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Abnormal Sleep Architecture and Hippocampal Circuit Dysfunction in a Mouse Model of Fragile X Syndrome

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Abstract—Fragile X syndrome (FXS) is the most common heritable cause of intellectual disability and single-gene cause of autism spectrum disorder. The *Fmr1* null mouse models much of the human disease including hyperarousal, sensory hypersensitivity, seizure activity, and hippocampus-dependent cognitive impairment. Sleep architecture is disorganized in FXS patients, but has not been examined in *Fmr1* knockout (*Fmr1*-KO) mice. Hippocampal neural activity during sleep, which is implicated in memory processing, also remains uninvestigated in *Fmr1*-KO mice. We performed *in vivo* electrophysiological studies of freely behaving *Fmr1*-KO mice to assess neural activity, in the form of single-unit spiking and local field potential (LFP), within the hippocampal CA1 region during multiple differentiated sleep and wake states. Here, we demonstrate that *Fmr1*-KO mice exhibited a deficit in rapid eye movement sleep (REM) due to a reduction in the frequency of bouts of REM, consistent with sleep architecture abnormalities of FXS patients. *Fmr1*-KO CA1 pyramidal cells (CA1-PCs) were hyperactive in all sleep and wake states. Increased low gamma power in CA1 suggests that this hyperactivity was related to increased input to CA1 from CA3. By contrast, slower sharp-wave ripple events (SWRs) in *Fmr1*-KO mice exhibited longer event duration, slower oscillation frequency, with reduced CA1-PC firing rates during SWRs, yet the incidence rate of SWRs remained intact. These results suggest abnormal neuronal activity in the *Fmr1*-KO mouse during SWRs, and hyperactivity during other wake and sleep states, with likely adverse consequences for memory processes.

Key words: Fragile X syndrome, *Fmr1* knockout mouse, sleep architecture, hippocampus, sharp-wave ripple, CA1 pyramidal cell, Gamma.

INTRODUCTION

Fragile X syndrome (FXS) is a neurodevelopmental disorder stemming from inactivation of the *FMR1* gene, which encodes the Fragile X Mental Retardation Protein (FMRP). FXS patients often meet criteria for intellectual disability and autism spectrum disorder. They display an array of signs and symptoms, many of which suggest hyperactivity of their underlying brain circuits. Sensory hypersensitivity has been observed in both FXS patients and the *Fmr1* knockout (*Fmr1*-KO) mouse model of FXS. This hypersensitivity has been linked to circuit hyperexcitability in somatosensory cortex in the *Fmr1*-KO mouse (Gibson et al., 2008; Hays et al., 2011; Gonçalves et al., 2013; Zhang et al., 2014). FXS patients and *Fmr1*-KO mice also exhibit cognitive impairments, abnormal hippocampal size and connectivity (Kates et al., 1997; Hessl et al., 2004; Testa-Silva et al., 2012; Hall et al., 2013; Molnár and Kéri, 2014; Haberl et al., 2015), decreased synaptic pruning in hippocampus (Pfeiffer and Huber, 2007), and seizure activity (Musumeci et al., 1988, 1999, 2000; Chen and Toth, 2001; Berry-Kravis, 2002) suggesting disturbed hippocampal circuit function.

Sleep dysfunction has also been found in children with FXS (Richdale, 2003; Kronk et al., 2009, 2010; Kidd et al., 2014). Abnormal sleep architecture has been observed in small cohorts of young and older pediatric FXS patients. A

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Abbreviations: BK, Large conductance potassium channel; CA1-PCs, CA1 pyramidal cells; CA3-PCs, CA3 pyramidal cells; DG, Dentate gyrus; EC2, Layer 2 of entorhinal cortex; EC3, Layer 3 of entorhinal cortex; EC5, Layer 5 of entorhinal cortex; *Fmr1*-KO or KO, *Fmr1* knockout; FMRP, Fragile X Mental Retardation Protein; FXS, Fragile X syndrome; HC, Home cage; HCN, Hyperpolarization-activated cyclic nucleotide-gated; Kv4.2, A-type potassium; LFP, Local field potential; LIA, Large-amplitude irregular activity; LTP, Long-term potentiation; NREM, Non-REM sleep; pcl, Pyramidal cellular layer; PSD, Power spectral density; QW, Quiet wakefulness; REM, Rapid eye movement sleep; SD, Standard deviation; SIA, Small-amplitude irregular activity; SWR, Sharp-wave ripple event; SWS, Slow-wave sleep; WT, Wild type.

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deficit of rapid eye movement sleep (REM) was the most consistent observation (Musumeci et al., 1995; Miano et al., 2008). Interestingly, reduced or abnormal timing of REM has been reported in patients with intellectual disability and autism spectrum disorders of other etiologies (Elia et al., 2000; Miano et al., 2004, 2007, 2008; Buckley et al., 2010; Angriman et al., 2015). Analogous studies have yet to be performed in *Fmr1*-KO mice.

Sleep is one of many processes regulated by circadian rhythms. Studies of circadian rhythm in the fruit fly model of FXS, *dFmr1 drosophila,* revealed complete loss of circadian rhythms, altered sleep durations, and sleep-dependent synapse remodeling (Inoue et al., 2002; Bushey et al., 2009; Donlea et al., 2011). Examination of circadian rhythms in the *Fmr1*-KO mouse has revealed mild perturbation of circadian running behaviors, and more dramatic effects on circadian rhythm with knockout of the gene paralog, *Fxr2* (Zhang et al., 2008). A recent behavioral study revealed decreased total sleep time in *Fmr1*-KO mice (Saré et al., 2017). While it is clear that loss of *FMR1* function affects circadian-entrained activity and sleep duration in flies and rodents, it is not known if the architecture of sleep, the progression sleep stages and their durations on a finer scale, is also affected in the mouse disease model as it is in FXS patients.

Sleep plays an important role in general homeostasis, cognition, and memory. Indeed, neural activity thought to mediate memory consolidation occurs during sleep (Ego-Stengel and Wilson, 2009; Girardeau et al., 2009). The most consistently observed abnormalities of hippocampus-dependent memory in the *Fmr1*-KO mouse are thought to involve memory consolidation, cognitive flexibility, and working memory (The Dutch-Belgian Fragile X Consortium et al., 1994; Kooy et al., 1996; D'Hooge et al., 1997; Yan et al., 2004; Seese et al., 2014; Radwan et al., 2016). A recent study highlighted the importance of memory consolidation by demonstrating intact short-term and impaired long-term spatial memory in *Fmr1*-KO mice (Babayan et al., 2012; Kramar et al., 2012; Seese et al., 2014).

Furthermore, myriad cellular and synaptic abnormalities have also been described within and between regions of the hippocampal circuit in the *Fmr1*-KO mouse. Dysregulation of voltage-gated ion channels in *Fmr1*-KO mice – including A-type potassium channels (Kv4.2), large conductance potassium channels (BK), and hyperpolarization-activated cyclic nucleotide-gated (HCN) channels – may promote neuronal and dendritic hyperexcitability (Deng et al., 2011, 2013; Gross et al., 2011; Lee et al., 2011; Brager et al., 2012; Zhang et al., 2014). More recently, hyperexcitability was observed in hippocampal neurons *in vitro* (Luque et al., 2017). *Fmr1*-KO mice have also been found to exhibit enhanced metabotropic glutamate receptor-mediated long-term depression (mGluR-LTD) (Huber et al., 2002; Dölen et al., 2007; Zhang et al., 2008; Michalon et al., 2012) and increased threshold of long-term potentiation (LTP) (Huber et al., 2002; Dölen et al., 2007; Lauterborn et al., 2007; Lee et al., 2011; Michalon et al., 2012), indicating a diminished tendency to strengthen hippocampal synapses. Directly linking altered synaptic plasticity and neuronal excitability to memory abnormalities and seizure predisposition is difficult. While discoordinated hippocampal neural activity coinciding with performance of a spatial memory task has been reported in *Fmr1*-KO mouse (Radwan et al., 2016), little is known about neuronal activity specifically implicated in memory consolidation within the anatomically intact *Fmr1*-KO mouse hippocampus.

Direct examination of the activity of individual neurons and neuronal populations during unrestricted behavior has greatly extended our understanding of hippocampal function. Sharp-wave ripples (SWRs) are high-frequency oscillatory events that occur during slow-wave sleep (SWS) and quiet wakefulness (QW) states. The events consist of ripple frequency band oscillations (100–250 Hz) superimposed on a lower frequency sharp wave. Powerful recurrent excitation in CA3 triggers massive discharges of CA3 pyramidal cells or pyramidal neurons (CA3-PCs) transmitted to CA1 pyramidal cells (CA1-PCs) and CA1 interneurons via Schaffer collaterals. CA1 is the main output region of the hippocampus, projecting to subiculum and many other cortical and subcortical regions. Complex interactions among pyramidal and interneurons generate ripple frequency activity within the CA1 pyramidal cellular layer (pcl). During these brief (50–350 ms) events, pyramidal neuron sequences corresponding to previous behavioral experiences are reactivated in a time-compressed manner. Hippocampal neural activity in the form of SWRs have been implicated in memory consolidation and other memory processes (Ego-Stengel and Wilson, 2009; Girardeau et al., 2009; Jadhav et al., 2012; Pfeiffer and Foster, 2013). In addition, abnormal SWRs have been observed in mouse models of schizophrenia, dementia, and mood disorders with known deficits of hippocampus-dependent memory (Suh et al., 2013; Witton et al., 2014; Altimus et al., 2015).

The hippocampus is a convergence point of sleep and memory. It exhibits dramatic state-dependent modulation of its activity and memory processing modes between different wake and sleep states (Wilson and McNaughton, 1994; Louie and Wilson, 2001; Lee and Wilson, 2002). To investigate this activity *in vivo,* electrophysiological studies of freely moving and behaving *Fmr1*-KO mice were necessary to assess neural activity with connections between intact brain areas and in true sleep states. We implanted microdrives containing tetrode arrays targeting the dorsal CA1 region of hippocampus and recorded 393 hippocampal pyramidal neurons and neuronal population activity while mice were in a familiar "home cage" (HC) environment. We used behavior and local field potential (LFP) data for identification of distinct wake and sleep states to (1) determine if the *Fmr1*-KO mouse models abnormal sleep architecture observed in FXS patients, (2) characterize hippocampal circuit activity in various physiological sleep states, and (3) determine if activity underlying hippocampal memory processes (e.g. SWRs, CA1-PC firing rates, etc.) is abnormal in the *Fmr1*-KO mouse.

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