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Abnormal hippocampal theta and gamma hypersynchrony produces network and spike timing disturbances in the *Fmr1*-KO mouse model of Fragile X syndrome

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ABSTRACT

Neuronal networks can synchronize their activity through excitatory and inhibitory connections, which is conducive to synaptic plasticity. This synchronization is reflected in rhythmic fluctuations of the extracellular field. In the hippocampus, theta and gamma band LFP oscillations are a hallmark of the processing of spatial information and memory. Fragile X syndrome (FXS) is an intellectual disability and the most common genetic cause of autism spectrum disorder (Belmonte and Bourgeron, 2006).

Here, we investigated how neuronal network synchronization in the mouse hippocampus is compromised by the *Fmr1* mutation that causes FXS (Santos et al., 2014), relating recently observed single-cell level impairments (Arbab et al., 2017) to neuronal network aberrations. We implanted tetrodes in hippocampus of freely moving *Fmr1*-KO and littermate wildtype (WT) mice (Mientjes et al., 2006), to record spike trains from multiple, isolated neurons as well as LFPs in a spatial exploration paradigm.

Compared to wild type mice, *Fmr1*-KO mice displayed greater power of hippocampal theta oscillations, and higher coherence in the slow gamma band. Additionally, spike trains of *Fmr1*-KO interneurons show decreased spike-count correlations and they are hypersynchronized with theta and slow gamma oscillations. The hypersynchronization of *Fmr1*-KO oscillations and spike timing reflects functional deficits in local networks. This network hypersynchronization pathologically decreases the heterogeneity of spike-LFP phase coupling, compromising information processing within the hippocampal circuit. These findings may reflect a pathophysiological mechanism explaining cognitive impairments in FXS and autism, in which there is anomalous processing of social and environmental cues and associated deficits in memory and cognition.

1. Introduction

Fragile X syndrome (FXS) is a monogenic intellectual disability that shows behavioral overlap with autism spectrum disorder (ASD) (Belmonte and Bourgeron, 2006), accounting for an estimated 5% of its prevalence (Budimirovic and Kaufmann, 2011). FXS arises from a triplet expansion of the *Fmr1* gene, silencing expression of the fragile X mental retardation protein (FMRP). FMRP binds mRNAs encoding approximately one third of pre- and postsynaptic proteins, most significantly targeting those involved in synaptic signaling pathways involved in long-term potentiation (LTP) and depression (LTD), CREB signaling, glutamate receptor regulation, and GABA receptor mediated inhibition (Darnell et al., 2011; Bhakar et al., 2012). FMRP silencing effectively leads to disturbed synaptic function and plasticity of both interneurons and pyramidal cells (Santos et al., 2014; Pilpel et al., 2009).

FXS is a promising target for obtaining a multi-dimensional understanding from genes, to microcircuits and networks, to cognitive

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impairment in neuropsychiatric disease due to its relatively simple genetic etiology (Fung and Reiss, 2016) and the development of rodent models (Mientjes et al., 2006; Berzhanskaya et al., 2017). Particularly affected in human patients and animal models is the hippocampus (Kates et al., 1997; Reiss et al., 1994), a structure essential for storing and consolidating experiences into long-term episodic and semantic memory.

Both animal (Kim and Fanselow, 1992; Morris et al., 1982) and human (Manns et al., 2003; Moscovitch et al., 2016) studies link the hippocampus to spatial, contextual, autobiographical and semantic memory. Single hippocampal neurons respond to the concept of given individuals, landmarks or objects (Quiroga et al., 2005). In FXS animal models, learning and memory deficits have been associated with dysfunction of the mechanisms underlying activity-dependent synaptic plasticity in the hippocampus (Bhakar et al., 2012; Huber et al., 2002).

Synaptic plasticity strongly depends on the precise temporal coordination of neuronal activity (Markram et al., 1997). This temporal coordination of neuronal activity is reflected in rhythmic oscillations of the local field potential (LFP) (Buzsáki et al., 2012). Neuronal oscillations have been associated with several cognitive and mechanistic processes through the brain, including neuronal communication and precise spike timing of activated neuronal groups (Bosman et al., 2014; Fries, 2015; Sejnowski and Paulsen, 2006). Hippocampal theta (4–8 Hz) chunks this experiential information in oscillation cycles (Skaggs and McNaughton, 1996; Gupta et al., 2012), and theta-nested gamma (20–100 Hz) oscillations induce synaptic plasticity, supporting memory consolidation processes (Bosman et al., 2014; Zheng et al., 2016; Colgin and Moser, 2010). Recently, abnormal gamma and theta phase-amplitude patterns of dendritic CA1 LFP oscillations were found in a mouse model of FXS (Radwan et al., 2016), related to an impaired excitatory-inhibitory equilibrium in FXS neuronal networks (Fenton, 2015; Contractor et al., 2015). However, it is unknown how these oscillatory dysfunctions affect the temporal coordination of spiking responses in these networks. Here, we hypothesize that compromised synaptic function in *Fmr1*-KO mice affects both the temporal coordination of cell ensembles and hippocampal oscillatory rhythms supporting neuronal synchronization. We evaluated this hypothesis using tetrode recordings the CA1 region of freely moving *Fmr1*-KO mice.

2. Material and methods

2.1. Subjects

We used four male *Fmr1*-KO (Mientjes et al., 2006) and four littermate wildtype (WT) control mice. All experiments were performed in accordance with Dutch National Animal Experiments regulations, were approved by the University of Amsterdam. Animals were received from the Erasmus MC Rotterdam breeding unit at an age of 8 weeks and group-housed until surgery. They were maintained on a regular 12-hour light-dark cycle (lights on: 8 am, lights off: 8 pm) and received water and food *ad libitum* throughout the experiment. To minimize bias due to possible undetected changes in environmental conditions, *Fmr1*-KO and WT animals were always studied in pairs; both recordings were done on the same day and counterbalanced per genotype. Therefore, the experimenter was not blind to genotype during the experiments: pairs of one *Fmr1*-KO and one WT mouse were implanted with a microdrive in each experiment. Once habituated to the experimenter and handling, mice underwent drive implantation surgery under buprenorphine-isoflurane anesthesia and were left to recover before the experiments.

2.2. Electrophysiological techniques

Six independently moveable tetrodes were loaded into a custom-made microdrive (Battaglia et al., 2009) and implanted over dorsal hippocampus (AP: −2.0 mm, ML: −2 mm; Fig. 1A). The tetrodes were advanced into the CA1 pyramidal cell layer guided by electrophysiological signals (sharp wave-ripple events) over the course of days following implantation surgery. Electrophysiological activity was recorded on an analog 27-channel Neuralynx data acquisition system at a 32 kHz sampling rate. Tetrode signals (bandpass filtered 0.6–6.0 kHz for single unit and 0.1–475 Hz for LFP) were referred to a nearby tetrode which was targeted to a location devoid of single unit activity. Single-unit data were preprocessed with Klustakwik (Harris et al., 2000) for automated spike clustering and the results were manually refined using Klusters (Hazan et al., 2006). The resulting spike trains were analyzed using custom-written MATLAB code. LFP analyses were done in MATLAB using FieldTrip (Oostenveld et al., 2011) and custom-made routines. Animal tracking position was extracted from video footage by Ethovision XT software (Noldus, Wageningen, the Netherlands) which was synchronized with the electrophysiological data

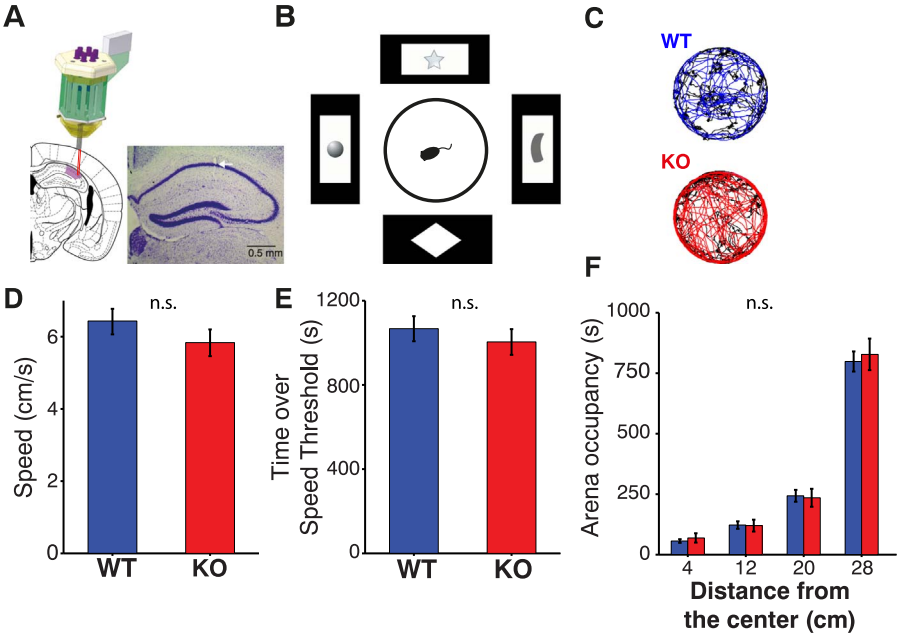

Fig. 1. Experimental setup and behavior.
(A) Left, Schematic of microdrive implantation target. Right, Coronal section showing the recording location (lesion) of a tetrode (arrow) in dorsal hippocampus CA1. (B) Schematic of the behavioral protocol. Animals freely explored a circular open field arena (middle) which was surrounded by four posters with geometric figures. (C) Accumulated trajectories of a WT (blue) and KO (red) animal exploring the arena during an example session in which the animal moved above threshold speed (3 cm/s). Inactivity periods (< 3 cm/s) are shown in black. (D) Average speed (> 3 cm/s) of WT and KO animals during arena exploration. (E) Average time per session where speed of WT and KO animals was > 3 cm/s. (F) Average time WT and KO animals spent at varying distances from the center of the arena (as a measure of thigmotaxis). Data (D–F) are represented as mean ± SEM.