	1.75 ± 0.08	2.16 ± 0.31

Table.3.2 – Peak frequency and peak power of the normalized LFP signal.

3.8 Memory impairment in epileptic mice is well correlated with a reduced level of theta coherence

The fact that epileptic mice had memory impairment despite contralateral CA1 staying intact suggests that synchronization between two hippocampi is crucial for memory. To test this idea we calculated the correlation between spatial memory performance and theta coherence. This analysis showed that theta coherence of two hippocampi is well correlated with epileptic mice's memory performance ($P < 0.05$, Fig. 3.7.G). We then examined if theta coherence is altered by FRDs or Interictal spikes (IISs). We calculated the coherence between hippocampi during focal rhythmic discharge (FRD), 2 s before the FRD onset, and 2 s after FRD offset. The theta coherence was reduced significantly following the FRD offset for 2 s (Fig. 3.7. E). We did the same comparison between 2s pre and post-IIS and we saw an increase in theta coherence after the IIS (Fig. 3.7.E). To test the idea that theta coherence reduction is driven by FRDs, or it persists regardless of epileptiform activities, we calculate theta coherence excluding the trials carrying FRDs. No difference was observed between theta coherence for the trials with FRD and trials with no FRD (519 trials with no FRDs, in n=6 mice, theta coherence= 0.71 ± 0.73; 572 trials with and with no FRDs, in n=6 mice, theta coherence= 0.71 ± 0.69, Fig.3.7.F). This result indicates that FRDs are not responsible for changes in the theta coherence. We also showed that the reduction in theta coherence is not emerging due to a decrease in theta power (Fig.3.7.F).

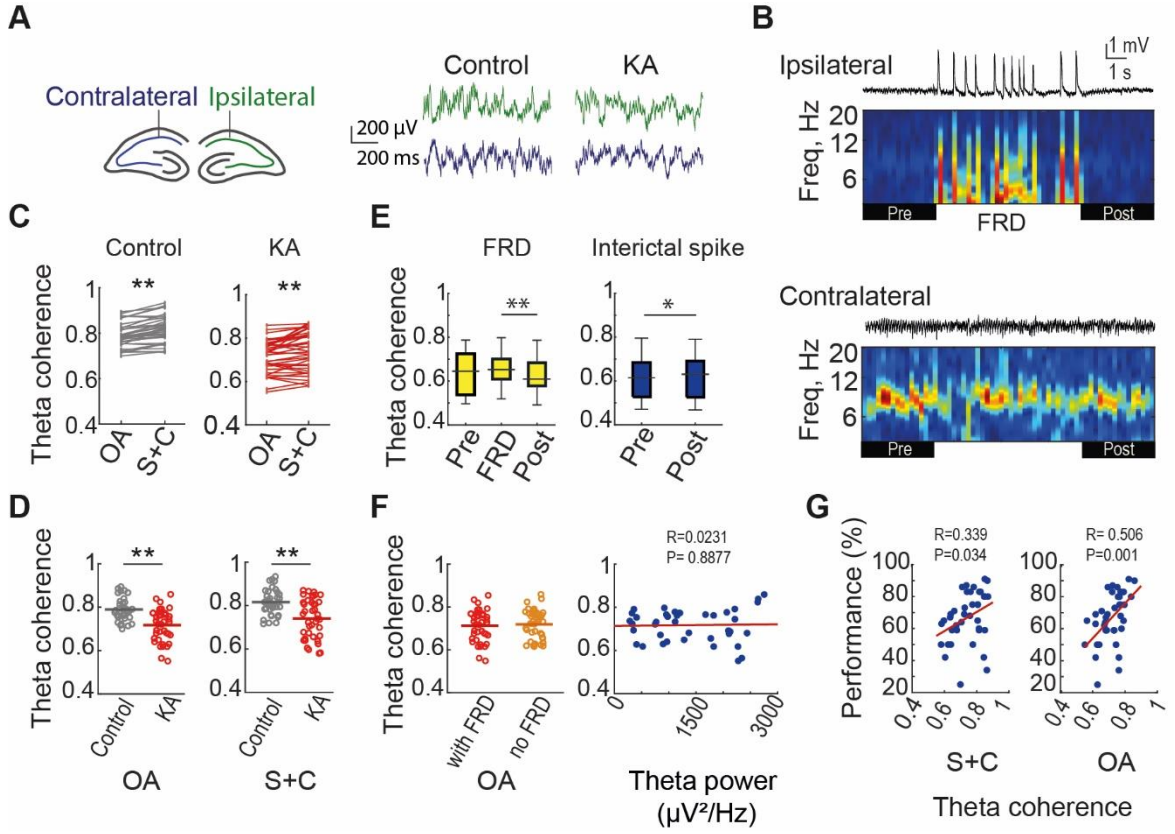

Figure.3.7 – CA1 theta coherence is reduced in KA mice.
A, Examples of CA1 LFP in control and KA mice. B, LFP signal from both hippocampi pre, post, and during an FRD. C, Theta coherence reduction in KA and control mice while the mouse is in the outer arm (OA) compare to stem and choice (S+C) zone ($P < 0.005$). D, Theta coherence is decreased in KA mice both in the outer arm (OA) and stem choice (S+C) zones. E, Theta coherence reduces after FRD offset ($P<0.005$) and increases after interictal spike ($P<0.05$). F, Theta coherence reduction in KA mice is not caused by FRDs or a decrease in theta power in the ipsilateral hippocampus. G, Theta coherence is significantly correlated with spatial working memory performance in KA mice.

3.9 Memory performance is not related to seizure burden in pre-behavioral sleep

Previously we showed that epileptiform events are occurring on the maze during task performance (Figure 3.7B). To study the direct effect of seizures on memory impairment we investigated whether memory impairment is caused by the high rate of seizures before running the memory task. We analyzed the quality of one-hour sleep sessions before performing the spatial working memory, measuring the rate of seizures and focal rhythmic discharges (FRDs). Seizures are defined as a set of ictal spikes in CA1 LFP that last longer than 10 s (explained in the materials and methods chapter) and FRDs are the sets

of interictal spikes that last more than 2 s. Analysis revealed no significant correlation between the burden of both FRDs or seizures and memory performance (n=6) (Fig.3.8.C). in order to avoid the correlation being driven by subjects this analysis was done across animals instead of sessions since the frequency of seizures is variable within epileptic mice. The same analysis was done for each animal across sessions and no correlation was seen between the seizure burdens and memory performance.

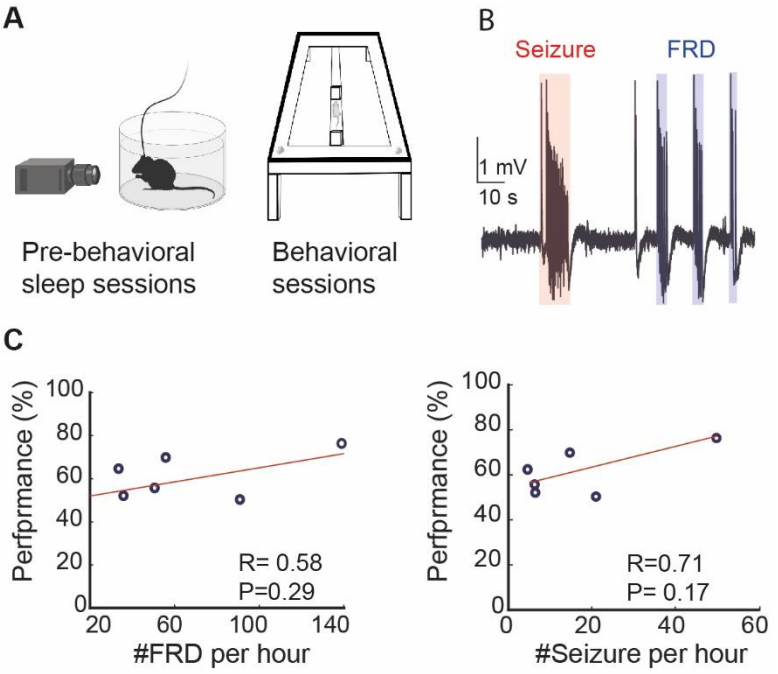

Figure.3.8 – Memory deficit in epileptic mice is not dependent on seizure burden during sleep sessions.

A, a schematic of the pre-behavioral sleep and figure-8 behavior sessions. B, examples of FRD (a set of interictal spikes > 2s) and (a set of interictal spikes > 10s) in a CA1 LFP recording. C, rate of seizures and FRDs are not correlated with spatial working memory performance obtained of figure-8 maze.

3.10 Good memory performance in epileptic mice is well correlated with FRDs being spatially stable

In order to address the direct interference of FRDs with spatial working memory in epileptic mice, we detected the FRDs in CA1 LFP recording that occurred while mice were performing the memory task. We saw that epileptic mice experienced frequent FRDs while performing the memory task (Fig.3.9.A) However, correlation analysis didn't show any relationship between FRD rate and memory performance (Fig.3.9.D). These data suggest that the deficit in spatial working memory in epileptic mice is not driven by the burden of FRDs. We also found that FRDs tend to occur at a particular area on the maze and

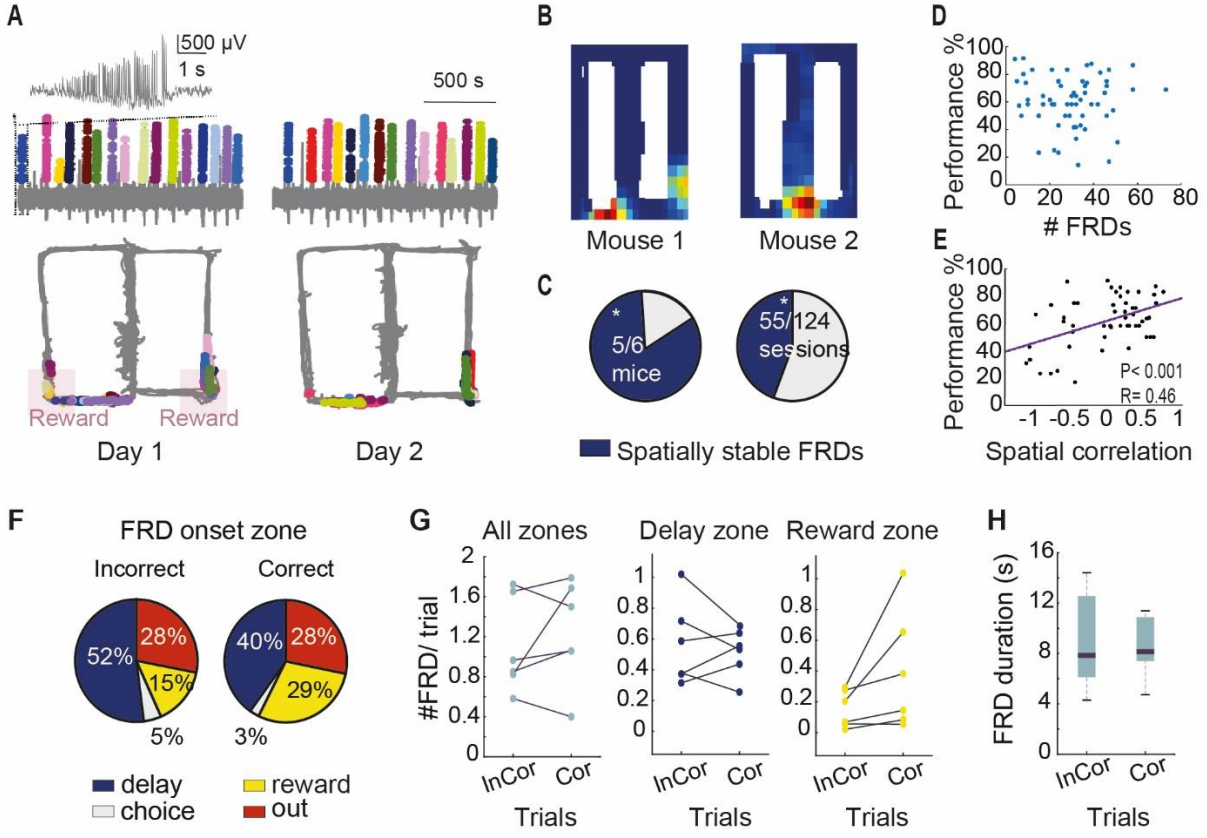

Figure.3.9 – FRDs occur frequently while mice performed the spatial working memory task.
A, CA1 LFP shows frequent FRDs while KA mice run the delay alternation task (DAT). FRD spatial map shows FRDs occur around reward zones over trials and days. B, FRDs spatial heat map for DAT session for two KA mice. C, FRDs are spatially stable for 5/6 KA mice and 55/124 DAT sessions. D, Memory performance is not correlated with FRD burden. E, Memory performance is significantly correlated with FRDs being spatially stable. F, The proportion of FRD onsets for all parts of the maze is shown. The proportion of FRD onset on the reward zone in correct trials is more compared to incorrect ones. The proportion of FRD onset on the delay zone in correct trials is less compared to incorrect ones. G, Left, Rate of FRD on correct trials compared to incorrect trials. Middle and right, FRD occurrence on delay and reward zones are shown for incorrect and correct trials. H, The duration of FRD on correct trials compared to incorrect trials.

surprisingly maintain a similar spatial pattern over trials and days. To test whether this pattern would be expected by chance, we calculate the chance spatial correlation for FRDs during each DAT session (Fig.3.9.B). This analysis revealed that FRDs are spatially stable in 5/6 epileptic mice and 55/124 memory sessions (Fig.3.9.C). Interestingly, FRD spatial stability is well correlated with memory performance (Fig.3.9.E). This means that memory performance is not affected by the burden of FRDs but by the spatial pattern of

FRD occurrence in the maze. To investigate whether the initiation zone of the FRD impacted the animal's performance, we separated the correct and incorrect trials and plotted the distribution of the FRD onset zones(n=6) (Fig.3.9.F). Calculating the rate of FRD per trial did not reveal any significant difference between the FRD occurrence in the delay zone for incorrect trials compared to correct trials (onset FRD in delay zone for incorrect trial= 0.5649 ± 0.2703 FRD/trial, onset FRD in delay zone for correct trial= 0.518 ± 0.516 FRD/trial, Fig.3.9. G). We did the same analysis for the reward zone and no difference was observed between correct and incorrect trials (onset FRD in reward zone for incorrect trial= 0.153 ± 0.119 FRD/trial, onset FRD in reward zone for correct trial= 0.392 ± 0.388 FRD/trial, Fig.3.9. G). We didn't see any difference in the rate of FRD occurring during correct trials compared to incorrect trials regardless of the FRDs location on the maze (incorrect trial= 1.0999±0.4734 FRD/trial, correct trial= 1.2497 ± 0.5167FRD/trial, Fig.3.9. G). To investigate if longer duration FRDs can affect performance, we compared the duration of FRDs on correct trials and incorrect trials, but we didn't see any difference (incorrect trials FRDs duration= 8.84±3.87 s, n=506, correct trials FRDs duration= 8.44±2.45 s, n=256. Fig.3.9. H). This data suggests that the duration and burden of FRDs are not the responsible factors driving memory impairment in epileptic mice.

3.11 FRDs are similar to SWR

To investigate the FRD spatial preference in the maze, we calculated the fraction of FRDs for each part of the maze (delay, choice, outer arm, and reward). We saw that most FRDs are initiated in the delay and reward zone (Fig.3.10 C). This observation is similar to a large body of studies showing the occurrence of awake sharp wave ripple at the reward and delay zone. Therefore, we detected SWR in the CA1 LFP in control mice and quantified the proportion of SWR for each part of the maze. We observed that more than half of SWR occur at the delay and reward zone (Fig.3.10.E). We also did the same analysis for high frequency oscillation (HFO) in epileptic mice, which are associated with FRDs, and found that more than half of HFO occurs in delay and reward zones (Fig.3.10.D). This result suggests that FRDs might overlap in mechanism with SWR. To test this idea we looked at the FRD spectrum (Fig.3.10.A). interestingly, we found that

FRD onsets comprise fast oscillation similar to SWR. Finally, we measured the occurrence rate of SWR in control mice, and FRD, and HFO in epileptic mice in pre and post-behavioral sleep sessions. A significant increase was found in the rate of HFO in epileptic mice and SWR in control mice during post-behavioral sleep sessions compare to pre-behavioral sleep sessions and behavioral sessions, while no such change was observed in the FRD rate (Fig.3.10.C, D, and F). Altogether, this data suggested that hippocampal discharges overlap in mechanism with sharp wave ripples.

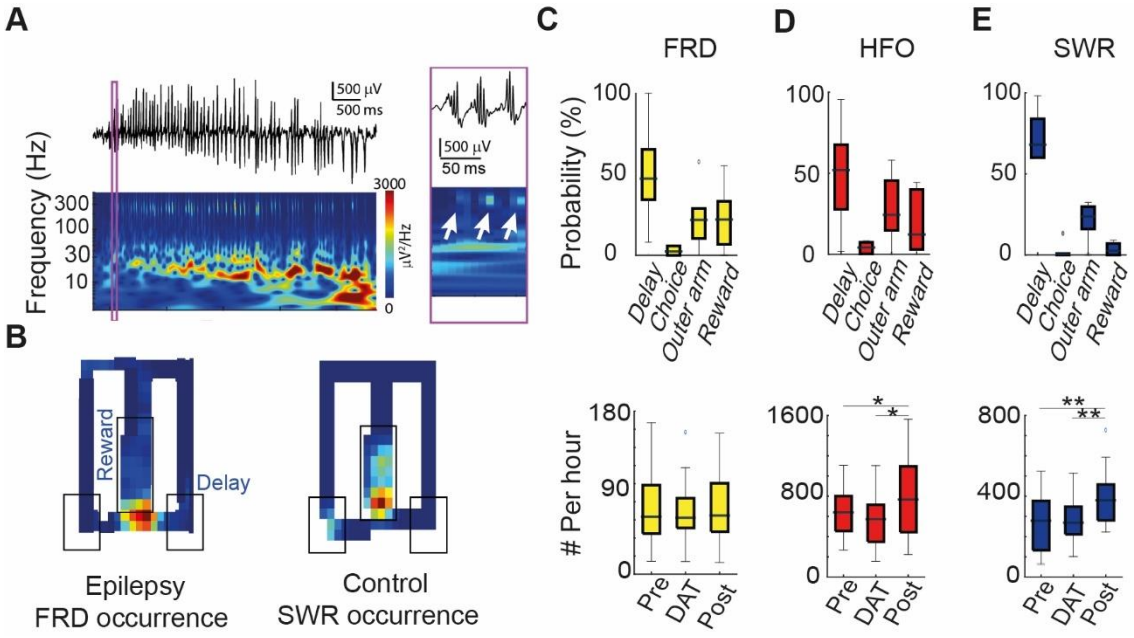

Figure.3.10 – FRDs in KA mice overlap in mechanism with SWR in control mice.
A, FRD onset comprises multiple fast ripples (FR). B, The heat map of FRD occurrence in a DAT session for KA mouse and SWR in control mouse. C, The most fraction of FRDs occur on the delay and reward zones. FRD rate in KA mice is not different during pre, post, and behavior sessions. D, the most fraction of HFOs occur on the delay, outer arm, and reward zones. HFO rate in KA mice is significantly more post-behavior sessions compared to pre and behavior sessions ($P< 0.05$). E, the most fraction of SWRs occur on the delay and outer arm zones. SWR rate in control mice is significantly more post-behavior sessions compared to pre and behavior sessions ($P< 0.005$).

3.12 FRDs shift their spatial field on the maze following the change of reward zone

The spatial pattern of FRD occurrence in the maze suggests that FRDs mainly initiate in the zones where mice stay still, like the delay and reward zone, or consume rewards. To

test this idea, we changed the timeline of the experiment so we could record from CA1 while mice were learning the task including the alternation roles and the location of rewards and delay zones. The idea was to investigate whether the spatial stability of FRDs was accompanied by learning the reward and delay zones or not. The preliminary data from one KA-induced epilepsy mouse showed that FRDs didn't emerge during the training including habituation, forced alternation, and continuous alternation. FRDs were detectable in CA1 LFP recording since the third day of DAT. They occurred in the delay zone and preferably in the left reward zone for the next 5 days. On the last day of DAT (8th day) FRDs mainly occurred at both reward zones. To investigate if FRD occurrence is related to reward, the mouse was placed in the maze after the post-behavioral sleep session on the last day while there was neither a reward nor any memory task-related object on the maze. The mouse was free to explore the maze without consuming any reward. Despite the absence of reward and memory task rules, the mouse experienced FRDs when it was around the previous reward zones. This observation could not be explained by mice occupation in zones of the maze (Fig.3.11.B). Later, we trained the mouse to perform the task on the same maze but in the opposite orientation and shifted reward/delay locations. We saw that FRDs developed at new reward locations and persisted at previous reward locations. These preliminary data indicate that the FRD spatial map developed gradually along with learning the memory task rules and concepts. Most importantly, these data suggest that the FRD occurrence map on the maze is not necessarily related to consuming the reward (Fig.3.11). This experiment is an ongoing project, being followed by Dr. Laura Ewell lab at University of California – Irvine

3.13 FRD classification

Our previous finding regarding FRDs overlapping in mechanism with SWR raised the idea that various types of FRDs might occur during memory task sessions compared to pre and post-behavioral sleep sessions. To investigate this idea, we used RhythSOM a computational tool written in MATLAB (by Valero et al., 2017) to classify FRDs. Since the FRD waveforms are varied across animals, the classifier was run for each mouse individually. We then distinguish clusters that were common between memory and pre/post-behavioral sleep sessions as common clusters and the ones that occurred just

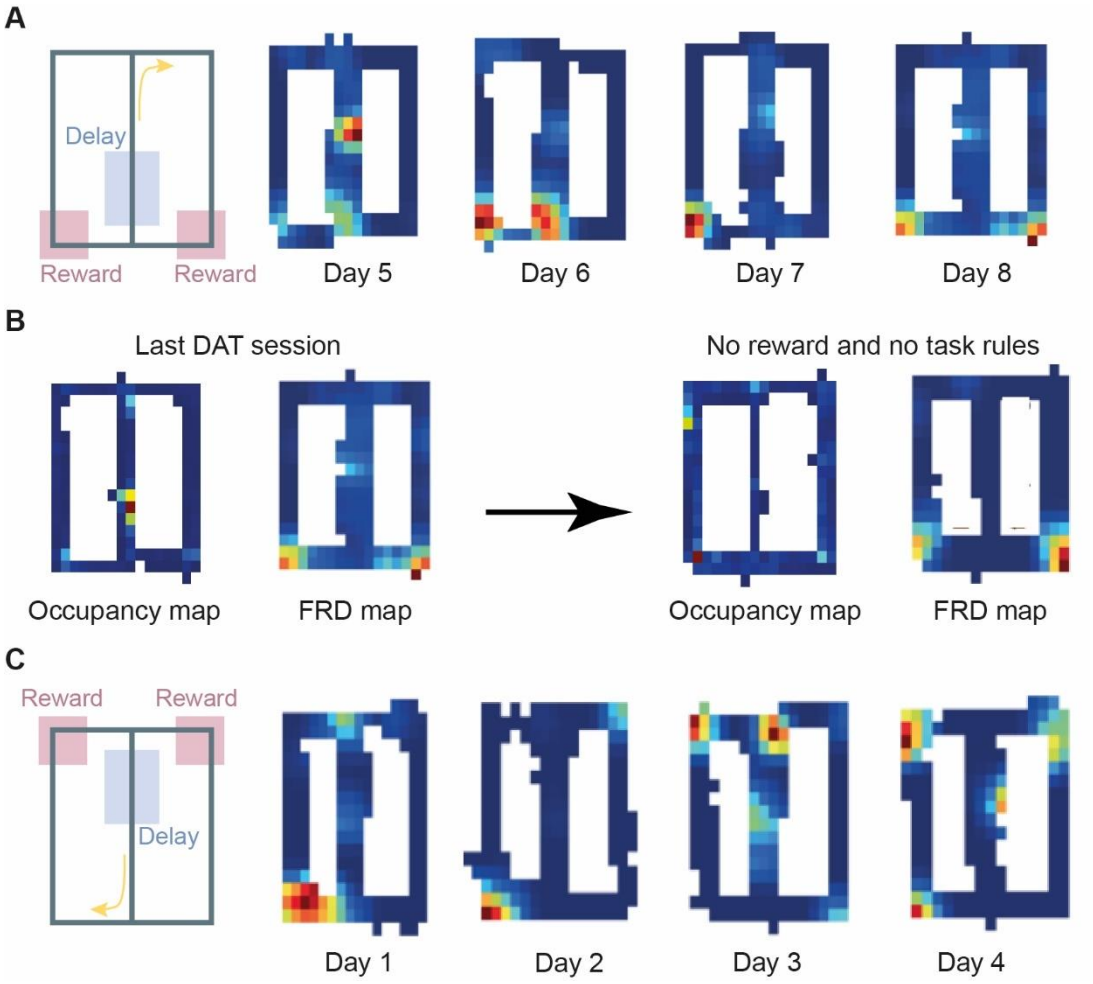

Figure.3.11 – FRDs spatial preference shifting after switching the reward zones.
A, Schematic representation of figure-8 maze and zone locations. Heat maps of FRD occurrence on the maze for four consecutive days of DAT sessions. B, Left, occupancy map of KA mouse performing the DAT (8^{th} day) and FRD map for the same sessions. Right, occupancy map of the KA mouse running on the maze (8^{th} day, after DAT session) while there are no reward or memory rules. FRD maps are similar in both sessions. C, Schematic representation of the shifted reward and delay zones as well as in the opposite orientation for running the task. FRD heating map for four days of performing DAT with the newly introduced maze ordinations. FRD spatial map developed gradually along with learning the memory task rules and concepts.

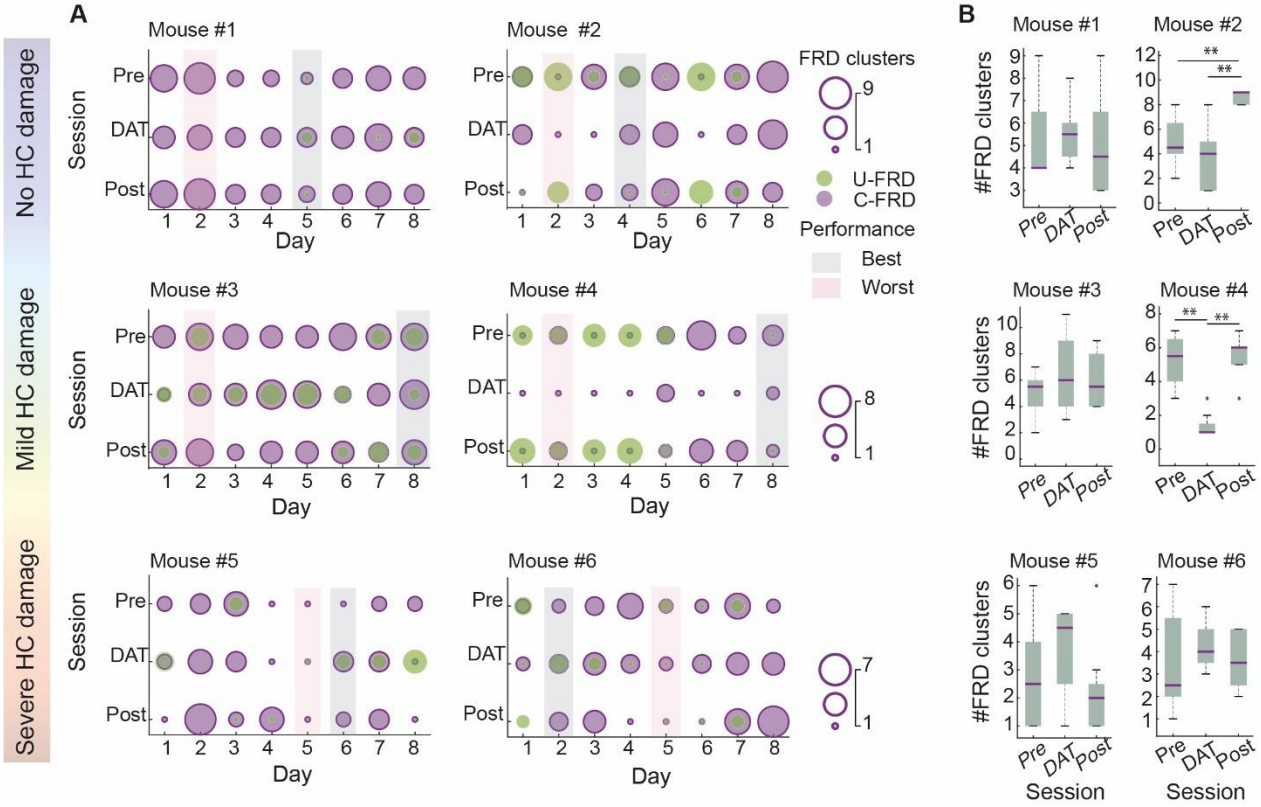

Figure.3.12 – FRD clusters for 8 days of recording including figure-8 maze and pre/post-behavioral sleep sessions
A, FRD clusters for individual mice are shown for all recording sessions. Unique FRDs (U-FRD) are FRD clusters that only occur on behavior or sleep sessions. Common FRDs are the clusters of FRD that are common between behavior and sleep sessions. Top, mice have no hippocampus damage. Middle, mice with mild HC damage. Bottom, mice with severe hippocampus damage (also see Fig. 3.4). The best and worst performance of the mice in 8 days of performing the DAT are shown for all the mice. B, Box plots of the number of all FRD clusters obtained from behavior and pre/post-behavioral sleep sessions are shown for each mouse. # mouse 2 with no HC damage showed a significant increase in the number of FRD clusters in post-behavioral sleep sessions compared to pre-behavioral sleep and behavioral sessions ($P < 0.005$). # mouse 4 with mild HC damage showed a significant increase in the number of FRD clusters in pre and post-behavioral sleep sessions compared to the behavioral session ($P < 0.005$).

during memory or pre/post-behavioral sleep sessions as unique clusters (average number of all FRD clusters =8.83 ± 1.16, n=6). KA mice were divided based on the HC damage (Fig. 3.12 also see Fig. 3.4). Since the FRD waveforms are diverse across mice, it was impossible to define a pattern that is appliable to all the KA mice and can explain the memory deficits with regards of the alternation in FRD cluster numbers or other features.

The only significant changes that we observed in the number of FRD clusters occurred in two KA mice during the different recording sessions (Fig. 3.12). To investigate the possibility of unique FRD clusters in memory impairment, we quantified the number of FRDs that belong to different FRD clusters for the days with the best and worst memory performance (Fig.3.12. A&B). Comparing FRD rates during pre and post-behavioral sleep sessions for the best and worst performance didn't show any meaningful difference.

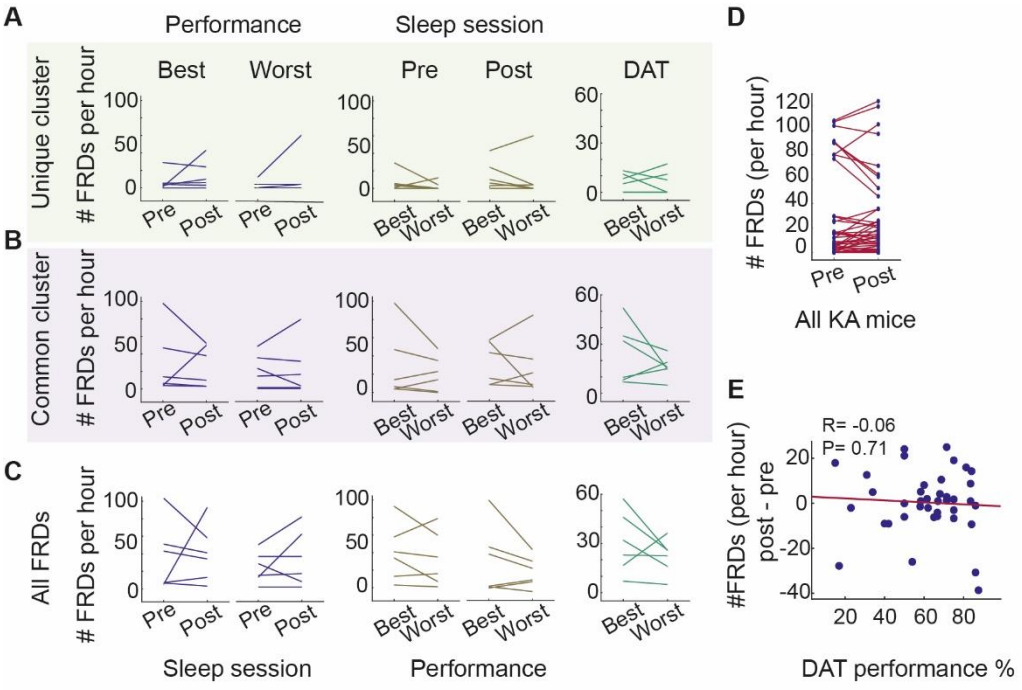

Figure.3.13 – FRD clusters.
A, left, the rate of FRDs belonging to the unique FRD cluster didn't change on the days with the best and worst performance in pre and post-behavioral sleep sessions. Middle, the rate of FRDs belonging to the unique FRD cluster didn't change in pre and post-behavioral sleep sessions for the best and worst performance. Right, the rate of FRDs belonging to the unique FRD cluster didn't change on the delay alternation task (DAT) session for the day with the best performance compared to the day with the worst performance. B, Same figures as A, but for the rate of FRDs belonging to the common FRD clusters. C, Same figures as A, but for all the FRDs. D, The rate of FRDs belonging to the most prevalent cluster in DAT is shown for pre/post-behavioral sleep. E, Subtracted FRD rate of the most prevalent cluster in DAT in pre-behavioral sleep from post-behavioral sleep session is not correlated to the mice's memory performance.

The same analysis was done for all the FRDs but no difference was observed (Fig.3.13.C). Following the previous result showing an increase in HFO and SWR rate in post-behavioral sleep sessions (Fig. 3.10), we expected to see an increase in the number of FRDs belonging to the most prevalent FRD cluster in DAT sessions in post-behavioral sleep sessions compared to pre-behavioral sleep sessions. However, comparisons of the FRD numbers in pre and post-behavioral sleep sessions didn't show any difference (Fig. 3.13.D). Further, we subtracted the FRD rate of the most prevalent cluster in DAT in pre-behavioral sleep from post-behavioral sleep sessions and correlated with memory performance. Still, no correlation was observed. Together, these data suggest that the occurrence of FRDs belonging to the most prevalent cluster of DAT pre/post-behavioral sleep sessions doesn't support memory performance.

In summary, we showed that spatial memory impairment in epileptic mica is not driven by epileptiform activities. Our data showed that theta power is reduced in ipsilateral CA1 in KA mice but not in contralateral CA1. Theta coherence between two CA1 is significantly decreased in KA mice and it is correlated to memory performance. We also found that epileptic mice experience frequent FRDs while running the memory task. These FRDs are spatially modulated. Interestingly the memory performance is not affected by the burden or duration of FRDs but by the timing of them.

4. Discussion

Cognitive impairments such as memory deficits are a common comorbidity of TLE that affect 70-80 % of TLE patients (Sillanpaa et al., 2004; Kerr, 2012). Among those impairments, memory impairment is the most common one which has been directly associated with hippocampal sclerosis and seizure burden (Ozkara et al., 2004; Helmstaedter and Kockelmann, 2006; Aldenkamp and Arends, 2004; Elger et al., 2004). The fact that memory deficits present in the latent (silent) period and interictal periods, and also in patients with no sign of lesion in the brain (Hort et al., 1999; Dinkelacker et al., 2016; Rayner et al., 2019) suggests that memory impairments are not always seizure driven or dependent on a structural lesion. In this study we used in vivo LFP to record from CA1 while KA-induced epilepsy mice ran a spatial working memory task, to study the CA1 network changes underlying memory deficit in TLE.

4.1 Spatial working memory deficit in epileptic mice

In order to study the hippocampal role in memory deficits in KA-induced epilepsy mice, we used a hippocampal dependent spatial working memory task: the delayed alternation task (DAT) (Racine and Kimble., 1965). Although epileptic mice had a memory deficit in the delay alternation task, they showed no impairment in performing the continuous alternation task (CAT), which is not hippocampal dependent. This finding suggests the impairment might be caused by hippocampal scelorsis. To address this idea, we categorized the KA mice based on hippocampal damage. KA mice with no significant damage (n=3) also did not show any significant impairment in spatial working memory but memory was impaired in mice with modest and severe hippocampal damage (n=4). However, KA mice were able to improve their memory performance after the 4th day of running the task. This data is in line with a study of TLE patients showing a significantly lower performance in the first days of verbal recalls and a virtual reality task based on real life experience (correctly bought products) (Grewe et al., 2014). These results reveal an

improvement in spatial navigation and memory over 8 days of performing the task. A possible explanation for such observation is that epileptic mice develop a hippocampal independent strategy over time, allowing them to improve memory performance.

The idea that memory is impaired as a result of seizures, both in the chronic phase (recurrent seizures) and status epilepticus has been studied in animal models and humans. It is fairly simple to investigate the possible relationship between seizure burden and memory deficit using widely accessible EEG and a memory task. In addition to immediate seizure interfering with memory, it is known that post ictal states usually lead to a decreased memory ability (Boukhezra et al., 2003). However, our findings don't support this idea, since we didn't see any correlation between the burden of sub-clinical seizure during pre-task long sleep or while running the task. Such correlation analysis is challenging since the frequency of seizures is variable within epileptic mice. In fact, the mice with more frequent seizures did not show significant damage in the hippocampus and didn't have memory impairment compared to the control mice. Moreover, correlation analysis within each mouse (across 8 sessions of DAT) did not show any relationship between memory and seizure burden. Among the seven KA mice, two of them had clinical seizures (stage V) while performing the DAT. Interestingly, the performance was not less than the animal's average memory performance during the same trial and the following day (mouse 1: average performance= 50.3% -+22.7%, performance on the trial with stage V seizure = 67%; mouse 2: average performance= 55.7% -+13.7%, performance on the trial with stage V seizure = 58%).

These findings suggest that the seizure burden is not the sole factor driving memory impairment in epilepsy. In fact seizures, themselves are the result of pathological and morphological changes that lead to the reorganization of neuronal circuitry and abnormal neuronal activities. This newly reshaped hippocampal network is most likely the subject of seizures as well as memory impairment. Next, we will discuss the CA1 network changes in epileptic mice that are involved in memory impairment, including changes in theta and sharp wave ripples.

4.2 Theta power reduction is related to memory deficit in epileptic mice

Hippocampal theta rhythms are present during exploration and REM sleep. As the animal runs through a place field, the place cell fires on the peak of the theta cycle. As the animal transverses the entire place field, the place cell fires on earlier and earlier phases of the theta cycle (Harris et al., 2003; O'Keefe and Recce, 1993). This phenomenon, so called theta phase precession, provides consistent frames for place cells to organize spatial information that is comprehensible for hippocampus memory process. Therefore, the capability of the hippocampus to encode spatial information while animals explore the environment relies on place code, providing the puzzle pieces, and theta phase precession, as the consistent matching frame to integrate puzzle pieces and create the whole picture and eventually build an animation representing the environment.

Degraded place coding, place cell instability, and reduced information content have been shown in different TLE models in animals (Masala et al., 2022; Shuman et al., 2020; Zhou et al., 2007; Lenck-Santini and Holmes., 2008). On top of that, a disturbance in phase precession in epileptic animals is thought to be related to memory deficits (Lenck-Santini and Holmes., 2008; Shuman et al., 2020). Given that hippocampal theta rhythm plays a key role in memory, it is not surprising that decreased theta power could disturb memory encoding and result in memory impairment (Winson., 1978; Yamaguchi et al., 2004; Buzsaki, 2006). Theta power reduction is one of the major alternations in epileptic hippocampal networks and it's known to be correlated to spatial deficits (Inostroza et al., 2013; Chauvière et al., 2009). Investigation of such changes is not possible in humans due to the lack of control groups. However, In humans, unsuccessful memory formation during encoding is associated with decreased theta oscillations (Fell et al., 2006). It is found that in epileptic patients theta power decrease after interictal spikes (Fu et al., 2018) which is consistent with animal studies showing a reduced theta power following interictal spikes and pHFO (Ewell et al., 2019). In this study, we also showed a decrease in theta power in epileptic mice while performing the spatial memory task. The fact that this observation stayed intact after excluding the trials carrying FRDs, short sub-clinical seizures, suggests that this reduction is caused by the reorganization of the hippocampal circuitry underlying TLE. These alternations could interrupt the spatial encoding by

disturbing the puzzle frame coordinations, which would lead to a defective, nonstable, and noisy animation.

Although theta power reduction on contralateral CA1 in epileptic mice didn't reach a significant level compared to control mice, it may still not be sufficient to prevent memory impairment. These data suggest memory encoding might require adequate theta rhythms in both hippocampi. In the other words, although one hippocampus is making the proper animation, it is not sufficient to form a memory since two animations are not synchronous. to test this idea we calculated theta coherence between two hippocampi. Notably, we showed the coherence of the bilateral CA1 theta band is significantly less in epileptic mice compared to the control group. The fact that theta coherence reduction is independent of theta power decrease on the ipsilateral side suggests that the quantity of the animations created by both hippocampi is not as important as the animations being synched together for memory encoding. We show for the first time that memory performance is correlated with the synchronization of two hippocampi during theta oscillation in epileptic mice. Following the idea of memory impairment being correlated to theta coherence, we hypothesized that both hippocampi are required to be highly synched so the animal can choose the correct side of the maze to visit. Interestingly, we showed that theta coherence between two hippocampi is significantly higher in the choice zone compared to the outer arm of the maze, both in KA and control mice. One explanation is that theta sequences, the sequence of place cells firing orders by theta phase precession, is not only related to the animal's current location but also reflects near future or upcoming trajectory while running a memory task (Wikenheiser and Redish., 2015). Consequently, both hippocampi may need to function as an integrated structure so the correct choice is made.

Such desynchronization between hippocampi is found in humans, on the scale of several minutes to hours before seizures (Mormann et al., 2003). Therefore, we calculated the synchronization for theta band pre and post-epileptiform events, interictal spikes and FRDs. Our finding showed a decrease in theta coherence preceding the interictal spikes (scale of 2s). We didn't see a similar theta coherence reduction before the onset of FRDs. Interestingly, our data showed a theta coherence increase during the FRDs, which is in line with the previous study done in TLE intrahippocampal KA mice (Meier et al., 2007). However, this increase might be a result of high amplitude spikes of the FRDs overlapping

with theta frequency. Since the main aim of is the Meier study was to address the mechanism leading to seizures and to develop an approach to predict the seizures, they did not investigate the hippocampi synchronization following the seizures. Here, we also measured the theta coherence after the FRDs offset and saw no difference during the 2s following and preceding the FRDs. This data suggest that the disrupted synchrony between two hippocampi over the theta frequency is not the result of seizures, but pathological changes underlying TLE.

Neuronal loss and circuit reorganization in the hippocampus, entorhinal cortex, and possibly in upstream structures such as the septum in TLE are involved in theta power and coherence reduction. The medial septum is one of the brain regions that is shown to be affected by seizures originating in the hippocampus, especially GABAergic neurons (Garrido Sanabria et al., 2006; Turski et al. 1986). Given the fact that septohippocampal activity is crucial for theta generation, such changes could lead to altered theta oscillation in the hippocampus. Change in intrinsic and synaptic properties of oriens-lacunosum-moleculare (O-LM) interneurons, which play an important role in theta generation, results to shift firing from theta to gamma frequency (Dugladze et al., 2007). This change is thought to be related to increased excitatory input in these interneurons. This alternation leads to an imbalanced phase-locked firing of the interneurons causing altered network dynamics such as theta power reduction (Inostroza et al., 2013). This imbalance in activity timing might be the result of miscoordinated input arriving at stratum lacunosum molecular (SLM) and molecular layer (ML) from the entorhinal cortex. Structural change in entorhinal such as cell loss in layer III MEC, which is normally strongly theta modulated, and laminar expansion of layer II synapses (perforant pathway) can lead to disrupted theta oscillation in the hippocampus (power reduction) and can also interrupt the hippocampi synchronization over theta rhythm (Deshmukh et al., 2010; Henriksen et al., 2010). Degeneration of commissural projections to the contralateral hippocampus, especially mossy cells and inhibitory cells in the hilus can be also responsible for reduced theta coherence (Bouilleret et al., 1999; Riban et al., 2002).

Altogether our data showed that theta power reduction and coherence between hippocampi interfere with the encoding step of the memory coding. Next we discuss the CA1 network dynamics during the second step of memory coding, memory consolidation.

4.3 Memory consolidation plays a key role in initiating the FRDs

Studying the memory deficit in epilepsy has been mainly focused on memory consolidation, the fundamental process of forming long term memory. This phase of memory formation is known to be accomplished during sharp wave ripples, SWRs (150–250 Hz), a high-frequency oscillation in the hippocampus. During SWRs, the hippocampus replays the same sequences that previously were created by the hippocampus during the active exploration several times but in a compressed time scale. Such a process leads to a high amount of synchronization, indeed the most synchronous network event in the healthy mammalian brain. The recurrent collateral system of CA3 pyramidal cells provides the proper hippocampal circuits that make the hippocampus able to produce such rhythmic cell firings. Slight alternation in CA3 circuits is sufficient to convert the physiological network to a synchronized network in pathological conditions. Pathological high-frequency oscillations (pHFOs) in epileptic animals, with a similar frequency signature to ripples in healthy animals, have been the center of a large body of epilepsy research. pHFOs have been proposed to play an important role in memory deficits. During consolidation, the encoded memory needs to be replayed in the proper sequence that matches the encoding phase (which can be thought of as rewatching the encoded animation). In this metaphor, cell firings at a misplaced point are similar to randomly playing picture frames that lead to a noisy/erroneous animation (Valero et al., 2017). Replying such disorganized animation that does not precisely represent the previous experience of the animal, and thus disturbs the memory process. In this study, we addressed the role of epileptiform events during both encoding, theta state, and memory consolidation, non-theta state with a high chance of SWR, in memory impairment.

Studies on human patients have shown that about 70-80% of seizures occur during sleep (Lanigar and Bandyopadhyay., 2017). Surprisingly our data showed that epileptic mice experience frequent FRDs while running a memory task as well as during sleep. Interestingly, memory impairment is neither related to the seizure burden during long pre-behavior long sleep nor while running the task. Our data showed that FRD frequency and duration are not different for incorrect trials compared to correct trials.

Although most seizures in epileptic patients are spontaneous, a small fraction of patients (5%) have reflex seizures. Reflex seizures are known to be triggered by specific stimuli

(Das and Luczak., 2022) such as flashing lights, hot water, reading, and even toothbrushing (Miller et al., 2010; Navarro et al., 1973; Meghana et al., 2012). Interestingly there are rare reflex seizures that are not evoked by extrinsic stimuli but by a particular intrinsic brain state such as abstract reasoning, chess, decision making in chess, calculation, spatial task, and thinking (Tatsuzawa et al., 2010; Forster et al., 1975; Wilkins et al., 1982; Goossens et al., 1990). Following this idea, we separated maze zones with respect to the memory coding phases. Our data from control mice showed that most SWRs occured in the delay zone and the outer arm when mice approachedreward zones. In the DAT, the delay and reward zones as the area which are mainly involved in memory consolidation. In contrast,the outer arm and choice zones are thought to be more involved in encoding and thus involve theta phase processing. Less than 4 % of FRDS occured in the choice zone, therefore we exclude that zone for further analysis. Notably, our data showed that FRDs mainly occur in delay, reward, and the outer arm (mostly when the animal approaches the reward). This spatial pattern suggested that FRDs might be triggered by the mechanism involved in memory consolidation, awake ripples. To investigate the neuronal network mechanism responsible for that similarity, we focused on the LFP spectrum preceding the FRD onset. Our data showed that many FRD onsets comprise fast oscillation similar to SWR which is in line with the patient's data showing an increase of HFOs shortly before the seizure that is largely localized to the seizure focus region (Fisher et al., 1992; Khosravani et al., 2009). A possible explanation for these observations is that a reduction in inhibitory activity preceding a seizure in the focal microcircuits could result in the pHFOs. Later, at seizure onset, these local circuits become bigger relating to seizure propagation (Bragin et al., 2005, Bragin et al., 2007, Engel et al., 2009).

An increase in ripple rate during post-behavior sleep sessions compared to the pre-behavior sleep sessions supports the role of SWRs in memory consolidations. As there is new memory related activity that should be consolidated over the post-behavior session. However, comparing the FRD frequency during pre and post-behavior sleep sessions in KA mice didn’t show any difference. In other words, memory consolidation (SWRs), is most likely not the only stimuli to evoke seizures in epileptic mice. This data is in line with clinical studies showing that patients may experience both spontaneous and reflex seizures. In addition, we did the same analysis for ripple-like events including ripples, HFO

in the FRD onsets, and pHFO, in the KA mice group compared to ripples for control groups. In terms of the spatial pattern of occurrence, ripple-like events in KA mice and ripples in control mice are similar, meaning they occur mainly in the delay zone, outer arms, and reward zone. However, the number of ripple-like events also increases during post-behavior long sleep compared to behavioral and pre-sleep behavior sessions. These findings suggest that SWRs and ripple-like events in epileptic mice emerge from the same neuronal population, but with altered neuronal dynamics. As we discussed before, memory consolidation relies on cell firing in a proper sequence(rhythmic activity of neurons). Although the participated neurons might be similar in both events, in TLE the physiological rhythmic activities become pathological synchronous activities (Ewell et al., 2019; Valero et al., 2017).

SWRs are internally originated oscillations generated by CA3 strong depolarization of population bursts that later transferred to the CA1 through the Schaffer collaterals (Buhl and Buzsaki., 2005; Leinekugel et al., 2002). Recent studies have shown that CA2 is also involved in SWRs generation (Oliva et al., 2016). Further investigation of precious neuronal mechanisms underlying the physiological and pathological ripples requires single-unit recordings from CA2 and CA3 neurons. Though our experiment can not prove the overlap in such similarity in neuronal scale, an ongoing project by Dr. Brittney Boublil at Dr. Laura Ewell's lab has provided another piece of evidence supporting the idea showing CA3 pyramidal neurons are activated during focal hippocampal discharges.

4.4 Memory impairment in TLE mice is related to FRDs spatial stability

Triggers of reflex seizurea needs to be consistent. Earlier we suggested that FRDs on the maze might be evoked by memory consolidation. In order to address the consistency of that hypotheses we calculate the FRD spatial stability for each session. In simple words, we investigate whether FRDs tend to occur at a particular place on the maze every time the mice run through it. We found that FRDs are spatially stable in more than half sessions. Interestingly, FRDs being spatially stable is correlated to memory performance meaning that the timing of the FRD occurrence plays a more important role than their frequency or

duration. Many cells get activated during FRDs, and such untimely population activity could interfere with the ongoing memory coding process since the accurate timing of cell firing is essential for both memory encoding and consolidation. The interference extent could be minimized if the FRD occurrence is spatially limited to a particular part of the maze throughout the behavior session. Further, we investigated if FRDs in a particular zone could be related to the correctness of the trial. We saw that FRDs originated in the delay zone during the incorrect trials are more frequent compared to the correct trials. Although it did not reach the significant levels and it is driven by the subjects (3/6 KA mice). In contrast, FRDs on reward zones are more frequent in the correct trials compared to the incorrect ones. Recent findings showed that CA1 place cells shift their firing place field toward the rewards (Jarzebowski et al., 2022; Kaufman et al., 2020). In addition to this overrepresentation of reward zones, many place cells are active shortly (few spikes) close to the goal location rather than their place field (Lenck-Santini et al., 2002). The increased population activity around the reward can be sufficient for pathological circuits in CA1 in TLE mice to evoke a FRD.

Yet, one could argue that the animal spends more time in the reward zone of the correct trials to consume the reward, so the most frequent FRDs are most likely related to the awake SWRs. To address that argument, after the last session of DAT the KA mouse was placed in the maze, while there was no reward and no task rules. Interestingly we saw that FRDs are still present around the 'previous reward zone' although there was neither reward consuming nor alternation behavior related to the memory task. Although the mouse spent more time around the reward zone, some other parts of the maze have been equally occupied despite no FRDs being recorded there. Furthermore, we changed the reward zones to the other side of the maze as well as the orientation for running the task. The idea was to test if FRDs move to the new reward location or persist in the old reward location. We saw that FRDs started to be initiated in the new reward zones on the 2nd day of the recently introduced maze orientation while they were still present at the old reward zone. Even though the mouse was consuming the rewards at the newly introduced reward locations and not searching for a reward at the old reward location. In addition to rewards being represented by by CA1 place cells, there are also dedicated reward-associated cells. Reward cells become active at multiple reward sites. We expect that reward celsl are unlikely to be driving FRD initiation because they are very sparse (1%-5% of CA1

cells) and also get active near the newly introduced reward very quickly (after 2-3 trials) (Gauthier and Tank., 2018). The persistence of FRDs on the old reward site could be explained by the fact that the place field remains unchanged after moving the goal location (Speakman and O'Keefe., 1990). Further investigation requires single unit recording to address place cell activities around the reward zone and their dynamic following the reward moving and their role in triggering reward FRDs.

So far we suggested that FRDs spatial stability could be associated with memory consolidation, awake SWR, and neuronal hyperactivity near the reward location. However, there are 28% of all FRDs initiated on the outer arm of the maze, not directly related to either of the previous interpretations. To date, there is just one study showing the spatially triggered network hyperexcitability. Boehringer and colleagues showed a spatially evoked network hyperexcitability in CA1 following silencing the CA2, which is associated with hyperexcitability in the recurrent CA3 circuits. These short events (1.82 ± 0.10 s) occurred on a linear track, which is not goal orientated, and mimicced the properties of place cells, both spatial and directional information (Figure 4.1). These events were associated with hippocampal theta. However, they alsocomprised a HFO locked to the rising phase of the events, and therefor could also be related SWR driven by CA3 (Wilson and McNaughton, 1994).

Despite the cell loss in CA1 and CA3, we did not see a neuronal loss in the CA2 region which is in line with previous studies showing that CA2 neurons survive during epileptogenesis (Freiman et al., 2021). There is much similarity of the hyperexcitable events in CA1 following silencing the CA2 with FRDs. (1) Both are spatially modulated, (2) both comprise HFO, and (3) both are thought to be associated with CA3 hyperactivity. The recurrent excitatory circuit of CA3 is known to be essential for SWRs generation and sequential firing during the SWRs (Ecker et al., 2022; Buzsáki, 2015; Davoudi and Foster, 2019). The ongoing project at Dr. Laura Ewell showing the activation of CA3 pyramidal neurons during FRDs supports this idea. Although CA3 neurons are highly vulnerable to epileptogenesis, the surviving neurons play important role in transferring the hyperactivity of granule cells hyperactivity to remaining CA1 neurons (Ma et al.,2006). Even with similarities in the frequency band, SWRs, and pHFOs could have different initiation mechanisms. pHFO can be generated with sparse pyramidal neuron firings, as well as

principal cell activities (Ylinen et al., 1995; Bragin et al., 2011). Therefore, the overrepresentation of reward location can also trigger the pHFO generation by providing sufficient neuronal activity.

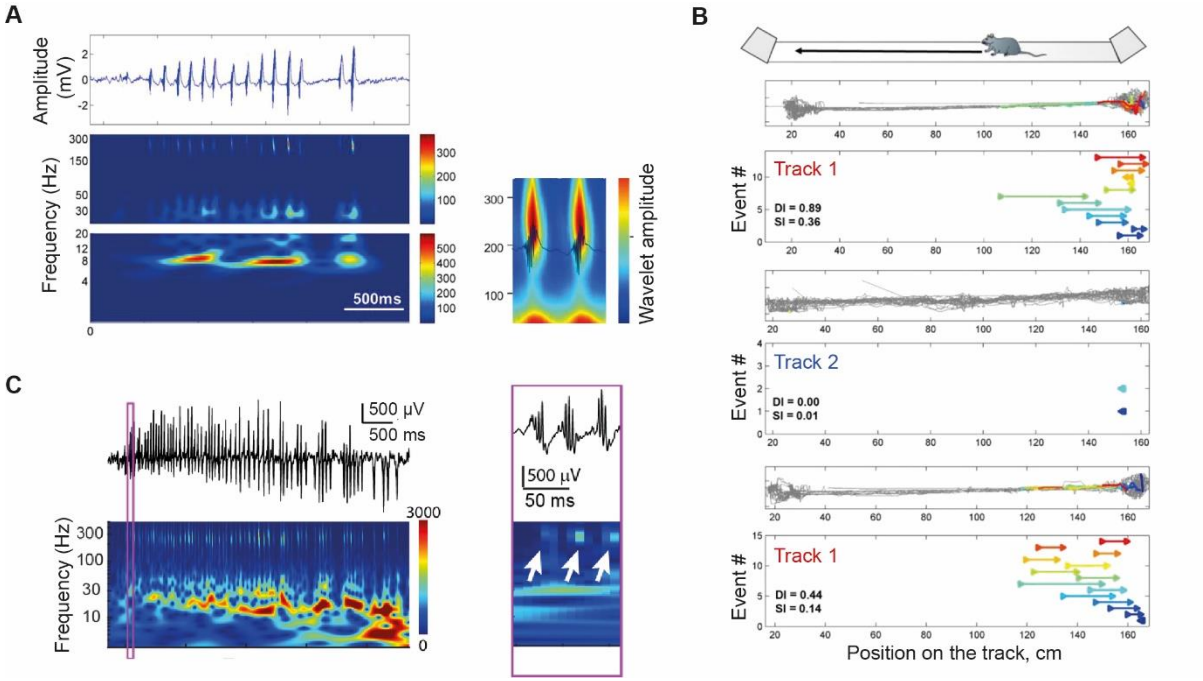

Figure.4.1- Spatially modulated hyperexcitability in CA1.

A, Examples of a CA1 spatially triggered network hyperexcitability event in mice with chronically silenced CA2. Wavelet transform shows HFO accompanying an increase in theta power.

B, network hyperexcitability events are spatially triggered, and context specify. Events have similar spatial maps on track 1 but not on track 2 (A and B are adapted from Boehringer et al., 2017)

C, Example of a spatially stable FRD recorded from epileptic mouse CA1 while running the spatial working memory task comprising HFOs.

Mechanistic and spectrum overlaps of SWRs and pHFOs can provide a useful lens to investigate the memory deficit. However, our data suggested that this lens shouldn't be restricted to the memory consolidation phase, where the SWRs play role. Since pHFOs are not limited to consolidation-related behavior phases such as resting. They occur during exploration or theta state and suppress the theta oscillation for several hundred milliseconds (Ewell et al., 2018; Fu et al., 2018).

Not all the FRDs in KA mice are proceeded by HFO in our study. One explanation is that different seizure onsets involve diverse neuronal networks. Inetrneuron networks are known to be involved in seizures with hypersynchronous-onset (HYP) (Lévesque et al., 2012). Low-voltage-fast onset (LVF) seizures are generated by principal networks. They both are associated with HFO but HYPs are associated with fast ripple bands (250-500 Hz) whereas LVFs are associated with ripple bands (80-200 Hz)(Avoli et al., 2016; Lévesque et al., 2012). Classifying the FRDs in order to address if there are distinct types of FRDs involved in memory encoding and consolidation can be useful to understand their interference with memory.

4.5 FRD classification

We showed that epileptic mice experience frequent FRDs during the behavior and sleep sessions. Despite this high FRD occurrence, half the recorded KA mice did not have memory impairment (3/6), though note that we observed robust memory impairment in the larger behavioral cohort. Our data also supported that FRDs are spatially modulated in most animals and that good performance is correlated to FRDs being spatially stable. Therefore, we asked if there are different 'types' of FRDs, particularly occurring in the behavior sessions, or at a specific part of the maze and during incorrect trials rather than correct ones. To classify FRDs, we used RhythSOM a computational tool to classify oscillations based on their waveforms (Valero et al., 2017). It is challenging to classify the FRDs for all KA mice together since the FRD waveforms are varied across animals. Therefore we classified the FRDs for each mouse individually. Most FRD clusters were common between behavior and sleep sessions. In 4/6 epileptic mice, half of the memory sessions had FRD clusters that occurred specifically in that session and not in pre/post-behavior sleep sessions. Among all FRD clusters, none of them were related to memory performance or particular maze zone and memory process steps.

Following the data showing FRD onsets consisting of HFO, it may be more beneficial to classify the FRD *onsets* across all the epileptic mice. Such analysis could provide insight into the seizure initiation mechanisms related to memory processing in the hippocampus. Combining the outcomes with in vitro studies to address the generation mechanism for

each possible type of FRD onsets could furthermore help to understand the role of the hippocampus in impaired working memory in TLE.

4.6 Conclusion

In this study, we aimed to address the CA1 network dynamics underlying spatial memory deficits in KA-induced epileptic mice. We found theta power reduction in the hippocampus of the KA injected side (right hippocampus) while the contralateral hippocampus remained healthy. Despite the sufficient theta provided by one hippocampus, memory impairment was still impacted. Furthermore, our data also showed synchronization between two hippocampi across the theta frequency band (6-12Hz) is reduced in epileptic mice. Notably, the magnitude of this reduction is correlated to memory performance. Furthermore, we found that memory deficits in epileptic mice are not related to epileptiform event burdens or duration. For the first time, we showed that FRDs (subclinical seizures longer than 2 s) are spatially modulated, meaning they tend to occur at a particular part of the maze. Interestingly, the spatial stability of FRDs is related to good memory performance. One interpretation is that the hippocampus is more capable of tolerating pathological epileptiform events and maintaining functionality as long as FRDs are limited to the a consistent location on the maze. Moreover, our data suggest that FRDs are originating from a similar neuronal network to SWRs in healthy mice. To support this hypothesis we provided two pieces of information: first, FRDs in KA mice and ripples in the control group have a similar spatial pattern, meaning they largely occur in the delay zone, reward zones, and the outer arms (mostly near to the reward zones). Second, FRD onsets comprise the HFO that overlaps with SWRs in the frequency band (100-300 Hz). However, not all the FRD onsets involve HFO which suggest that it is highly likely that more than one single factor is responsible for FRD generation. Further studies, including single-unit recording from CA3 and CA1 as well as in vitro studies aiming to address the mechanism involved in generating various FRDs, are required for a comprehensive understanding of spatially modulated FRDs mechanism.

5. Abstract

Around 50 million people worldwide suffer from epilepsy. Seizures are not the only feature of epilepsy that patients suffer from. Despite the fact that other comorbidities of epilepsy such as memory deficits widely impact a patient's life, they are not the target of common treatments since they have been associated with hippocampal sclerosis and seizure burden.

In this study, we used in vivo electrophysiology to investigate the hippocampal neuronal network changes underlying memory deficits in temporal lobe epileptic mice, the most common form of pharmacoresistant epilepsy in adults. We aim to show seizures and memory impairments are two separate symptoms of temporal lobe epilepsy (TLE).

Kainic acid (KA) epilepsy-induced mice showed a deficit in performing a hippocampal-dependent spatial working memory task. Our data showed that memory deficits in epileptic mice are not related to epileptiform event burdens or duration. Furthermore, we showed that epileptic mice experience frequent focal rhythmic discharges (FRDs, subclinical seizures longer than 2 s). For the first time, we showed that these FRDs are spatially modulated. Interestingly, FRDs being spatially stable is correlated to memory performance meaning that the timing of the FRD occurrence plays a more important role than their frequency or duration. Moreover, our data suggest that FRDs overlap with sharp wave ripples (SWRs) in spectrum features and the occurrence zone on the maze. This finding suggests that FRDs and SWRs are originating from a similar neuronal network to SWRs in healthy mice.

Investigating the CA1 network changes underlying TLE, we found a theta power reduction in the hippocampus of the KA injected side (right hippocampus) while the contralateral hippocampus remained healthy. We also showed synchronization between two hippocampi CA1 across the theta frequency band is reduced in epileptic mice. This theta coherence reduction is correlated to memory performance.

6. List of figures

7. List of tables

8. References

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