Lost in place
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General discussion
6. General discussion

Tetrode recording in Fmr1-KO hippocampal CA1 during spatial exploration

The hippocampus is linked to autobiographical memory, which includes spatial and contextual memory, in both animal (Kim 1992)(Morris 1982) and human (Manns 2003)(Moscovitch 2016) studies. Accordingly, the activity of hippocampal neurons can encode abstract representations of multi-sensory perceptual information. Correspondingly, hippocampal dysfunction is a component of intellectual pathologies such as FXS and commonly comorbid autism-spectrum disorders, in which memory is disrupted (Reiss 1994)(Salmond 2005), ultimately contributing to anomalous processing of social and environmental cues and associated deficits in memory and cognition (Happé 2006).

In this thesis, we describe multiple electrophysiological manifestations of abnormal hippocampal spatial information processing in Fmr1-KO mice, characterizing how the neurodevelopmental phenotype of FXS affects learning and memory formation in vivo. Hippocampal CA1 pyramidal neurons of the Fmr1-KO mouse model of FXS have shown morphological abnormalities and impaired synaptic plasticity linked to mGluR5 expression in previous studies (Levenga 2011)(Krueger 2011). These animals are also impaired in hippocampus-dependent spatial tasks, implying spatial memory deficits (Mineur 2002)(Guo 2012)(Kooy 1996)(D’Hooge 1997)(van Dam 2000)(Yan 2004), consistent with findings in human patients of FXS (Cianchetti 1991). Thus, we explored the consequences of the FMRP deficit in Fmr1-KO mice on processing of multi-sensory information underlying representation of place by hippocampal neurons.

Hippocampal neurons (place cells) fire in spatially restricted locations during spatial exploration (place fields) and together form a cognitive map of the environment. Successful abstraction of environmental information into a cognitive map depends on synaptic plasticity, reflected in place field stability, synchronization of local neuronal network activity, and offline processing events (Moser 2015). We determined the consequences of the FMRP deficit in FXS on these measures of neuronal information processing underlying spatial cognition, by recording in vivo hippocampal CA1 single cell and network activity in freely moving Fmr1-KO mice and WT controls, using an implantable microdrive supporting six individually moveable tetrodes that was developed in our lab (Battaglia 2009). Recordings were done as the mice explored an open field arena that was surrounded by visual cues, as well as in rest periods immediately before and after exploration. We found no behavioral differences in exploratory behavior (running speed, coverage of the arena, thigmotaxis) between genotypes. We characterized the following in vivo hippocampal neurophysiological information processing impairments resulting from the Fmr1 mutation, the implications of which will be discussed in greater detail after this summary.
Pyramidal cell impairments in Fmr1-KO hippocampal CA1
To abstract environmental information into a cognitive map, it is imperative that the activity of hippocampal CA1 place cells remain stably spatially specific. We find that correlation and specificity of Fmr1-KO place cell activity in spatial exploration is significantly reduced compared with WT: the FMRP deficit leads to impaired stability of place fields and impaired spatially-restricted firing of place cells (Chapter 2). Neither are improved by mGluR5 antagonism (Chapter 3), although it should be noted that the impaired stability found in Chapter 2 could not be reproduced in the cell sample studied here, so our data do not support the influential hypothesis that glutamatergic hyperstimulation underlies hippocampal deficits in FXS.

Interneuron impairments in Fmr1-KO hippocampal CA1
We looked in more detail into hippocampal CA1 neuronal activity processing spatial information to characterize the pathophysiological mechanisms explaining this biomarker of cognitive impairments in this animal model of FXS. In Chapter 4 we describe that, compared to WT, Fmr1-KO interneuronal spiking is less correlated to other interneurons and to pyramidal cells, demonstrating a temporal discoordination of the variance of spontaneous cell assembly activity. An important role of hippocampal CA1 interneurons is to synchronize the local neuronal network, which is conducive to synaptic plasticity. We find that Fmr1-KO interneurons are hypersynchronized with ongoing theta and gamma network oscillations compared to WT (Chapter 4) which might reflect an aberrant temporal precision of neuronal activity.

Network impairments in Fmr1-KO hippocampal CA1
Indeed, stronger interneuronal locking to local network oscillations in Fmr1-KO mice was concomitant with higher power of theta oscillations and higher coherence of slow gamma compared to WT (Chapter 4). These findings reflect abnormalities in excitatory/inhibitory coupling between neurons, which might underlie functional deficits in local networks, compromising information processing within the hippocampal circuit.

Offline processing impairments in Fmr1-KO hippocampal CA1
Replay of hippocampal neuronal activity in SWRs during rest periods subsequent to spatial exploration might aid consolidation of spatial information, promoting the integrity and solidity of the hippocampal cognitive map. We find that the oscillation frequency of SWR events is decreased in Fmr1-KO mice compared to WT (Chapter 5), possibly affecting the integrity of downstream synaptic plasticity underlying memory consolidation.
Implications of impaired $Fmr1$-KO hippocampal CA1 spatial information processing

Spatial information processing is a key function of the hippocampus, with a tight link to (or coincident with) its role in memory [O’Keefe 1978]. The discovery of place cells in rodents [O’Keefe 1971] has provided one of the best models for elucidating how neural circuits process information in vivo. In fact, spatially selective neuronal activity in the hippocampus is found in humans as well [Ekstrom 2003], suggesting that rodent place cells parallel mechanisms of spatial memory in humans. Extraordinarily, human place cells tied to locations of certain objects can become active during free recall of the objects, mirroring rodent place cell reactivation during replay [Miller 2013]. It is proposed that mechanisms of declarative memory have evolved from mechanisms of spatial navigation [Buzsáki 2013]. It follows that anomalous place cell activity in disease models might be characteristic of cognitive impairment in learning and memory disorders in humans, providing fertile ground for translational research [Shen 1997]. During environmental exploration, hippocampal place fields arise from a complex process of integration of information from different modalities. These multisensory inputs are combined into a ‘scaffold’ of internally generated representations, which depend to a large extent on self-motion information [O’Keefe 1978][McNaughton 2006]. The metric for this self-motion is provided by so-called grid cells, which are neurons that have multiple firing fields in all environments, that tessellate the space in a periodic, hexagonal grid-patterned firing [Hafting 2005]. Grid cells are found throughout the medial entorhinal cortex (MEC) and pre- and para-subiculum, but are most abundant in MEC layer 2, where grid-like firing can be found in both types of principal neurons (stellate and pyramidal cells) [Domnisoru 2013]. Grid cells are part of a wider network of spatially modulated MEC neurons, such as head-direction and border cells, that fire depending on the direction the animal is facing, and at borders delineating the environment, respectively [Sargolini 2006][Savelli 2008]. Direct inputs from MEC layer 2 are necessary for CA1 place field formation [Brun 2008] and possibly serve as a path integrator for self-localization of the animal in the environment [McNaughton 2006]. Place cell firing sequences are found while rodents run on a wheel during delay periods between alternating (left/right) runs in a figure-8 maze, i.e. place cells can be active when there is body movement but no physical change in environmental location or in the surroundings [Pastalkova 2008]. Place fields can therefore be generated based on self-motion cues. This internally generated map accumulates errors and drifts over time when visual or other guiding sensory feedback is lacking, and is realigned by external spatial cues [Gothard 1996b][Markus 1994]. Attention to spatial cues can mediate rapid spatial encoding by place cells [Monaco 2014] and can improve place field stability [Kentros 2004]. In Chapter 2, we show that $Fmr1$-KO CA1 pyramidal cells are similar to
those of WT, but that their temporal stability is impaired. This instability might reflect deficits in integration of external streams of information in the self-motion based map, either due to a lack of attention paid to the cues, or (not mutually exclusive) because their input to CA1 from MEC might be constrained.

Indeed, direct inputs from MEC layer 2 to the hippocampus might be compromised in FXS, contributing to impaired stability of place fields. For instance, Fmr1-KO CA1 pyramidal cell plasticity might be affected by enhanced mGluR5-dependent LTD (Huber 2002), or by diminished LTP as a consequence of higher dendritic expression of H-channel subunit HCN1 (Brager 2012). Higher expression of the HCN1 gene may impair spatial memory and plasticity in CA1 pyramidal neurons by affecting the ability of the entorhinal cortex to excite their distal dendrites (Nolan 2004). Freed from this constraint, HCN1-KO mice show increased stability of hippocampal place fields (Hussaini 2011). Amongst other possible factors, the temporal instability we find in Fmr1-KO place fields might be interpreted within this context as an increase in HCN1-mediated control over CA1 pyramidal cell plasticity from entorhinal inputs, which affect the sensory information-dependent updating of the self-motion based map. Over-expression of the HCN1 subunit might not, however, account for all of our observations of impaired Fmr1-KO spatial information processing: like our findings in Fmr1-KO, place fields are enlarged in HCN1-KO mice (Hussaini 2011), perhaps as a consequence of enlarged grid fields also found in these animals (Giocomo 2011). This implies the involvement of additional FMRP-mediated synaptic plasticity effects to fully explain our results.

In Chapter 3 we examined the effect of acute mGluR5-antagonism, hypothesized to attenuate the increased mGluR5-dependent LTD found in previous studies, on place field stability in Fmr1-KO mice. Place field stability was not improved in this study. However, probably due to the small sample size of this study, we were unable to replicate the place field stability impairments observed in Chapter 2. Based on these results we thus cannot conclusively reject hyperstimulation of glutamatergic pathways as a key factor in Fmr1-KO spatial mapping impairments. Future studies are required to assess whether impaired spatial representation in Fmr1-KO mice is truly caused by degraded MEC inputs, and whether this is mediated by abnormalities in glutamatergic transmission.

Alternatively, sensory information-dependent updating of the self-motion based map may be affected by disrupted network mechanisms regulating the flow of information from MEC to hippocampus. Spatial representation in CA1 is constructed from a balance between encoding information on the animal’s current position (inputs from MEC) and retrieval of these representations (stored in CA3). During spatial exploration,
neuronal network activity in CA1 couples with CA3 through slow (20-50 Hz) gamma oscillations mediating activation of representations of upcoming trajectories, whereas it is temporally aligned with MEC through fast gamma (50-100 Hz) oscillations coding ongoing trajectories (Colgin 2009){Zheng 2016}. We might hypothesize then, that the integrity of fast gamma coupling between CA1 and MEC is degraded in absence of FMRP, and that this alters entorhinal inputs to the hippocampus, potentially resulting in the failure to elicit spike-timing dependent plasticity (Cabral 2014a){Benchenane 2011}{Battaglia 2011}, affecting the stability of spatial representations.

Indeed, the fast gamma that couples CA1 and MEC neuronal networks is ideally suited to induce synaptic plasticity supporting consolidation of incoming spatial information from MEC (Csicsvari 2003){Bieri 2014}, and NMDA receptor-associated LTP is required for the stable representation of this spatial information in CA1 (McHugh 1996){Kentros 1998}{Ekstrom 2001}{Cabral 2014a}. In fact, place field size increases when NMDA receptor signaling is disturbed in hippocampal CA1 pyramidal cells (McHugh 1996), possibly by impairing the incorporation of current spatial information from MEC into the stored representation (Cabral 2014b). Hippocampal NMDA receptor subunit composition might be altered in FXS (Edbauer 2010), affecting this type of plasticity, and in fact, LTP is impaired in Fmr1-KO mice (Godfraind 1996){Zhao 2005}{Shang 2009}{Chen 2014}. An interesting future direction would be to determine whether impaired spatial representation in Fmr1-KO mice might be caused by abnormal NMDA receptor signaling in CA1, degrading potentiation of current environmental information carried by MEC inputs.

Rodent hippocampal activity is organized by theta (4-8 Hz) oscillations, which ‘chunk’ together experiential information (Gupta 2012). Hippocampal theta oscillations accompany exploratory behavior and are implicated in spatial learning and memory (Hasselmo 2005). During place field traversals, corresponding place cells fire consistently at a particular phase of the theta rhythm, shifting systematically forward on each theta cycle. This phenomenon is referred to as theta phase precession (O’Keefe 1993). It is proposed that theta phase precession helps the animal determine its location with greater precision, by using the phase relationship of place cell firing in addition to place cell firing. Additionally, the compressed repetition of the temporal sequence of place cell firing within individual theta cycles might allow for LTP mechanisms, aiding memory formation (Skaggs 1996b). An interesting topic for future investigation of the functional consequences of the neuronal network abnormalities that we find in Fmr1-KO mice therefore would be to evaluate how they are linked to place cell function, for instance by studying theta phase precession. Our data is not fit for this type of analysis, as in our paradigm, animals freely explored an open field arena; this type of
exploration does not yield enough repetitions of spatial trajectories and place cell firing sequences to obtain reliable statistics. The relationship between place cell and neuronal network activity could be evaluated in an experiment that requires the animal to move across the same trajectory in the same way multiple times, such as in a linear running track.

Gamma oscillations are nested within theta oscillations, and their coupling to particular phases of these theta cycles is important for successful memory performance (Tort 2009). We find that compared with WT, Fmr1-KO interneuron firing is hypersynchronized with ongoing theta and gamma oscillations, and that the power of theta oscillations is abnormally high (Chapter 4). Our experiment was not designed to identify different types of interneuron, so we cannot discern with certainty what cell type our population of recorded cells is comprised of. However, since our recordings were targeted to the CA1 pyramidal cell layer, it is likely our recordings contain spikes recorded from basket cells. These interneurons are involved in the hyperexcitability of the FXS neuronal network (Cea-Del Rio 2014). The bodies of basket cells lie in the pyramidal cell layer, where they provide perisomatic inhibition to local pyramidal cells, and are involved in the local generation of gamma oscillations (Gloveli 2005). CA1 interneuronal synchronization of local neuronal network activity is conducive to synaptic plasticity (Csicsvari 2003). Our findings thus might reflect a mechanism through which the FMRP deficit in Fmr1-KO may cause aberrant temporal precision of neuronal activity, and thereby aberrant synaptic plasticity. A previous study described greater hippocampal CA1 coupling between the phase of theta oscillations and the amplitude of gamma oscillations in these mice, concomitant with impairments in a cognitive discrimination task (Radwan 2016). These findings imply that altered theta might effect changes in CA1 gamma oscillations, perhaps altering their ability to induce synaptic plasticity supporting memory consolidation.

In Chapter 2 we found no difference between Fmr1-KO and WT place field stability when we removed a subset of visual cues surrounding the arena, whereas WT place fields were significantly more stable than those of Fmr1-KO before this change in the environment. Thus, Fmr1-KO place cells might rely more on internally generated representations than on external cues. This might be reflected in the increased phase consistency of Fmr1-KO spikes to slow gamma in the LFP we describe in Chapter 4, which may preferentially synchronize CA1 with CA3 (over MEC via fast gamma) compared with WT. Our findings align with recent work showing that cognitive inflexibility of Fmr1-KO mice in a place avoidance task can be explained by slow gamma-mediated recollection of inappropriate information dominating fast gamma oscillations in CA1 (Dvorak 2018).
Neurons that were active during spatial exploration subsequently fire in offline SWR events, which may be optimally suited to induce LTP in the efferent synapses of CA1 neurons. This mechanism is thought to aid in the transfer of information to neocortex for long-term storage (McClelland 1995) and to be required for spatial memory (Girardeau 2009) (Maingret 2013). Local CA1 interneurons are believed to synchronize pyramidal cell spiking during SWRs. Abnormal patterns of interneuronal activity similar to the ones described in Chapter 4 might underlie the decreased intra-ripple frequency of Fmr1-KO SWR we observe in Chapter 5. Alternatively, the integrity of SWR events in Fmr1-KO might be affected by disrupted sleep observed in these animals (Saré 2017). The affected SWRs may, in turn, compromise spatial memory consolidation in Fmr1-KO mice, leading to the impaired stability of CA1 place fields described in Chapter 2. In conclusion, the data presented in this thesis describe the following effects of Fmr1-KO in hippocampal CA1 related to spatial exploration: impaired place mapping stability in principal cells, altered neuronal network activity, abnormal participation of interneurons therein, and aberrant sharp-wave ripple activity. The future directions mentioned in this discussion will help determine how these phenotypes affect synaptic plasticity and neuronal communication between CA1 and connected brain areas, and how they contribute to the devastating cognitive symptoms of FXS.

Clinical implications of spatial information processing deficits in Fmr1-KO mice

Our experiments were not designed to provide behavioral evidence that spatial navigation is affected by the information processing impairments we describe. Behavioral phenotypes of FXS animal models have mostly been subtle, whereas we show that hippocampal CA1 place cell and neuronal oscillation activity patterns provide an opportunity to characterize the effects of FMRP deficit on the processing and abstract representation of information in a brain area crucial for declarative memory. In an experiment mentioned above, place cell activity was observed while rodents ran on a wheel in between alternating left/right runs on a figure-8 maze (Pastalkova 2008). In the same experiment however, place cell activity was not observed during control wheel runs unrelated to navigation in the maze, i.e. when memory was not required. Our observation of place fields in Fmr1-KO mice freely exploring an environment therefore implies that these mice are indeed mapping space, and that anomalous activity underlying this process provides characteristics of cognitive impairment in FXS on which potential therapeutic interventions may be tested.

An influential line of research has identified hyperstimulation of glutamatergic pathways as a possible target for treatment in FXS (Bear 2004), and many studies have found attenuation of mGluR5 signaling can rescue certain neuronal and pathophysiological
Phenotypes in Fmr1-KO [Yan 2005] [Dölen 2007] [de Vrij 2008]. Deficits of a cognitive nature, however, have proven difficult to establish and test reliably in animal models of FXS. We examined the effect of acute mGluR5-antagonism in Chapter 3, and did not find it to improve Fmr1-KO place field stability. Interestingly, despite their success on the above-mentioned phenotypes, recent pharmaceutical studies in adult human patients are in line with our results, failing to show efficacy of mGluR5-antagonists (basimglurant and mavoglurant) to improve behavioral endpoints [Scharf 2015]. We therefore propose that evaluating the effects of FXS treatment on the neuronal mechanisms underlying hippocampal spatial mapping in the mouse provides a more accurate assay for testing cognitive outcomes than has previously been employed. Currently, Novartis is recruiting participants for a new clinical trial (ClinicalTrials.gov Identifier: NCT02920892), studying the effects of mavoglurant in children with FXS (ages three to six) to assess its effects on a cognitive outcome measure (language learning). Since the FMRP deficit not only acutely affects synaptic transmission, but also leads to more pervasive neuronal network alterations throughout development, starting treatment of any kind at a younger age might prove more beneficial than in adulthood. However, interfering in glutamatergic signaling pathways in a growing brain might alter its function in other ways. We therefore suggest assessing the cognitive effects of mavoglurant in young Fmr1-KO mice before testing in children.

GABAergic dysfunction in FXS provides another possible target for treatment. FMRP regulates expression of the GABA\textsubscript{A} receptor subunit \(\delta\) [Miyashiro 2003] and many abnormalities of the GABAergic system are found in FXS [Paluszkiewicz 2011]. Chronic administration of a GABA\textsubscript{A} receptor agonist (arbaclofen) can correct certain neuronal and pathophysiological phenotypes in Fmr1-KO mice [Henderson 2012]. Despite this success, arbaclofen did not improve behavioral endpoints in a human patient study [Berry-Kravis 2012]. Since Fmr1-KO mice show decreased hippocampal GABAergic signaling [D’Antuono 2003] and \(\beta\) and \(\delta\) subunit expression of the GABA\textsubscript{A} receptor [El Idrissi 2005] [D’Hulst 2006], we suggest evaluating the effects of future pharmacological agents modulating GABAergic signaling in FXS on neuronal mechanisms underlying hippocampal spatial mapping might provide a valuable predictor of its efficacy. If proven effective, in this case it might also prove beneficial to begin treatment of FXS patients at an early age, circumventing the developmental phenotype of FRMP deficit. However, as with glutamatergic pathways, altering GABAergic signaling pathways in the growing brain is not without risk, it will likely affect normal brain function.

Due to the developmental abnormalities engendered by the FMRP deficit in FXS, it might not be realistic to completely rescue its phenotype through short term, acute treatment. Instead it might prove more effective to detect FXS and intervene at an
early stage. As FXS is caused by a monogenic mutation, the advent of gene editing technologies (such as CRISPR-Cas9), if applied early enough, might rescue the naturally occurring FMRP deficit completely. Once these techniques have proven safe and effective for human use, such technology could be used to introducing a copy of the intact Fmr1 gene into the genome of a patient, or even in germline or embryonic cells. This type of genome editing obviously brings with it its own legal and ethical challenges which lie outside the scope of this thesis.

A compelling and less controversial direction for treatment of FXS is childhood environmental enrichment. In general, animals kept in stimulating environments show reduced anxiety (Benaroya-Milshtein 2004), improved spatial learning and memory (Schrijver 2002), and altered synaptic morphology (Greenough 1985). Rearing Fmr1-KO mice in environmentally enriched cages rescues spike-timing dependent LTP in prefrontal cortex, dendritic abnormalities in visual cortex, and deficits in open-field exploration, possibly via FMRP-independent pathways activating glutamatergic mechanisms of neuronal plasticity (Meredith 2007) (Restivo 2005). Environmental factors can even positively influence cognitive outcomes of children with FXS (Dyer-Friedman 2002). It would be interesting to see whether rearing conditions can rescue the impaired characteristics of Fmr1-KO hippocampal spatial mapping we describe in this thesis.