Sustained correction of associative learning deficits after brief, early treatment in a rat model of Fragile X Syndrome

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Mattia Maroso, PhD
Associate Editor
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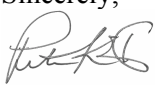
Please find attached our revised submission entitled "Sustained correction of associative learning deficits following brief, early treatment in a rat model of Fragile X Syndrome" (manuscript number aao0498), for consideration for publication in Science Translational Medicine. As per our discussions, we have made all of the editorial changes you requested, with the exception of those discussed with you. We thank you for all your help in preparing the manuscript and look forward to hearing form you soon.

Sincerely,



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Sustained correction of associative learning deficits following brief, early treatment in a rat model of Fragile X Syndrome.

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Overline: Fragile X Syndrome

One sentence summary: Brief, early treatments with lovastatin provides long-lasting rescue of associative memory deficits in a rat model of Fragile X.

Abstract

Fragile X Syndrome (FXS) is one of the most common monogenic forms of autism and intellectual disability. Preclinical studies in animal models have highlighted potential of pharmaceutical intervention strategies for alleviating the symptoms of FXS. However, whether treatment strategies can be tailored to the developmental time-window that defines the emergence of a particular phenotype is unknown. Similarly, whether a brief, early intervention can have long-lasting beneficial effects, even after treatment cessation, is not known. To address these questions, we first examined the developmental profile for the acquisition of

associative learning in a rat model of FXS generated on a Long Evans Hooded background. Associative memory was tested using a range of behavioral paradigms that rely on an animals' innate tendency to explore novelty. *Fmr1* knockout (KO) rats showed a developmental delay in their acquisition of object place recognition but no difference in the acquisition of object or object-context recognition relative to their littermate controls. Furthermore, *Fmr1* KO animals could not identify novelty in the object-place-context recognition paradigm at any age tested (up to 23 weeks of age). Treatment of *Fmr1* KO rats with lovastatin between 5 and 9 weeks of age, during the normal developmental period this associative memory capability is established, prevents the emergence of deficits but has no effect in wildtype animals. Moreover, we observe no regression of cognitive performance in the FXS rats over several months post-treatment. This restoration of the normal developmental trajectory of cognitive function is associated with the sustained rescue of both synaptic plasticity and altered protein synthesis. The findings provide proof-of-concept that the impaired emergence of the cognitive repertoire in neurodevelopmental disorders may be prevented by brief, early pharmacological intervention.

Introduction

Fragile X Syndrome (FXS) is a major heritable cause of intellectual disability (ID) and one of the most common single gene causes of autism spectrum disorder (ASD), with 30-50% of boys clinically diagnosed with ASD (28814540). It affects approximately 1:4,000 boys and 1:6-8,000 girls. FXS has numerous co-occurring conditions including anxiety disorders, sensory hypersensitivity and seizures (28814540). FXS is usually diagnosed around 3 years of age as a result of a delay in language development (2). However, early diagnosis through genetic screening suggests early symptom development in agreement with data from cellular phenotypes in rodent models (2-4). FXS is typically caused by an expansions of a trinucleotide repeat (CGG) in the promoter region of the gene that leads to silencing of the gene and no protein expression (5), although de novo mutations that are predicted to alter protein function also cause FXS (6, 7).

There is abundant preclinical evidence that an array of functional impairments in FXS arise from a disruption of cellular biochemistry and physiology that is correctable with pharmacological interventions (for reviews see, 2; 8-10). Furthermore, based on knowledge of critical periods in sensory system development and language acquisition (11, 12), it has been suggested that therapeutic success would be improved by starting treatments early,

before major symptoms develop (13). Further, it is not known whether effective treatments would need to be maintained throughout life.

Numerous clinical trials have taken place for FXS including the development of novel compounds as well as drug repurposing (14). There are numerous advantages of drug repurposing in the treatment of disease, including speed of translation and cost implications associated with new drug development (15). When assessing the feasibility of initiating chronic treatments in infancy, an obvious concern is safety. For these reasons, there has been interest in the possibility of repurposing drugs with a known safety profile in children. Two examples of such candidates are arbaclofen, a γ-aminobutyric acid B ($GABA_B$) receptor agonist used for the treatment of spasticity in cerebral palsy, and lovastatin, an HMG-CoA reductase inhibitor used for the treatment of hypercholesterolemia. These compounds have been shown to correct the alterations in synaptic plasticity, neuronal morphology and excitability and behavioral alterations in a mouse model of FXS (16, 17). The precise mechanisms underlying this rescue in *Fmr1* knockout (KO) mice is not known; however, increasing $GABA_B$ activation with arbaclofen alters the excitability of neurons and reduces presynaptic release of glutamate leading to a reduction in postsynaptic glutamate receptor activation, including the metabotropic glutamate receptor 5 (mGluR5) (18); two key cellular processes thought to underlie FXS pathophysiology (9). Lovastatin regulates the membrane association of Ras and subsequently mildly reduces the downstream activation of the extracellular signal-regulated kinases 1/2 (ERK1/2) (17). A recent human study evaluating the effects of arbaclofen in child and adolescent with FXS suggested that age is indeed a critical variable in determining the effect of the treatment; Arbaclofen had therapeutic effects in children that were lost in adolescent/adults with FXS (19). Phase 1 clinical trials with lovastatin in FXS were promising (20) and a follow up studies have not been reported yet (NCT02680379, NCT02998151, NCT02642653). The combined age range in these trials are 8-55 years of age.

As noted above, a key question for the potential treatment of FXS is the age at which treatment should begin to maximize effectiveness. In this study, we tested the hypothesis that lovastatin treatment restricted to early development could permanently prevent impairments in cognitive function in an animal model of FXS. We chose lovastatin as it can be orally administered in food, it has been shown to ameliorate circuit deficits in *Fmr1* KO mice and it has a known safety profile in children (17).

Key to addressing this question is knowledge of the development of cognitive abilities in animal models. Robust behavioral paradigms independent from reward-based learning that can be used repeatedly in the same animals are therefore required. Therefore, to assess cognition across juvenile development in rats, we utilized a battery of spontaneous object exploration "novelty preference" tasks. These tasks are based on rodents' innate preference to explore novel over familiar stimuli in their environment (21), and can be used to infer memory for the familiar stimulus (22). The four task variants used in the current study test the ability to discriminate between novel and familiar objects (object recognition, OR; 22), novel and familiar configurations of objects and local contextual cues within the testing box (object-context recognition, OCR; 23, 24), novel and familiar configurations of objects and their spatial locations within the testing box (object-place recognition, OPR; 25) or novel and familiar configurations of objects, their spatial locations and the local context (object-place-context recognition, OPCR; 25). These tasks were chosen for several reasons. First, we have previously reported that whereas adult *Fmr1* KO rats show intact short-term recognition (2-5 min) of objects (OR), object-context associations (OCR) and object-place associations (OPR), unlike their wildtype (WT) littermates they do not show intact short-term memory for object-place-context configurations in the OPCR task (26). Therefore, we could assess whether treatment during juvenile development would ameliorate the deficits in OPC memory in *Fmr1* KO rats. Second, the tasks are based on spontaneous behavior, and do not depend on acquisition of task rules and stimulus-reward contingencies. This means that the same animal can be tested on multiple occasions (with different objects) to assess recognition memory at different ages. Third, the spontaneous exploration protocol has been used successfully in juvenile rats to characterize the ontogeny of recognition memory abilities in rats. These studies have shown that object recognition memory emerges before postnatal day 17 in the LEH rat (27), whereas object-context recognition emerges at around P17 or P26 depending on the types of cues used to define context (28). In contrast, object-place recognition (29) and object-place-context recognition (30) emerge later during juvenile development. Finally, short term (2-5 min) recognition memory in the four different task variants described above is thought to depend on different overlapping brain circuits (31). Whereas the complete circuitry supporting recognition memory in each task is not fully resolved, it is generally agreed that the perirhinal cortex is required for object recognition (OR), whereas the entorhinal cortex, hippocampus and medial prefrontal cortex (mPFC) are not (although there is debate concerning the role of the hippocampus at longer retention intervals, and also its normal role in the intact brain) (23-25, 31-34) . For the version of the OC task described here (in which context is signaled by local

non-polarizing cues), the postrhinal cortex and lateral entorhinal cortex (LEC) are necessary (24, 34, 35). The LEC together with its connections with the mPFC are required for memory in the OP task (33, 34), whereas for the OPC task the hippocampus is required, together with the LEC and its connections with mPFC (25, 32, 33).

In this study, we examine the developmental emergence of associative memory using object exploration tasks in a new rat model of FXS. We then examine whether deficits in associative memory can be prevented by early treatment with lovastatin and whether they persist after treatment termination. Finally, we examine whether lovastatin treatment corrects alterations at cellular levels in hippocampus and mPFC, two regions known to be involved in the tasks used in the study and known to have altered function in FXS (36, 37).

Results

WT LEH rats show distinct developmental trajectories in different spontaneous object exploration tasks

As a first step to determining the emergence of cognitive abilities in rats, we conducted a longitudinal study to examine the developmental time-course over which WT LEH rats exhibit non-associative and associative recognition memory capabilities by examining their performance on a battery of four spontaneous exploration-based tasks from 4 weeks old to adulthood. In all tasks, memory is infered based on the amount of time an animal spends preferentially exploring the novel object or novelty based on an object's location and/or context (Fig. 1A). At 4-6 weeks old, rats exhibit novelty preference in both the non-associative OR task and the associative OCR task (Fig. 1B, C), indicating memory of objects and object-context associations. Memory was observed at all subsequent ages tested for these tasks. In contrast, novelty preference was not observed in the associative OPR and OPCR tasks at 4-6 weeks of age but was present at 7-9 weeks and at later ages (Fig. 1D, E). This indicates that the circuits necessary for these associative memory processes are not fully mature until 7 weeks of age. Total exploration time did not differ between time points for any task (Fig. 1B-E).

Loss of Fragile X protein (FMRP) leads to selective deficits in object-place and object-place-context memory in *Fmr1* KO rats

We generated an *Fmr1* KO rat line on the outbred LEH strain (Fig. 2A) to determine whether the developmental trajectory of associative memory was altered as a result of chronic deletion

of the *Fmr1* gene. In *Fmr1* KO LEH rats, no *Fmr1* transcripts were detected by reverse transcription-PCR (Fig. 2B). This was also confirmed by RNA sequencing. Similarly, no protein was detected using either western blotting or immunohistochemistry (Fig. 2C, D) confirming the complete absence of FMRP. In agreement with previous findings in the mouse (33) and Sprague-Dawley (SD) rats (26), FMRP is expressed throughout postnatal development (Fig. 2E).

At a behavioral level, our results showed that adult (3-4 months old) *Fmr1* KO rats on the LEH background had selective deficits in the OPCR task, but intact novelty preference in the OR, OCR and OPR tasks (fig. S1). This agrees with our previous findings in *Fmr1* KO SD rats (26), indicating that this pattern of cognitive deficits is shared across outbred strains of adult rats. Repeated testing during juvenile development (4-23 weeks) revealed an emergence of OR and OCR ability in *Fmr1* KO LEH rats undistinguishable from that seen in WT animals (Fig. 2F, G). In contrast, the ability of *Fmr1* KO rats to show significant memory in OPR was delayed by 2-4 weeks compared to WT rats (Fig. 2H). As predicted from our findings in adult rats (26) (fig. S1), the *Fmr1* KO rats did not exhibit object-place-context associative memory on the OPCR task at any age (Fig. 2I). For each of the four tasks, the total time exploring objects in the sample and test phases did not differ between genotypes across different time points (fig. S2), indicating that the deficits were not secondary to changes in motivation or ability to explore objects.

Transient treatment with lovastatin restores wildtype-like developmental trajectory of object-place and object-place-context memory in *Fmr1* KO rats and has sustained effects on memory

Previous studies in the mouse model of FXS have shown that continuous, chronic treatments can correct behavioral phenotypes such as hyperactivity in an open field (17, 38), susceptibility to audiogenic seizures (16, 17) and impairments in startle response and inhibitory avoidance (38). However, the effects of a transient treatment, initiated early in postnatal life on cognitive impairments are still mostly unexplored. We next tested whether a transient administration of the drug lovastatin to juvenile rats would correct the cognitive deficits described above. Lovastatin was chosen for three reasons. First by inhibiting the ERK signaling pathway (fig. S3), lovastatin has been shown to correct the excessive protein synthesis that is a core pathophysiology of FXS, and numerous cellular and circuit-based phenotypes associated with the loss of FMRP in mice. Second, lovastatin is an FDA approved drug for the treatment of a

familial form of hypercholesterolaemia (39) and has a known safety profile in children. Third, lovastatin can easily be administered through the diet (17).

We tested the effect of administering a five-week oral free-feeding laboratory chow protocol, with and without lovastatin (100mg/kg), initiated at 4 weeks, prior to the emergence of associative recognition in the OPR and OPCR tasks in WT rats (Fig. 3A). Treatment condition (lovastatin vs control diet) had no effect on food intake, and weight gain did not differ between genotypes or treatment conditions (fig. S4). Moreover, total object exploration time did not differ between experimental groups (fig. S5). However, the lovastatin treatment restored the normal developmental emergence of OPR and OPCR abilities in *Fmr1* KO rats without altering that of WT rats (Fig. 3B, C). Indeed, at 7-9 weeks of age both WT and *Fmr1* KO animals on lovastatin showed similar novelty preference as WT animals on control chow in the OPR and OPCR tasks, whereas *Fmr1* KO animals on control chow showed no evidence of memory on these tasks at that age (Fig. 3D). OR and OCR ability, which are not impaired in the *Fmr1* KO rats, were not affected by the lovastatin treatment in either genotype (fig. S6). Next, to determine whether the ability of *Fmr1* KO rats to perform OPCR requires continuous exposure to lovastatin, we terminated the treatment at 9 weeks of age and tested the rats again at 14 and 23 weeks of age. Treated *Fmr1* KO rats maintained their ability to perform the OPCR task even at 14 weeks, and also at 23 weeks of age, 5 and 14 weeks after termination of lovastatin treatment respectively (Fig. 3E). Moreover, the animals' ability to perform the OPR remained unaffected following removal of lovastatin (see Fig. 3B). These results suggest that the restoration of the developmental trajectory of associative recognition performance by lovastatin treatment persists long after treatment termination.

Early, brief lovastatin treatment corrects increased basal protein synthesis levels in *Fmr1* KO hippocampus 15 weeks after drug removal

We next tested whether the same brief lovastatin exposure produced sustained correction of the elevated protein synthesis that is known to result from the loss of FMRP (40-42). Since OPCR is a hippocampus-dependent task (32) we examined basal protein synthesis in hippocampal slices from the rats used in our behavioral study, harvested at 24 weeks of age. *Fmr1* KO rats on control diet showed an increase in basal protein synthesis relative to WT rats even at this old age. The temporary lovastatin treatment between 4 and 9 weeks of age corrected basal protein synthesis in *Fmr1* KO rats without affecting WT rats (Fig. 3F).

Lovastatin prevents the emergence of prefrontal cortex plasticity deficits associated with the loss FMRP

We next tested whether the same regimen of brief early lovastatin treatment could prevent the emergence of an age-dependent phenotype in synaptic plasticity associated with the loss of FMRP (43). As mPFC connections are required for OPCR (referred to by Chao et al. as object-context preference task) (33), and *Fmr1* KO mice exhibit an age-dependent deficit in long term potentiation (LTP) of synaptic responses in this region (43), we assessed LTP in the mPFC of *Fmr1* KO rats. Our results show that whereas LTP at layer 2 to layer 5 synapses of mPFC is not affected by the loss of FMRP at 4-6 weeks of age (Fig. 4A), LTP in *Fmr1* KO rats was impaired at 10-12 weeks of age (Fig. 4B). Lovastatin treatment from 5 weeks of age prevented the emergence of this LTP deficit as measured in 10-12 week-old animals (Fig. 4C).

Finally, lovastatin has previously been shown to rescue the exaggerated mGluR-dependent LTD found in mouse hippocampus at 1 month of age (17). To determine whether a similar effect on hippocampal LTD was present in our animals, we first examined whether LTD was exaggerated at 4-5 weeks and at 9-10 weeks (the age at which lovastatin is terminated in our experiments). Although we confirmed that Dihydroxyphenlyglycine (DHPG) induced LTD is exaggerated in *Fmr1* KO LEH rats at 4-5 weeks of age, no difference was found at 9-10 weeks. Therefore, the effects of lovastatin treatment in our paradigm could not be examined. These data suggest that the downstream mechanisms supporting mGluR-dependent LTD change during postnatal development (fig. S7).

Discussion

This study sought to test the hypothesis that early and transient therapeutic intervention can produce long lasting benefits on cognition in a rat model of FXS. Our results demonstrate that early lovastatin treatment for 5 weeks, initiated before the capabilities for object-place and object-place-context associations emerge, restored the normal developmental trajectory of these cognitive abilities in *Fmr1* KO rats. Furthermore, the ability to perform associative recognition in these tasks persisted for at least 14 weeks after the end of treatment. These findings indicate that brief, early treatment not only prevents the emergence of cognitive deficits, but also that the beneficial effects on cognition are sustained long after the end of treatment, suggesting that lovastatin might rescue the normal development of the neural circuits underlying these behaviors, and that FMRP, lacking in these animals, might not be needed for

their maintenance. Hence, treating rats during an early developmental time window prior to or during the development of cognitive abilities had long-lasting effects on cognition.

Natural history studies of the symptomatology of individuals with neurodevelopmental disorders provide valuable insights into the mechanistic basis of the developmental trajectory of these disorders. A challenge for animal studies modelling these disorders is to effectively capture developmental trajectories, especially for cognitive abilities (44, 45). Ideally this requires longitudinal behavioral testing to assess cognitive ability across development in the same animal. In this study, we demonstrate distinct developmental trajectories for different types of recognition memory in WT Long Evans hooded rats using a battery of four spontaneous exploration-based tasks. The ability to exhibit non-associative memory for objects and associative object-context memory is apparent by 4-6 weeks of age [consistent with previous findings (28)]. In contrast, we find that the ability to exhibit associative object-place and object-place-context memory does not emerge until 7 weeks of age [but see (30)]. Whereas the *Fmr1* KO rats showed intact memory at each time point in the early-developing object and object-context tasks, loss of FMRP specifically affected the later-developing cognitive abilities, with a delay in the object-place task, and inability to show memory in the object-place-context task at any age. OPR and OPCR require the coordination of number of intact brain circuits including prefrontal cortex (32-34). The observed abnormalities in OPR and OPCR are paralleled by an age-dependent deficit in LTP in the prefrontal cortex. This late appearance of LTP deficit in *Fmr1* KO LEH rats suggests that the role of FMRP in mediating this form of plasticity changes over postnatal development. A similar conclusion can be drawn from our finding that mGluR-dependent LTD in CA1 is increased in *Fmr1* KO rats at 1 month but not 2 months of age. It will be interesting in future experiments to determine whether the targets of FMRP change over this period or whether the mRNAs associated with polyribosomes differ (46) (see below).

The characterization of developmental trajectories of cognitive function in rodent models of neurodevelopmental disorders is important, as it provides a temporal framework for designing experiments to determine whether potential therapeutic interventions either prevent the emergence of deficits or reverse established deficits. Based on this framework we treated animals with lovastatin starting at 4 weeks of age and showed that this intervention prevented the emergence of cognitive deficits in object-place and object-place-context recognition usually seen at 7-9 weeks in *Fmr1* KO rats. Furthermore, even after treatment was terminated at 9 weeks, these cognitive abilities remained intact for at least another 3 months (the last time-point tested). This is particularly important, as untreated *Fmr1* KO rats were unable to show

OPC recognition at any age tested. Lovastatin also prevented the emergence of age-dependent deficits in FMRP-dependent synaptic plasticity in the prefrontal cortex of *Fmr1* KO rats.

To investigate the mechanisms underlying the effect of lovastatin treatment, we next examined basal protein synthesis in the hippocampus. The increase in protein synthesis in rat hippocampus at 4 weeks of age (26) persisted until 6 months of age and was normalized by transient lovastatin treatment from 4-9 weeks. This extends previous findings from the mouse model of FXS showing that acute lovastatin application to hippocampal slices corrects deficits in basal protein synthesis (17) by demonstrating that oral lovastatin administration can reverse this deficit once it has appeared. More importantly, this reversal lasts for 4 months after removal of the lovastatin suggesting that transient inhibition of the ERK signalling pathway is sufficient to promote a long-term reset of protein synthesis in FXS. This effect appears to be inconsistent with the idea that increased protein synthesis in the hippocampus of *Fmr1* KO rats is a direct consequence of the loss of FMRP binding to their target RNAs (18). An alternative explanation is that the increased basal protein synthesis observed in *Fmr1* KO hippocampus actually reflects a compensatory response to the absence of FMRP, and this is no longer needed following appropriate treatment. Such an interpretation would suggest that most of the excess signal in the protein synthesis assay arises from translation of mRNAs that are not direct FMRP targets, which is supported by our recent study (46), using the Translating Ribosome Affinity Purification (TRAP) assay which detects mRNAs associate with ribosomes. We found that FMRP target mRNAs were underrepresented in the ribosome bound pool in *Fmr1* KO CA1 hippocampal pyramidal neurons relative to WT controls (46). Furthermore, *Chrm4* mRNA, which encodes the muscarinic acetylcholine receptor 4 (M4), showed an increase in ribosome association and stimulation of M4 normalized many *Fmr1* KO phenotypes. These findings suggest that the increase in translation in *Fmr1* KO neurons is a compensatory response to the deletion of FMRP rather than a direct result, at least at the ages tested in these studies (46).

Treatment with lovastatin may remove the trigger for these compensatory changes by preventing the developmental emergence of the cellular or circuit dysfunction associated with the loss of FMRP. Cellular and circuit excitability dysregulation has been shown in a range of brain regions and cell types in *Fmr1* KO mice (9, 10). Since circuit activity is known to regulate early neural development, lovastatin could be exerting its disease-modifying role through its regulation of mGluR-dependent neuronal excitability. This hypothesis is supported by our previous findings that acute lovastatin application rescues both audiogenic seizures, and mGluR-dependent epileptiform activity in acute brain slices in *Fmr1* KO mice (17).

Furthermore, acute lovastatin treatment rescued the increase in mGluR-dependent LTD in CA1 in *Fmr1* KO mice (17).

It is important to note the limitations of this study that can be explored in the future. First although there was no alteration in weight gain between genotypes or treatment group, we have not been able to measure the dose of lovastatin received by each animal. Furthermore, we have not defined whether a critical period exists for effectiveness of lovastatin treatment or defined an effective minimal treatment duration and we have focused on associative learning paradigms that rely on an animals' inherent attraction to novelty. Whether the beneficial effects of lovastatin can be generalized to other ages and other forms of learning is unknown, but we note that previous studies have reported this to be the case (17). Whether these findings will directly translate to clinical outcome for individuals with FXS is unknown. However, in this context it is important to remember that our treatment regimen was initiated in 1-month old rats. Although it is difficult to accurately estimate the corresponding age in humans, it is clear that this is much earlier than the majority of FX individuals enrolled in ongoing trials with lovastatin (NCT02680379, NCT02998151, NCT02642653).

In summary, using assays of cognitive ability that rely on an animal's natural exploratory behavior we have been able to demonstrate that early, brief treatment restores the normal developmental trajectory of associative memory acquisition that persists well into adulthood. This rescue is paralleled by physiological and biochemical rescue in the prefrontal cortex and hippocampus, respectively. The findings provide proof-of-concept evidence that Fragile X Syndrome, and perhaps neurodevelopmental disorders more generally, may be amenable to transient, early intervention to permanently restore normal cognitive developmental trajectories.

Materials and Methods

Study design

The purpose of this study was to test the hypothesis that early therapeutic intervention can produce long lasting benefits on cognition in a rat model of FXS. For behavioral studies four groups of rats were used: WT control, WT treatment, KO control, KO treatment; N=12 per group based on power calculations on published data from the lab (Langston and wood) (Effect size=1.34, α=0.05, power=0.85). Littermates were housed in mixed-genotype cages (3-4 rats per cage), and cages were randomly assigned to control or treatment conditions [in line with the ARRIVE guidelines (47)]. For the protein synthesis experiments sample sizes were chosen

based on power calculations on published data from the lab (17, 26, 42) (Effect size=2.12, α=0.05, power=0.91); WT control, WT treatment, KO control, KO treatment; N=6 per group (at least two slices from each rat used to produce the value for each animal) were used for metabolic labelling. In the same fashion for in vitro electrophysiology sample sizes were chosen based on power calculations on published data from the lab (17, 26, 42) (Effect size=1.45, α=0.05, power=0.82); WT control-N=9, WT treatment-N=7, KO control-N=9, KO treatment-N=8; (at least two slices from each rat used to produce the value for each animal). Experimenters were blind to the genotype and the treatment during both data collection, scoring of behavioral data, and data analysis. For behavioral testing, we excluded data from trials in which animals showing very low object exploration (less than 5 s of exploration per object or 15 s total object exploration during the sample phase, or less than 15 s total object exploration in the test phase). No effect of genotype was observed on the number of trials excluded due to insufficient exploration. Testing was always performed during the light phase of the cycle. All animal experiments were approved by the University of Edinburgh veterinary services prior to their start and were performed in accordance with the guidelines established by European Community Council Directive 2010/63/EU (September 22, 2010) and by the Animal Care (Scientific Procedures) Act 1986.

Animals and treatment

Male LE-*Fmr1*$^{em1/PWC}$, hereafter referred to as *Fmr1* KO, and WT littermates, bred in-house and kept in a 12h/12h light dark cycle with ad libitum access to water and food were used. Colony founders were produced by Sigma Advanced Genetic Engineering (SAGE) Labs, using Zinc finger nuclease (ZFN)–mediated disruption of *Fmr1* (48) with a targeted construct containing coding sequence for eGFP; resulting founders did not express FMRP or eGFP. Pups were weaned off their dams at postnatal-day 22 (P22) and housed in mixed genotype cages with littermates, 3-4 animals per cage. Animals were genotyped by PCR. Ad libitum standard laboratory chow was provided until P29 On P29 the diet was changed to either control or lovastatin-enriched (100mg/kg) (Bioserv) diet which was restocked and weighed once daily. At P64, animals were returned to ad libitum standard laboratory chow until the end of experiment (P164). Rats' weight and consumption per cage was monitored throughout the dosing period (P29-P64) to ensure that diet did not have any adverse effects on their growth.

RNA isolation and RT-PCR

Total hippocampus RNA was isolated from 4-month olds rats (3 WT and 3 *Fmr1* KO) using RNeasy Lipid Tissue Kit (Qiagen) as per manufacturer's instructions. 2 µg total RNA was used for cDNA synthesis using SuperScriptIII (Invitrogen) with oligo(dT) and random hexamers. PCR was performed using GoTaq Green master mix (Promega). *Fmr1* primers span exons 1 to 4 (Fmr1_e1F: CGA GGA AGG ACG AGA AGA TG and Fmr1_e4R: CAC CCT TTA TCA TCC TCA C; amplicon 284bp). Primers to GFP (GFP_F: ACG TAA ACG GCC ACA AGT TC and GFP_R: ATG CCG TTC TTC TGC TTG TC; amplicon 421bp) and 18S (18S F: GTG GAG CGA TTT GTC TGG TT and 18S R: CAA GCT TAT GAC CCG CAC TT; amplicon 321bp) and cDNA from a GFP transgenic mouse were used as positive controls.

Immunoblotting

Hippocampus extracts from *Fmr1* KO rats and controls (n=3/age for developmental expression; n=3/genotype at P14 to verify loss of expression) were prepared in RIPA buffer containing protease inhibitors (Complete EDTA-free), immunoblotted using primary antibodies raised to the C-terminal half of FMRP (1:5000; AbCAM ab69815), GFP (1:5000; AbCAM ab6673) and b-actin (1:10,000; Sigma AC-74) and imaged as previously described in (36).

Immunohistochemistry

Histology was performed as previously described (49). Coronal sections were reacted with an antibody raised to the N-terminal half of FMRP (1:1500, Millipore MAB2160).

Spontaneous object exploration tasks

Apparatus: Animals were tested in a rectangular polycarbonate testing box (76 cm long × 45 cm wide × 60 cm tall) with removable walls and floor inserts that could conform to two contexts. Context 1: white textured wallpaper and wood-effect linoleum floor. Two Dual Lock (3M) re-sealable fasteners were attached to the floor 9cm from the box walls at north-east and north-west locations, used to keep the two objects firmly attached to the floor in the same locations for every trial. Context 2: matt blue painted walls and black rubber textured floor insert, with holes cut to gain access to the Dual Lock re-sealable fasteners where the objects were attached. The testing box was placed on a table surrounded on 3 sides by a black curtain, with one opening at the south side of the box (where subjects were placed). A lamp in the north-west corner and large high contrast poster on the north-east corner were used as external cues, which remained in the same position and orientation throughout experiments. The external

environment was kept as consistent as possible and a radio on low volume was used to mask potentially distracting noises. A variety of objects were used which were between 8x8x8cm and 11x11x11cm, non-porous, and easily cleaned. Each object was only used once per animal. Objects were cleaned between trials with 70% ethanol solution and unscented baby wipes (Huggies).

Handling and habituation: Starting from P21 (a day before weaning), animals were handled daily in the animal house and experimental room for 7 days prior to experiments. Task-specific habituation was performed in the two days prior to experiments (P26-27) within the experimental apparatus box to familiarize the animals to the apparatus and type of objects. On P26, the animals were habituated to both context configurations in cage groups (30 minutes per context) in the morning, and individually (10 minutes per context) in the afternoon. Between exposures to contexts, rats were placed in an opaque holding bucket. On P27, animals were individually habituated twice (morning and afternoon both context configurations each time) but this time 2 different objects were fixed in the positions they would encounter objects during testing (10 minutes per context, with objects). These objects were not used again during testing. During the habituation sessions, subjects were left undisturbed to explore.

Testing in spontaneous object exploration tasks: Rats were tested on 4 different object exploration tasks (object recognition, object context recognition, object-place recognition and object-place-context recognition) over a two-day period, and testing was repeated in the same rats at different ages (P28&P29, P35&P36, P42&P43, P49&P50, P56&P57, P63&P64, P70&P71, P77&P78, P98-P105, P164&P165). The general procedures for each task were the same.

For each phase of every task, the experimenter prepared the appropriate context configuration and attached two cleaned appropriate objects to the appropriate locations within the box using the DualLock re-sealable fasteners. Each animal was then removed from the home cage and placed in the box facing the south wall of the apparatus. The sample phase(s) and test phase were each 3 min long during which animals were free to explore. An overhead black and white camera (Panasonic) was used to monitor the exploring rat around the testing box. The video signal was fed into a DVD recorder and a computer on the desk of the experimenter which was 2 m away from the testing box. The computer ran in-house timing software (National Instruments, LabView) whereby the press of a key on the computer mouse would activate a timer. This was performed manually by the experimenter who observed the behavior of the rat

via the computer screen and recorded the amount of time the rat was engaged in exploration of each object. After 3 min, the animal was either placed in the opaque holding bucket (30cm diameter containing standard bedding) for 2 minutes (after a sample phase) or returned to the home cage (after a test phase). Exploration was defined as the animal actively exploring an object with its snout within 2 cm of the object and performing actions such as sniffing and whisking. Exploration was not scored when animal was not actively exploring object (i.e. climbing or resting on an object). Novel object positions, test phase contexts, context order in OCR and OPCR, and objects, were counterbalanced across genotypes, tasks and time points to ensure that the final results were as unbiased as possible.

Object Recognition (OR)

Object Recognition (OR, Fig. 1A) is a two-phase non-associative recognition task. In the sample phase, two identical objects are available in either context 1 or 2. In the test phase, two objects (one identical to the objects from the sample phase and one novel object) are available in the same context as the sample phase. This task is used to test whether the animal can detect object novelty and discriminate between two non-identical objects. Higher exploration of the novel than the familiar object is indicative of memory for the familiar object.

Object-Place Recognition (OPR)

Object-Place Recognition (OPR, Fig. 1A) is a two-phase associative-recognition task. In the sample phase, two non-identical objects are available in either context 1 or 2. In the test phase two objects (both identical to one object from the sample phase) are available in the same context as in the sample phase. This task is used to test whether an animal can associate a specific object with a location in space. Higher exploration of the object that is in a different location than it was experienced in the sample phase is indicative of object-place memory.

Object-Context Recognition (OCR)

Object-Context Recognition (OCR, Fig. 1A) is a three-phase associative-recognition task. In sample phase 1, two identical objects are available in either context 1 or 2. In sample phase 2, a different pair of identical objects are explored in the other context. In the test phase, two objects (one identical to the objects from sample phase 1, and the other identical to the objects from sample phase 2) are available in either context 1 or 2. This task is used to test whether an animal can associate an object with a surrounding context. Higher exploration of the object which is in a different context than it was experienced in the sample phase is indicative of object-context memory.

Object-Place-Context Recognition (OPCR)

Object-Place-Context Recognition (OPCR, Fig. 1A) is a three-phase associative-recognition task. In sample phase 1, two non-identical objects are available in either context 1 or 2. In sample phase 2, objects identical to those in sample phase 1 are available but the objects have swapped locations and are in the other context. In the test phase, two identical objects (identical to one of the objects in sample phases 1 and 2) are available in one of the two contexts. This task is used to test if the animal can associate an object to a location in a specific surrounding context (episodic-like memory). Higher exploration of the object which is in different location in that context than it was in the sample phase is indicative of object-place-context memory.

***In vitro* electrophysiology**

mPFC LTP

Subjects were anaesthetized using isoflurane and decapitated. The brain was quickly dissected out and placed in ice-cold (<4°C) modified slicing aCSF solution (in mM: NaCl 86, NaH_2PO_4 1.2, KCl 2.5, NaHCO3 25, D-glucose 24, Sucrose 75, $CaCl_2$ 0.5, $MgCl_2$ 7). 300µm coronal slices containing prelimbic mPFC were cut in ice-cold modified slicing aCSF solution using a vibratome and transferred to a holding chamber containing warmed recording aCSF solution (in mM: 124 NaCl, NaH_2PO_4 1.2, KCl 2.5, $NaHCO_3$ 25, D-glucose 20, $CaCl_2$ 2, $MgCl_2$ 1) where they were maintained at 35°C for 30 mins. Slices were left to recover for a further 30 mins at room temperature prior to the start of experimentation.

Slices were then placed in a submerged recording chamber heated to 31°C and perfused with pre-warmed carbogenated recording aCSF solution at a rate of 4-5ml/min. A recording electrode was placed in layer 5 of the prelimbic mPFC and stimulating electrode was placed in layer 2/3. Stimulating and recording electrodes were staggered to prevent direct antidromic stimulation. Synaptic responses were evoked every 30 secs using a bipolar nichrome stimulating wire attached to a constant current stimulus isolator delivering a 200µs pulse. Following acquisition of a 20min stable baseline, LTP was induced using 5x 500ms trains of 300Hz stimulation at 3 min intervals. Responses were then recorded for 60 min post tetanization.

Signal waveforms were amplified 1000x, low-pass filtered at 4kHz and digitized at 20kHz. fEPSP slopes were normalized to baseline values. Magnitude of LTP was calculated from 40-60min post-tetanus time points. Normalized data were averaged across experimental groups and reported as mean ± SE.

DHPG-induced LTD

Horizontal hippocampal slices (400 μm) prepared from P21 to P32 animals were incubated in oxygenated ACSF at 31°C for 30 min, then stored at room temperature until recording. An incision was made through CA3 prior to recording. Slices were continuously perfused in an interface chamber with 30 ± 1°C ACSF saturated with 95% O2-5% CO2 at 4–5 ml/min. mGluR-LTD was induced using dihydroxyphenylglycine (S-DHPG; 50 μM) for 5 min. LTD magnitude was calculated by dividing the average fEPSP slope from 50 to 60 min post-DHPG application by the average fEPSP slope during the 20 min baseline before DHPG application.

Metabolic labelling.

Hippocampal slices were prepared from age-matched male WT and *Fmr1* KO rats in an interleaved fashion as previously described (17, 26, 42). Briefly, hippocampi were rapidly isolated and 500 μm slices prepared from the dorsal half using a Stoelting tissue slicer. Slices were recovered for 4 h in 32.5°C ACSF (in mM: 124 NaCl, 3 KCl, 1.25 NaH_2PO_4, 26 $NaHCO_3$, 10 dextrose, 1 $MgCl_2$, 2 $CaCl_2$, saturated with 95% O_2 and 5% CO_2), then incubated for 30 min with 25 μM ActD to block transcription. To measure protein synthesis, slices were then transferred to fresh ACSF containing 10 μCi/ml ^{35}S-Met/Cys (Perkin Elmer) and incubated for 30 min. After labelling, slices were homogenized, and labelled proteins isolated by TCA precipitation. Samples were read with a scintillation counter and subjected to a protein concentration assay (Bio-Rad). Final data were expressed as counts per minute (CPM) per μg protein, normalized to the ^{35}S-Met/Cys ACSF used for incubation, and the average incorporation of all samples analysed in that experiment (8 samples per experiment, 4 WT and 4 *Fmr1* KO). All aspects of the experiments were performed blind to genotype.

Statistical analysis.

For all experiments, the researchers conducting the experiments, and scoring and analyzing the data were blinded to the genotype of the rats and to treatment group. For the lovastatin dosing experiments, mixed-genotype cages were randomly allocated to lovastatin and control diet groups. Statistical analysis was performed using GraphPad (Prism 6.0), SPSS 16.0 software or R v.3.4.4 (R Core Team, 2018); scripts were written and run using RStudio 1.0.153 (RStudio Team, 2016). Due to missing data because of our exclusion criteria linear mixed effect models (LMEs) were fitted to our longitudinal behavioral data using the R package lme4 v1.1-17 (50). Animal identity was included in models as a random effect and the variables of interest as fixed effects. To evaluate significance of effects using LMEs, the model without the variable of

interest (a reduced/null model) was compared to the model with the variable of interest using a likelihood ratio test. Where appropriate statistical significance was assessed using either two-way ANOVA or three-way ANOVA. Unpaired two sample t-tests followed by Bonferroni correction were used to compare differences between groups. One-sample t-tests were used to compare discrimination indicesagainst chance (DI=0) controlled for the false discovery rate using the Benjamini–Hochberg procedure (51). Rats were used as an experimental unit throughout the manuscript. In electrophysiological experiments were multiple slices were used from each animal (LTP/LTD experiments, protein synthesis) average value for each animal was used in the analysis. Results are presented as mean ± sem. Probabilities of $P < 0.05$ were considered as significant.

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Supplementary materials

Figure S1. Adult *Fmr1* KO LEH rats exhibit deficits in OPCR

Figure S2. Time exploring objects during test phase of spontaneous recognition tasks does not differ between genotypes

Figure S3. Rationale of lovastatin treatment in Fragile X syndrome

Figure S4. Lovastatin has negligible effect on the weight gain of rats

Figure S5. Time exploring objects during test phase of spontaneous recognition tasks does not differ between genotypes with or without lovastatin treatment

Figure S6. WT and *Fmr1* KO rats perform equally well in OR and OCR tasks throughout development with control and lovastatin diet

Figure S7. *Fmr1* KO LEH rats exhibit age specific increase of group I mGluR-LTD in CA1 of the hippocampus

Table S1. Statistical results from one-sample t-tests and post-hoc two-sample t-test for object exploration tasks throughout development in WT and *Fmr1* KO rats

Table 2. Statistical results from two-way ANOVA of exploration times in object exploration tasks throughout development in WT and *Fmr1* KO rats

Table S3. Statistical results from one-sample t-tests for object exploration tasks throughout development in WT and *Fmr1* KO rats with or without lovastatin treatment

Table S4. Statistical results from post-hoc two-sample t-tests for object exploration tasks throughout development in WT and *Fmr1* KO rats with or without lovastatin treatment

Table S5. Statistical results from two-way ANOVA of exploration times in object exploration tasks throughout development in WT and *Fmr1* KO rats with or without lovastatin treatment

Table S6. Statistical results from adult object exploration tasks, effect of lovastatin on food intake/weight gain, hippocampal basal protein synthesis and synaptic plasticity data

Table S7. Linear mixed effects model distribution tests of behavioural data

Table S8. Linear mixed effects modelling results of WT and *Fmr1* KO object exploration tasks throughout development

Table S9. Linear mixed effects modelling results of WT and *Fmr1* KO object exploration tasks throughout development with or without lovastatin treatment

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Author contributions: A.A., A.D.J., S.M.T., S.L., E.R.W., and P.C.K. conceived and designed the experiments. A.A. performed behavioural experiments and analysed the results. A.D.J. performed electrophysiology experiments and analysed the results. S.M.T. performed immunohistochemistry and molecular biology experiments and analysed the results. S.L. performed biochemistry experiments and analysed the results. T.S. performed electrophysiology experiments. O.D. performed statistical analysis. S.M.T. and P.C.K. designed the genetically modified rats. M.F.B., D.J.A.W. G.H.H. and E.K.O helped guide the research. A.A., A.D.J., S.M.T., E.R.W., and P.C.K. interpreted all the results and wrote the paper.

Competing interests:

M.F.B. holds a patent title ‘Methods of treating disorders with group I mGluR antagonists’ (US6890931B2). M.F.B. has served as a paid consultant to Q-State Biosciences, Vertex Pharmaceuticals, and Sunovion Pharmaceuticals.

Data and materials availability: The LE-*Fmr1*$^{em1/PWC}$ rat line are available from Prof. Peter Kind under a material transfer agreement with the University of Edinburgh. All data needed for the evaluation of the conclusions in the manuscript are present in the main manuscript and the Supplementary Materials.

Figure legends

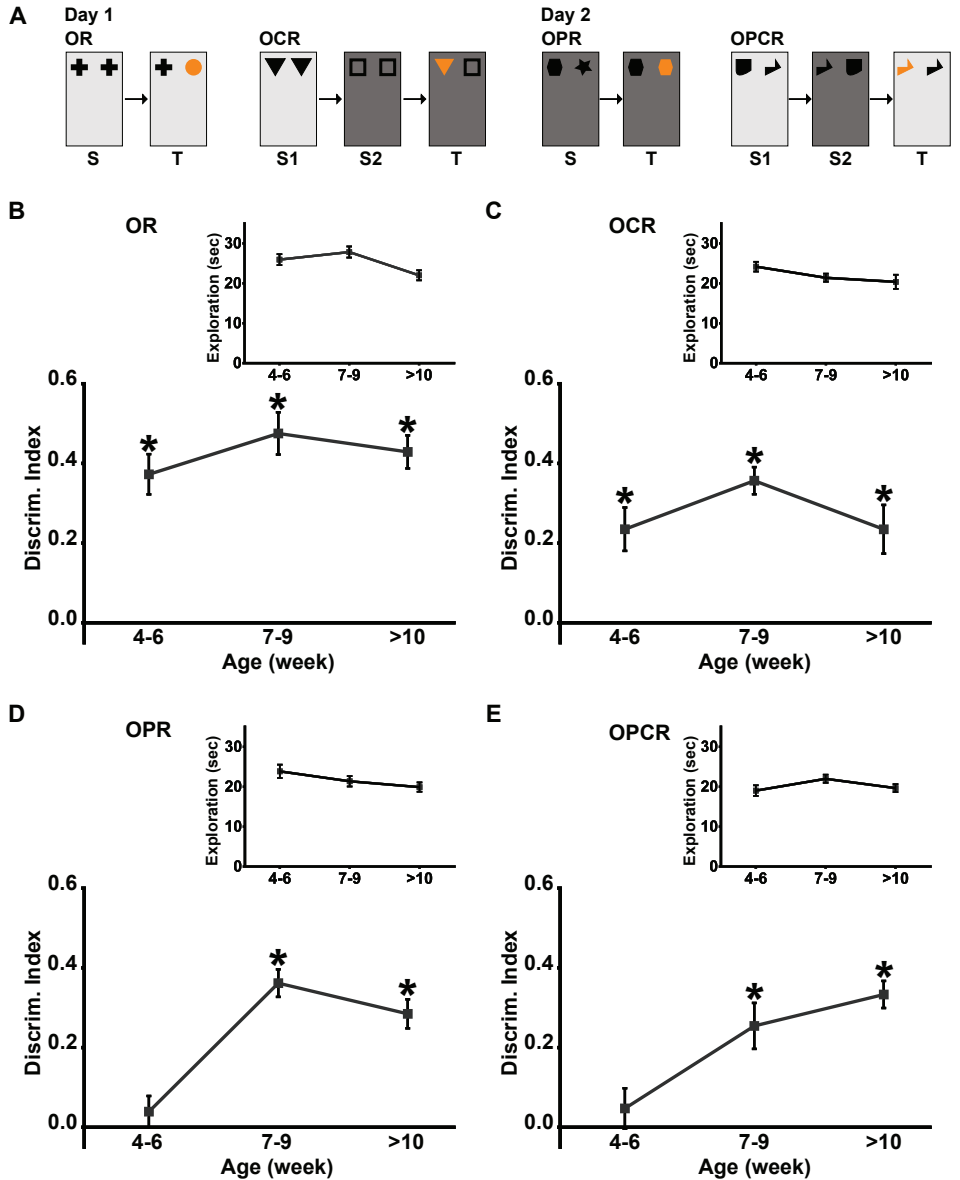
Figure 1. WT LEH rats show distinct developmental trajectories in different spontaneous object exploration tasks (**A**)**.** Schematic of the spontaneous object exploration tasks used. S = sample phase, T = test phase; Light and dark grey backgrounds denote distinct contexts; Orange icons denote novel object/object association. Discrimination index over time for WT LEH rats in (**B**) OR, (**C**) OCR, (**D**) OPR and (**E**) OPCR tasks. **Insert B-E** Total object exploration time during test phase for each task. For all tasks $N_{(4-6)}$=13, $N_{(7-9)}$=13, $N_{(>10)}$=11; * $p<0.05$ difference from chance (Discrimination Index =0) based on one-sample t-tests. p-values from one-sample t-tests have been corrected for false discovery rate using the Benjamini–Hochberg procedure. For details on t, df and p-values for one-sample t-tests see table S1.

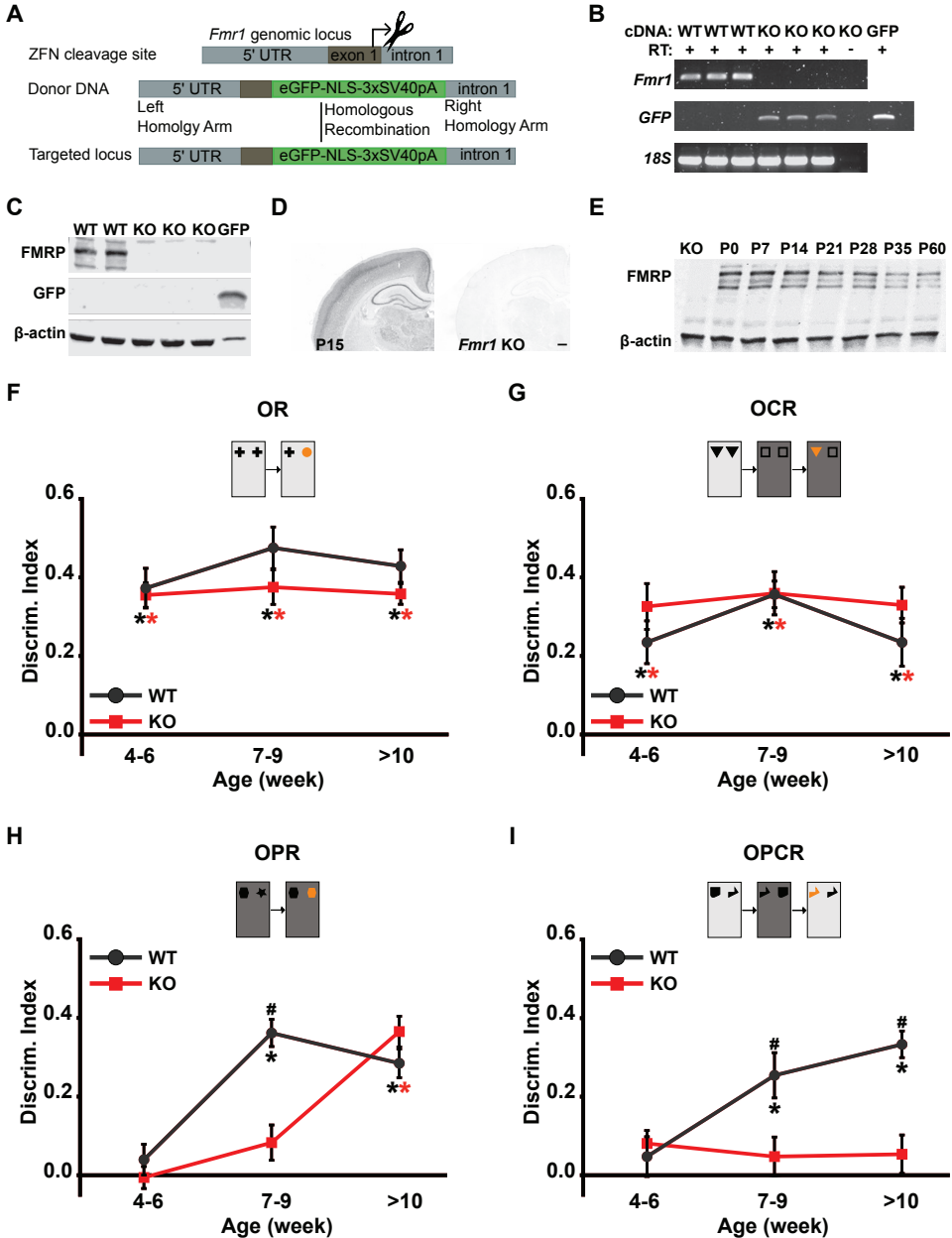
Figure 2. Loss of FMRP leads to selective deficits in object-place and object-place-context memory in *Fmr1* KO rats (**A**) Zinc finger nuclease (ZFN)–mediated disruption of *Fmr1*. Diagrams illustrate the target site for ZFN cleavage, donor DNA sequence including enhanced green fluorescent protein (*eGFP*) and a nuclear localization signal (NLS) flanked by 5' and 3' homology recombination arms for homology directed repair (HDR), and the resulting targeted locus (top); (**B**) RT-PCR for *Fmr1* and *eGFP* mRNA in WT and *Fmr1* KO rats. Lanes 1-3, samples from three WT rats; lanes 4-6 samples from three *Fmr1* KO rats; lane 7 –RT control from one *Fmr1* KO rat; lane 8 *GFP* positive control (bottom left); (**C**) Western blot of FMRP and GFP expression in WT and *Fmr1* KO rats. Lanes 1-2 samples from two WT rats; lanes 3-5 samples from three *Fmr1* KO rats; lane 6 positive control for GFP (bottom right). (**D**) Immunohistochemical localization of FMRP in P15 WT and *Fmr1* KO rats (top); scale bar: 500 μm. (**E**) Western blot analysis of FMRP expression in hippocampus homogenates from WT littermates over postnatal development compared with P15 *Fmr1* KO rat (bottom). Discrimination index at different ages for WT and *Fmr1* KO rats in (**F**) OR, (**G**) OCR, (**H**)

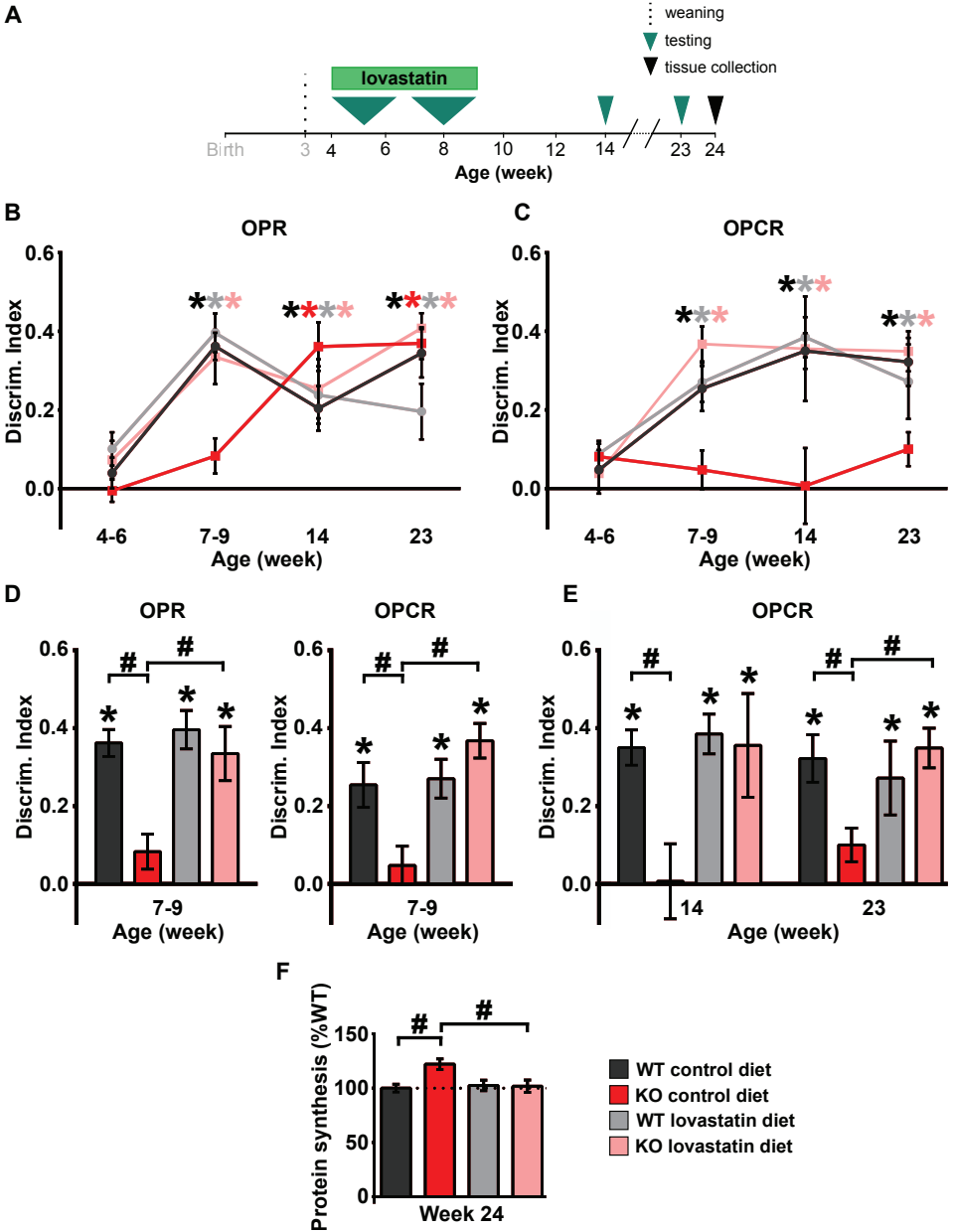
OPR and (**I**) OPCR task. For all tasks $N_{WT(4-6)}$=13, $N_{WT(7-9)}$=13, $N_{WT(>10)}$=11, $N_{KO(4-6)}$=12, $N_{KO(7-9)}$=12, $N_{KO(>10)}$=11; * $p<0.05$ difference from chance (Discrimination Index =0) black for WT and red for KO; # $p<0.05$ difference between groups. Linear mixed effect models (LMEs) were fitted to the data (for details see tables S7 and S8). p-values from one-sample t-tests and post-hoc two-sample t-tests have been controlled for the false discovery rate using the Benjamini–Hochberg procedure. For details on t, df and p-values, one-sample and post-hoc two-sample t-tests see table S1.

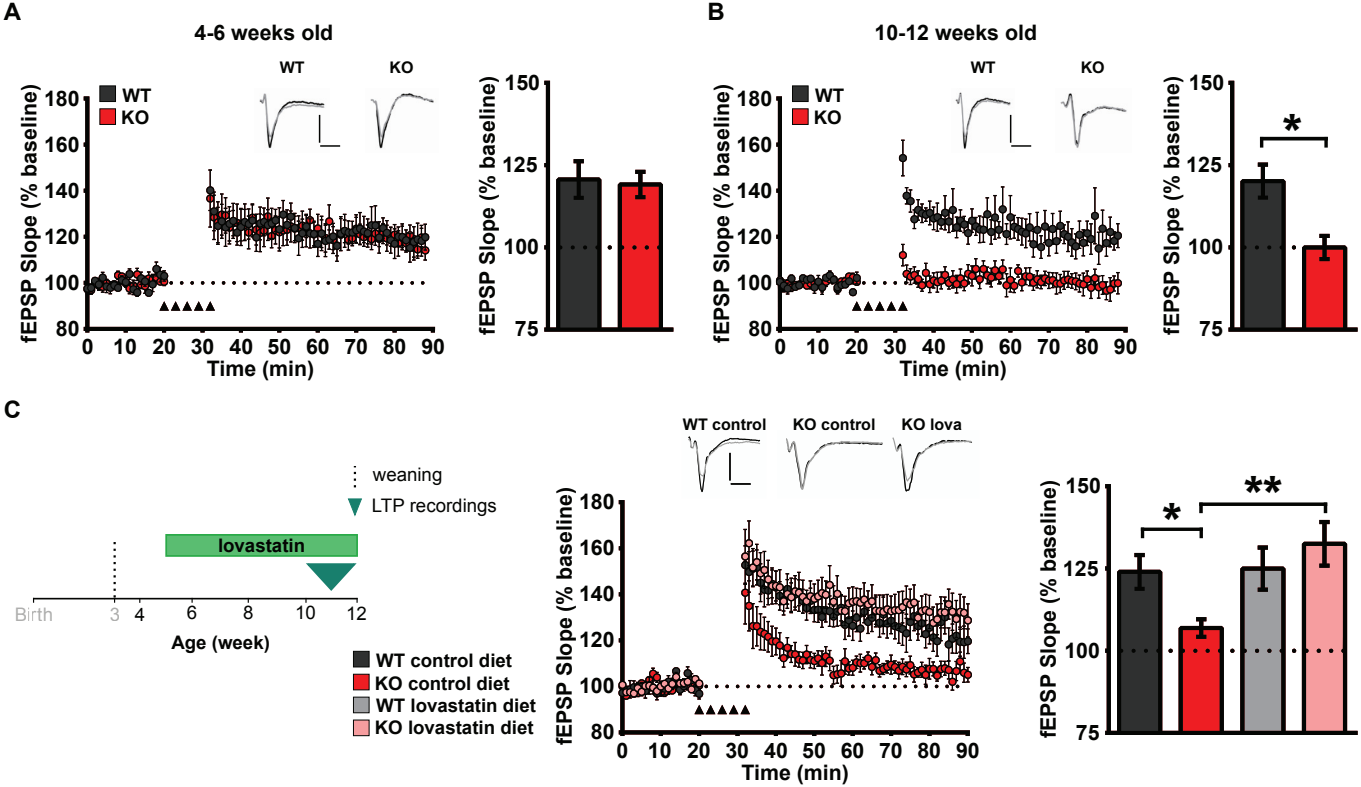
Figure 3. Transient treatment with lovastatin restores wildtype-like developmental trajectory of object-place and object-place-context memory in *Fmr1* KO rats and has sustained effects on both memory and cellular pathophysiology (**A**) Experimental time course for longitudinal assessment of cognitive development in WT and *Fmr1* KO rats treated with lovastatin between week 4 and week 9 of age. Discrimination index at different ages for WT and *Fmr1* KO rats treated with control or lovastatin diet in (**B**) OPR and (**C**) OPCR task. (**D**) Effect of lovastatin treatment on OPR (Left) and OPCR (Right) memory tested at 7-9 weeks of age in WT and *Fmr1* KO rats. (**E**) Effect of lovastatin treatment on OPCR memory in WT and *Fmr1* KO rats tested at 14 and 23 weeks of age. (**F**) Effect of lovastatin treatment on hippocampus basal protein synthesis levels in WT and *Fmr1* KO rats measured at 24 weeks of age, after behavioral testing was complete. Sample sizes: OPR 4-6 weeks $N_{WTcontrol}$=13, N_{WTlova}=12, $N_{KOcontrol}$=12, N_{KOlova}=12; OPR 7-9 weeks $N_{WTcontrol}$=13, N_{WTlova}=12, $N_{KOcontrol}$=12, N_{KOlova}=12; OPR 14 weeks $N_{WTcontrol}$=10, N_{WTlova}=11, $N_{KOcontrol}$=11, N_{KOlova}=8; OPR 23 weeks $N_{WTcontrol}$=11, N_{WTlova}=10, $N_{KOcontrol}$=11, N_{KOlova}=7; OPCR 4-6 weeks $N_{WTcontrol}$=13, N_{WTlova}=12, $N_{KOcontrol}$=12, N_{KOlova}=12; OPCR 7-9 weeks $N_{WTcontrol}$=13, N_{WTlova}=12, $N_{KOcontrol}$=12, N_{KOlova}=12; OPCR 14 weeks $N_{WTcontrol}$=11, N_{WTlova}=11, $N_{KOcontrol}$=11, N_{KOlova}=8; OPCR 23 weeks $N_{WTcontrol}$=10, N_{WTlova}=10, $N_{KOcontrol}$=11, N_{KOlova}=8; for protein synthesis N=6 for all groups. * $p<0.05$ difference from chance (Discrimination Index =0) black for WT and red for KO; # $p<0.05$ difference between groups. Linear mixed effect models (LMEs) were fitted to the behavioral data (for details see Tables S7 & S9) and two-way ANOVA with post-hoc two-sample t-tests was used to analyse effect of lovastatin on hippocampal protein synthesis levels (for details see table S6F). p-values from one-sample t-tests and post-hoc two-sample t-tests have been controlled for the false discovery rate using the Benjamini–Hochberg procedure. For details on behavioral data t, df and p-values for one-sample t-tests see table S3 and for post-hoc two-sample t-tests see table S4.

Figure 4. Lovastatin prevents the emergence of plasticity deficits associated with the loss FMRP. (**A**) Left panel: Time course plotting fEPSP slopes normalised to baseline following LTP induction in layer 2 to layer 5 synapses in the prelimbic mPFC taken from 4-6 week old WT and *Fmr1* KO rats. Right panel: Averages of fEPSP slopes normalised to baseline during the last 20min of the recording (70-90min). (**B**) Left panel: Time course plotting fEPSP slopes normalised to baseline following LTP induction in layer 2 to layer 5 synapses in the prelimbic mPFC taken from 10-12 week old WT and *Fmr1* KO rats. Right panel: Averages of fEPSP slopes normalised to baseline during the last 20min of the recording (70-90min). (**C**) Left panel**:** Experimental time course for assessment of effect of lovastatin treatment beginning at 5 weeks of age on WT and *Fmr1* KO plasticity in the prelimbic mPFC. Middle panel: Time course plotting averages of fEPSP slopes normalised to baseline following LTP induction in layer 2 to layer 5 synapses in prelimbic mPFC slices taken from 10-12 weeks old WT and *Fmr1* KO treated with either control or lovastatin diet. Right panel: Averages of fEPSP slopes normalised to baseline during the last 20min of recordings (70-90 min). Insets: Example traces showing synaptic responses during baseline (black trace) and 80-90 min (grey trace), Scale bar 0.5mV, 5ms. Sample sizes: LTP 4-6 weeks N=6, for WT and KO; LTP 10-12 weeks N=7, for WT and KO; for lovastatin effects on LTP $N_{WTcontrol}$=9, N_{WTlova}=7, $N_{KOcontrol}$=9, N_{KOlova}=8; *p<0.05, **p<0.01 difference between groups; Two-way ANOVA with post-hoc two-sample t-tests were used for data analyses (for details see Table S6G and H). p-values for post-hoc two-sample t-tests have been controlled for the false discovery rate using the Benjamini–Hochberg procedure.


A
Day 1
OR
S
T
OCR
S1
S2
T
Day 2
OPR
S
T
OPCR
S1
S2
T
B
OR
Exploration (sec)
30
20
10
0
4-6
7-9
>10
Discrim. Index
0.6
0.4
0.2
0.0
4-6
7-9
>10
Age (week)
C
OCR
Exploration (sec)
30
20
10
0
4-6
7-9
>10
Discrim. Index
0.6
0.4
0.2
0.0
4-6
7-9
>10
Age (week)
D
OPR
Exploration (sec)
30
20
10
0
4-6
7-9
>10
Discrim. Index
0.6
0.4
0.2
0.0
4-6
7-9
>10
Age (week)
E
OPCR
Exploration (sec)
30
20
10
0
4-6
7-9
>10
Discrim. Index
0.6
0.4
0.2
0.0
4-6
7-9
>10
Age (week)


A
Fmr1 genomic locus
ZFN cleavage site
5' UTR
exon 1
intron 1
Donor DNA
5' UTR
eGFP-NLS-3xSV40pA
intron 1
Left Homolgy Arm
Homologous Recombination
Right Homology Arm
Targeted locus
5' UTR
eGFP-NLS-3xSV40pA
intron 1
B
cDNA: WT WT WT KO KO KO KO GFP
RT: + + + + + + - +
Fmr1
GFP
18S
C
WT WT KO KO KO GFP
FMRP
GFP
β-actin
D
P15
Fmr1 KO
E
KO P0 P7 P14 P21 P28 P35 P60
FMRP
β-actin
F
OR
Discrim. Index
0.6
0.4
0.2
0.0
WT
KO
4-6
7-9
>10
Age (week)
G
OCR
Discrim. Index
WT
KO
4-6
7-9
>10
Age (week)
H
OPR
Discrim. Index
WT
KO
4-6
7-9
>10
Age (week)
I
OPCR
Discrim. Index
WT
KO
4-6
7-9
>10
Age (week)


A
weaning
testing
tissue collection
lovastatin
Birth
3
4
6
8
10
12
14
23
24
Age (week)
B
OPR
Discrim. Index
0.6
0.4
0.2
0.0
4-6
7-9
14
23
Age (week)
C
OPCR
Discrim. Index
0.6
0.4
0.2
0.0
4-6
7-9
14
23
Age (week)
D
OPR
OPCR
Discrim. Index
0.6
0.4
0.2
0.0
7-9
Age (week)
E
OPCR
Discrim. Index
0.6
0.4
0.2
0.0
14
23
Age (week)
F
Protein synthesis (%WT)
150
100
50
0
Week 24
WT control diet
KO control diet
WT lovastatin diet
KO lovastatin diet


A
4-6 weeks old
WT
KO
fEPSP Slope (% baseline)
Time (min)
B
10-12 weeks old
*
C
weaning
LTP recordings
lovastatin
Birth
Age (week)
WT control diet
KO control diet
WT lovastatin diet
KO lovastatin diet
WT control
KO control
KO lova
**

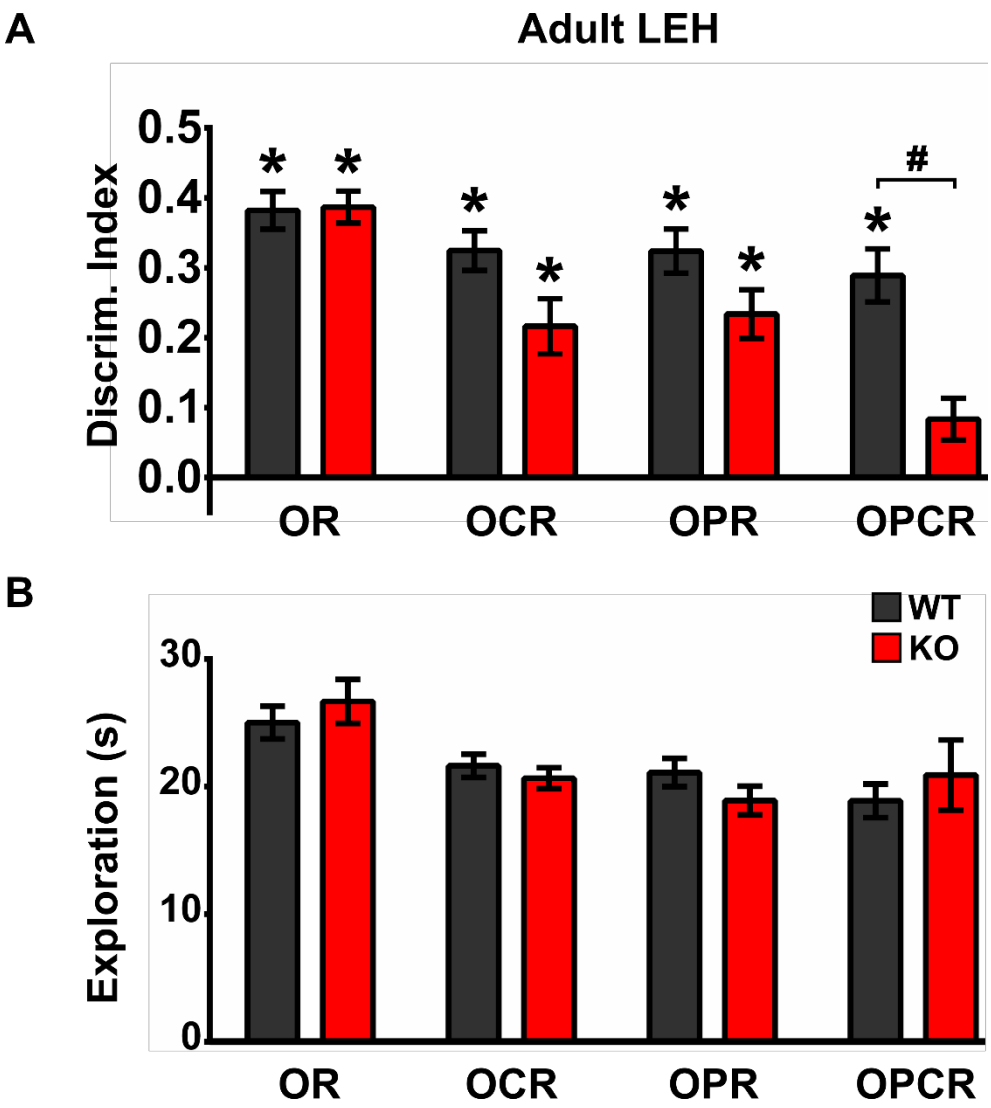


Figure S1. Adult *Fmr1* KO LEH rats exhibit deficits in OPCR. (**A**) Discrimination index of adult WT and *Fmr1* KO LEH rats in spontaneous object exploration tasks. (**B**) Total exploration times of adult WT and *Fmr1* KO LEH rats in spontaneous object exploration tasks. * $p<0.05$ difference from chance (Discrimination Index =0) from a one sample t-test and # $p<0.05$ difference between groups from two-way ANOVA with post-hoc two-sample t-test, for statistical details see table 6A and 6B.

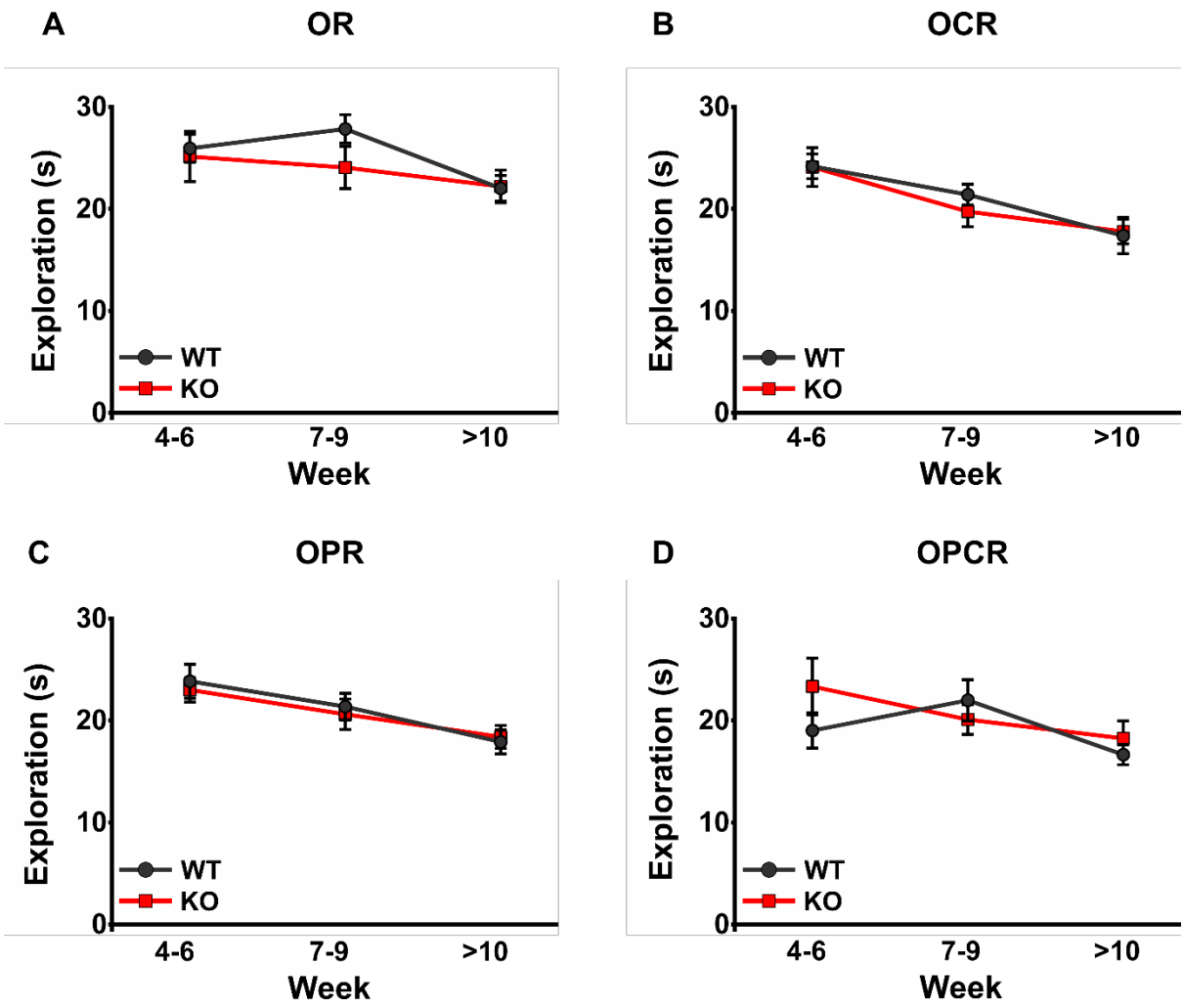


Figure S2. Time exploring objects during test phase of spontaneous recognition tasks does not differ between genotypes. Total object exploration over time of WT and *Fmr1* KO rats in (**A**) OR, (**B**) OCR, (**C**) OPR and (**D**) OPCR tasks. Two-way ANOVA was used for statistical analysis, for details see table S2.

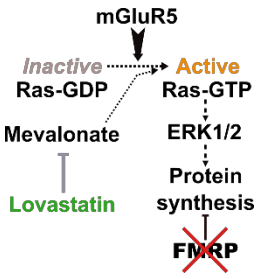


Figure S3. Rationale of lovastatin treatment in Fragile X syndrome. Lovastatin has been shown to indirectly downregulate the mGluR5 signalling pathway by limiting active Ras.

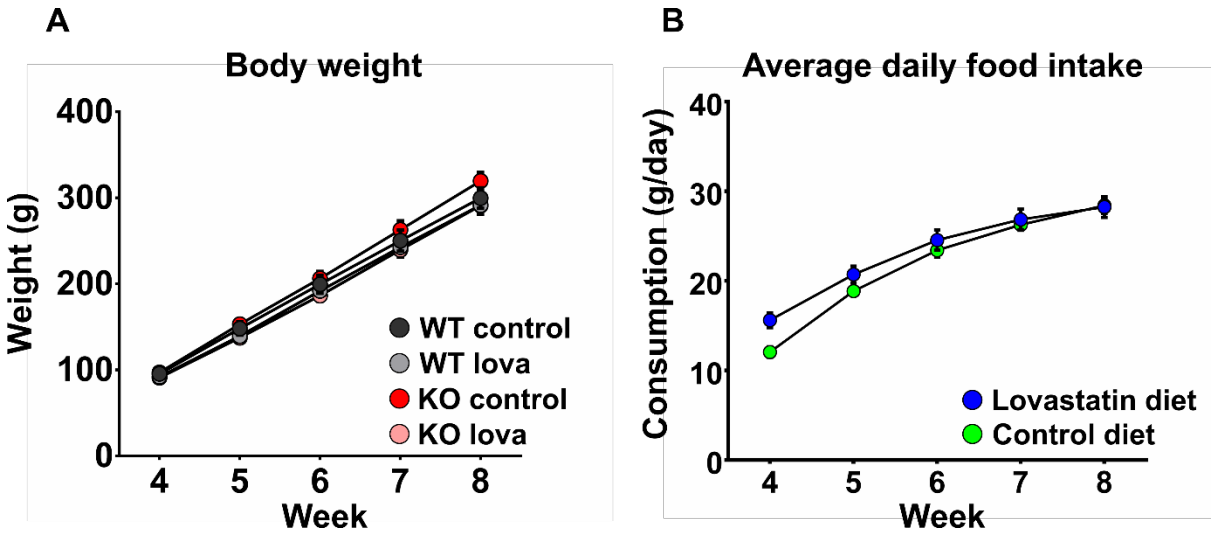


Figure S4. Lovastatin has negligible effect on the weight gain of rats. (**A**) Effect of lovastatin diet on WT and *Fmr1* KO rat body weight over time. (**B**) Effect of lovastatin diet on food consumption over time. Statistical analysis performed with three-way and two-way ANOVA, for details see table S6D and S6E.

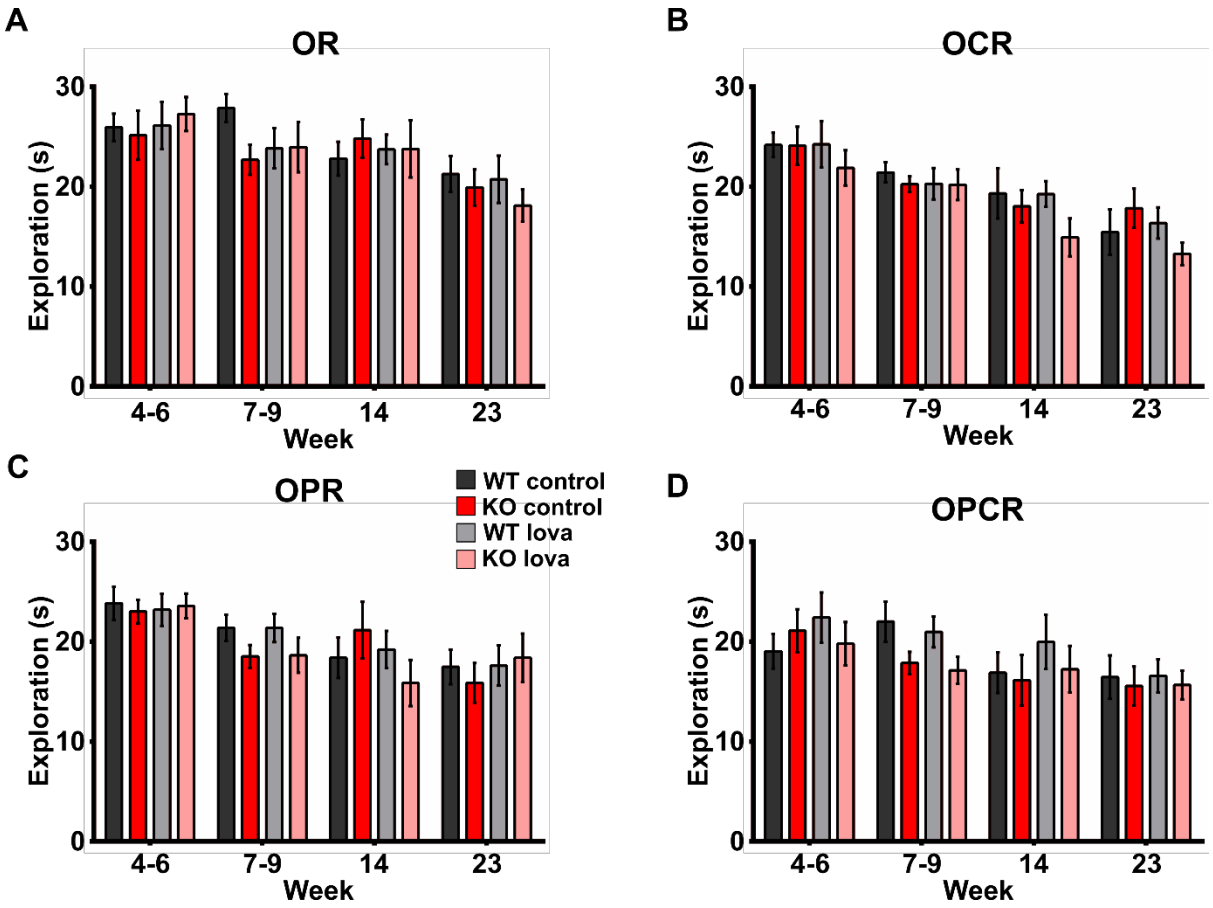


Figure S5. Time exploring objects during test phase of spontaneous recognition tasks does not differ between genotypes with or without lovastatin treatment. Total object exploration over time of WT and *Fmr1* KO rats on control and lovastatin diet in (**A**) OR, (**B**) OCR, (**C**) OPR and (**D**) OPCR tasks. Three-way ANOVA was used for statistical analysis, for details see table S5.

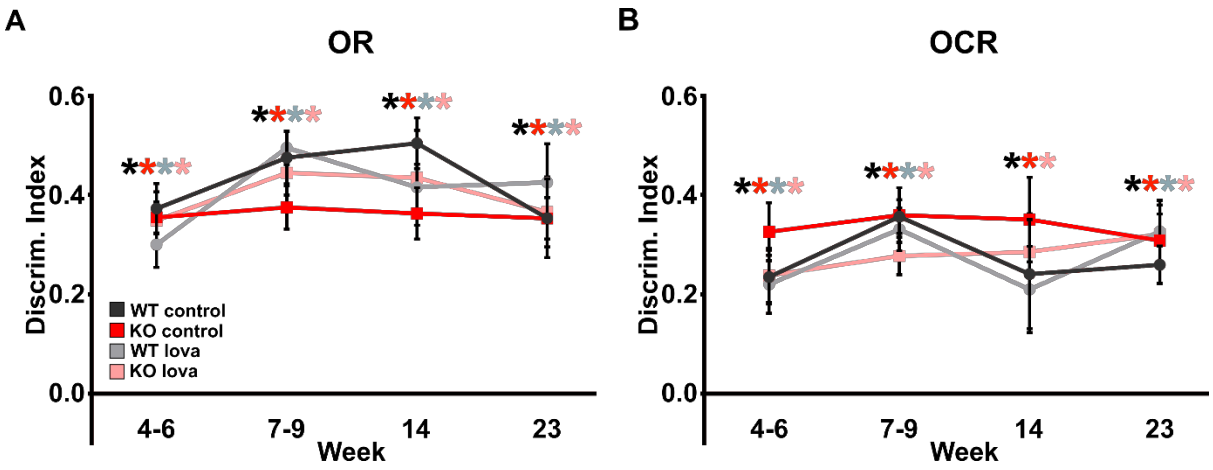


Figure S6. WT and *Fmr1* KO rats perform equally well in OR and OCR tasks throughout development with control and lovastatin diet. Discrimination index over time of WT and *Fmr1* KO rats on control and lovastatin diet in (**A**) OR and (**B**) OCR. * $p<0.05$ difference from chance (Discrimination Index =0) from a one sample t-test, for statistical details see table S3.

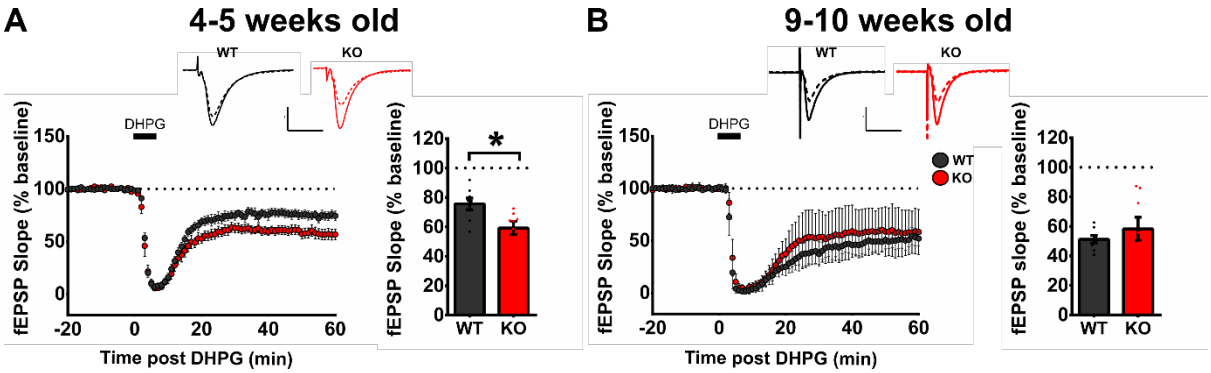


Figure S7. *Fmr1* KO LEH rats exhibit age specific increase of group I mGluR-LTD in CA1 of the hippocampus. Left panel: Time course plotting averages of fEPSP slopes normalised to baseline following LTD induction by 5min, 100µM DHPG wash-on in hippocampal slices taken from (**A**) 4-5 weeks and (**B**) 9-10 weeks old WT and *Fmr1* KO rats. Right panels: Averages of fEPSP slopes normalised to baseline at 40-60mins post DHPG wash-on. Insets: Example traces showing synaptic responses during baseline (solid trace) and 50-60 min (dotted trace), Scale bar 0.5mV, 25ms. * $p < 0.05$. Statistical analysis performed with two-way ANOVA and post-hoc two-sample t-test, for details see table S6C.

Table S1. Statistical results from one-sample t-tests and post-hoc two-sample t-test for object exploration tasks throughout development in WT and *Fmr1* KO rats

One-sample t-test against chance DI=0							
Genotype	Task	Week	n	t	df	p	B&H
WT	OR	4-6	13	7.386	12	<0.001	Significant
		7-9	13	8.970	12	<0.001	Significant
		>10	11	10.339	10	<0.001	Significant
	OCR	4-6	13	4.319	12	<0.001	Significant
		7-9	13	10.509	12	<0.001	Significant
		>10	11	3.837	10	<0.01	Significant
	OPR	4-6	13	1.007	12	0.330	
		7-9	13	10.459	12	<0.001	Significant
		>10	11	7.816	10	<0.001	Significant
	OPCR	4-6	13	0.937	12	0.367	
		7-9	13	4.416	12	<0.001	Significant
		>10	11	9.827	10	<0.001	Significant
KO	OR	4-6	12	11.348	11	<0.001	Significant
		7-9	12	8.543	11	<0.001	Significant
		>10	11	13.492	10	<0.001	Significant
	OCR	4-6	12	5.590	11	<0.001	Significant
		7-9	12	6.540	11	<0.001	Significant
		>10	11	7.254	10	<0.001	Significant
	OPR	4-6	12	-0.208	11	0.839	
		7-9	12	1.844	11	0.092	
		>10	11	9.368	10	<0.001	Significant
	OPCR	4-6	12	2.460	11	0.032	
		7-9	12	0.955	11	0.360	
		>10	11	1.091	10	0.301	
Post-hoc tests between groups							
	Task	Week		t	df	p	B&H
	OPR	4-6		0.940	21.342	0.358	
		7-9		4.920	21.109	<0.001	Significant
		>10		-1.512	19.907	0.146	
	OPCR	4-6		-0.556	20.329	0.584	
		7-9		2.722	22.752	0.012	Significant
		>10		3.895	16.839	<0.01	Significant

Table 2. Statistical results from two-way ANOVA of exploration times in object exploration tasks throughout development in WT and *Fmr1* KO rats

A	OR	
	Factor/Analysis	Two-way ANOVA
	Genotype	F(1,66) =1.05, p=0.31
	Age	F(2,66) = 2.79, p=0.07
	Genotype x Age	F(2,66) = 0.69, p=0.50
B	OCR	
	Factor/Analysis	Two-way ANOVA
	Genotype	F(1,66) =0.14, p=0.71
	Age	F(2,66) = 2.79, p<0.001
	Genotype x Age	F(2,66) = 0.28, p=0.76
C	OPR	
	Factor/Analysis	Two-way ANOVA
	Genotype	F(1,66) =0.11, p=0.74
	Age	F(2,66) = 7.29, p<0.01
	Genotype x Age	F(2,66) = 0.14, p=0.87
D	OPCR	
	Factor/Analysis	Two-way ANOVA
	Genotype	F(1,66) =1.41, p=0.25
	Age	F(2,66) = 2.38, p=0.10
	Genotype x Age	F(2,66) = 1.41, p=0.39

Table S3. Statistical results from one-sample t-tests for object exploration tasks throughout development in WT and *Fmr1* KO rats with or without lovastatin treatment

One-sample t-test against chance DI=0								
Diet	Genotype	Task	Week	n	t	df	p	B&H
Control	WT	OR	4-6	13	7.386	12	<0.001	Significant
			7-9	13	8.970	12	<0.001	Significant
			14	11	9.882	10	<0.001	Significant
			23	11	4.468	10	<0.01	Significant
		OCR	4-6	13	4.319	12	<0.01	Significant
			7-9	13	10.509	12	<0.001	Significant
			14	11	2.194	10	0.053	
			23	10	6.912	9	<0.001	Significant
		OPR	4-6	13	1.007	12	0.334	
			7-9	13	10.459	12	<0.001	Significant
			14	10	3.611	9	<0.01	Significant
			23	11	5.576	10	<0.001	Significant
		OPCR	4-6	13	0.936	12	0.367	
			7-9	13	4.416	12	<0.001	Significant
			14	11	7.713	10	<0.001	Significant
			23	10	5.266	9	<0.001	Significant
	KO	OR	4-6	12	11.350	11	<0.001	Significant
			7-9	12	8.543	11	<0.001	Significant
			14	11	7.009	10	<0.001	Significant
			23	11	8.474	10	<0.001	Significant
		OCR	4-6	12	5.590	11	<0.001	Significant
			7-9	12	6.540	11	<0.001	Significant
			14	11	4.120	10	<0.01	Significant
			23	11	5.766	10	<0.001	Significant
		OPR	4-6	12	0.208	11	0.839	
			7-9	12	1.844	11	0.092	
			14	11	5.871	10	<0.001	Significant
			23	11	9.137	10	<0.001	Significant
		OPCR	4-6	12	2.460	11	0.032	
			7-9	12	0.955	11	0.360	
			14	11	0.069	10	0.946	
			23	11	2.318	10	0.043	
Lovastatin	WT	OR	4-6	12	6.551	11	<0.001	Significant
			7-9	12	14.600	11	<0.001	Significant
			14	11	8.989	10	<0.001	Significant
			23	11	5.508	10	<0.001	Significant
		OCR	4-6	12	3.762	11	<0.01	Significant
			7-9	12	7.711	11	<0.001	Significant
			14	11	2.414	10	0.036	Significant
			23	10	5.216	9	<0.001	Significant
		OPR	4-6	12	2.372	11	0.047	
			7-9	12	8.050	11	<0.001	Significant
			14	11	3.272	10	<0.01	Significant
			23	10	2.765	9	0.022	Significant
		OPCR	4-6	12	2.729	11	0.037	
			7-9	12	5.414	11	<0.001	Significant
			14	11	7.537	10	<0.001	Significant
			23	10	2.866	9	0.019	Significant
	KO	OR	4-6	12	6.070	11	<0.001	Significant
			7-9	12	9.913	11	<0.001	Significant
			14	9	4.553	8	<0.01	Significant
			23	8	5.192	7	<0.01	Significant
		OCR	4-6	12	4.404	11	<0.01	Significant
			7-9	12	7.357	11	<0.001	Significant
			14	9	3.727	8	<0.01	Significant
			23	8	5.472	7	<0.001	Significant
		OPR	4-6	12	1.509	11	0.159	
			7-9	12	4.820	11	<0.001	Significant
			14	8	3.378	7	0.012	Significant
			23	7	10.647	6	<0.001	Significant
		OPCR	4-6	12	0.744	11	0.472	
			7-9	12	8.269	11	<0.001	Significant
			14	8	2.672	7	0.032	Significant
			23	8	6.853	7	<0.001	Significant

Table S4. Statistical results from post-hoc two-sample t-tests for object exploration tasks throughout development in WT and *Fmr1* KO rats with or without lovastatin treatment

Post-hoc tests between groups						
Task	Week	Comparison	t	df	p	B&H
OPR	7-9	WT control vs KO control	4.920	21.109	<0.001	Significant
		WT control vs WT lova	-0.570	20.068	0.575	
		WT control vs KO lova	0.348	16.219	0.732	
		KO control vs KO lova	-3.046	18.832	<0.01	Significant
OPCR	7-9	WT control vs KO control	2.722	22.752	0.012	Significant
		WT control vs WT lova	-0.206	22.775	0.838	
		WT control vs KO lova	-1.556	22.028	0.134	
		KO control vs KO lova	-4.806	21.739	<0.001	Significant
	14	WT control vs KO control	3.227	14.237	<0.01	Significant
		WT control vs WT lova	-0.513	19.726	0.614	
		WT control vs KO lova	-0.040	8.640	0.969	
		KO control vs KO lova	-2.125	13.625	0.043	
	23	WT control vs KO control	2.966	16.513	<0.01	Significant
		WT control vs WT lova	0.445	15.380	0.663	
		WT control vs KO lova	-0.338	15.949	0.739	
		KO control vs KO lova	-3.729	15.185	<0.01	Significant

Table S5. Statistical results from two-way ANOVA of exploration times in object exploration tasks throughout development in WT and *Fmr1* KO rats with or without lovastatin treatment

A	OR	
	Factor/Analysis	Three-way ANOVA
	Genotype	F(1,120) =0.56, p=0.46
	Age	F(3,120) = 6.19, p<0.01
	Diet	F(1,120) =1.27, p=0.26
	Genotype x Age x Diet	F(6,131) = 0.77, p=0.60
B	OCR	
	Factor/Analysis	Three-way ANOVA
	Genotype	F(1,118) =3.30, p=0.07
	Age	F(3,118) = 7.17, p<0.01
	Diet	F(1,118) =1.35, p=0.25
	Genotype x Age x Diet	F(6,129) = 1.57, p=0.21
C	OPR	
	Factor/Analysis	Three-way ANOVA
	Genotype	F(1,116) =0.05, p=0.83
	Age	F(3,116) = 2.03, p=0.14
	Diet	F(1,116) = 1.11, p=0.30
	Genotype x Age x Diet	F(6,127) = 0.85, p=0.53
D	OPCR	
	Factor/Analysis	Three-way ANOVA
	Genotype	F(1,118) =0.18, p=0.67
	Age	F(3,118) = 2.86, p=0.06
	Diet	F(1,118) = 2.15, p=0.15
	Genotype x Age x Diet	F(2,129) = 0.53, p=0.78

Table S6. Statistical results from adult object exploration tasks, effect of lovastatin on food intake/weight gain, hippocampal basal protein synthesis and synaptic plasticity data

A	Adult *Fmr1* KO rats show distinct deficit in OPCR	
	Factor/Analysis	Two-way ANOVA
Object Exploration Tasks	Genotype	F(1,30) =13.47, p<0.001
	Task	F(3,30) = 15.23, p<0.001
	Genotype x Task	F(3,30) = 4.28, p <0.01
	Sample Sizes	n(WT)= 16, n(KO)= 16
	One-sample t-tests (vs DI=0)	WT: OR t(15)=14.18, p<0.01; OCR t(15)=11.44, p<0.01; OPR t(15)=10.23, p<0.01; OPCR t(15)=7.6, p<0.01
		KO: OR t(15)=16.95, p<0.01; OCR t(15)=5.49, p<0.01; OPR t(15)=6.72, p<0.01; OPCR t(15)=2.79, p=0.055
	Two-sample t-tests WT vs KO	OR t(30)=0.13, p=0.99; OCR t(30)=2.23, p=0.13; OPR t(30)=1.91, p=0.26; OPCR t(30)=4.25, p<0.01
B	No difference in object exploration between adult WT and *Fmr1* KO rats	
	Factor/Analysis	Two-way ANOVA
Object Exploration Tasks	Genotype	F(1,30) =0.02, p=0.90
	Task	F(3,30) = 7.03, p<0.001
	Genotype x Task	F(3,30) = 0.93, p =0.43
C	Age dependent DHPG LTD in *Fmr1* KO rats	
	Factor/Analysis	Two-way ANOVA
LTD	Genotype	F(1,28) =0.83, p =0.37
	Age	F(1,28) =6.11, p <0.05
	Genotype x Age	F(1,28) =5.25, p <0.05
	Sample Sizes	Weeks 4-5: n(WT)= 8, n(KO)= 8; Weeks 9-10: n(WT)= 8, n(KO)= 8
	Two-sample t-tests WT vs KO	Weeks 4-5: t(14)=2.76, p<0.05; Weeks 9-10: t(14)=0.85, p=0.41
D	Average daily food intake	
	Factor/Analysis	Two-way ANOVA
Daily food intake	Diet	F(1,70) =6.14, p =0.02
	Time	F(4,70) =85.29, p <0.001
	Time x Diet	F(4,70) =1.26, p =0.30
E	No difference in body weight between WT and *Fmr1* KO developing rats	
	Factor/Analysis	Three-way ANOVA
Weight	Genotype	F(1,220) =0.93, p =0.34
	Time	F(4,220) =446.88, p <0.01
	Diet	F(1,220) =13.96, p <0.01
	Genotype x Diet	F(1,220) =2.85, p =0.93
	Genotype x Diet x Time	F(12,220) =0.30, p =0.99
F	Long lasting correction of excessive protein synthesis in *Fmr1* KO rats by lovastatin	
	Factor/Analysis	Two-way ANOVA
Protein synthesis	Genotype	F(1,20) =4.96, p <0.05
	Diet	F(1,20) = 3.46, p=0.08
	Genotype x Diet	F(1,20) = 5.68, p <0.05
	Sample Sizes	Control: n(WT)= 6, n(KO)= 6; Lovastatin: n(WT)= 6, n(KO)= 6
	Two-sample t-tests	WT control vs KO control: t(10)=3.63, p<0.01; KO control vs KO lova: t(10)=2.72, p<0.05;
G	Age dependent deficits in prefrontal LTP in *Fmr1* KO rats	
	Factor/Analysis	Two-way ANOVA
LTP	Genotype	F(1,24) =7.18, p <0.05
	Time	F(1,24) =5.67, p <0.05
	Genotype x Time	F(1,24) =4.52, p <0.05
	Sample Sizes	Weeks 4-6: n(WT)= 6, n(KO)= 6; Weeks 10-12: n(WT)= 7, n(KO)= 7
	Two-sample t-tests WT vs KO	Weeks 4-6: t(10)=0.22, p=0.83; Weeks 10-12: t(12)=3.29, p<0.01
H	Lovastatin prevents the emergence of plasticity deficits associated with the loss FMRP	
	Factor/Analysis	Two-way ANOVA
LTP	Genotype	F(1,29) =0.81, p =0.37
	Diet	F(1,29) =6.33, p <0.05
	Genotype x Diet	F(1,29) =5.41, p <0.05
	Sample Sizes	Control: n(WT)= 9, n(KO)= 9; Lovastatin: n(WT)= 7, n(KO)= 8
	Two-sample t-tests	WT control vs KO control: t(16)=2.94, p<0.01; KO control vs KO lova: t(15)=3.73, p<0.01;

Table S7. Linear mixed effects model distribution tests of behavioural data

			Distributions		
			Normal	Log-normal	Gamma
NOR	Development	Kolmogorov-Smirnov Test	0.0568	0.0638	0.0566
		Cramer-von Mises Test	0.0311	0.0525	0.0429
		Anderson-Darling Test	0.2472	0.3555	0.3029
		Akaike's Information Criterion	-64.4873	-64.4762	-64.8023
		Bayesian Information Criterion	-59.9340	-59.9229	-60.2490
	Lovastatin treatment	Kolmogorov-Smirnov Test	0.0419	0.0626	0.0550
		Cramer-von Mises Test	0.0471	0.1586	0.1119
		Anderson-Darling Test	0.3907	1.0735	0.7851
		Akaike's Information Criterion	-59.5055	-52.8802	-56.0099
		Bayesian Information Criterion	-53.7399	-47.1146	-50.2443
OCR	Development	Kolmogorov-Smirnov Test	0.0689	0.0915	0.0839
		Cramer-von Mises Test	0.0635	0.1136	0.0929
		Anderson-Darling Test	0.4474	0.7850	0.6471
		Akaike's Information Criterion	-38.7324	-34.3233	-36.1628
		Bayesian Information Criterion	-34.1791	-29.7700	-31.6095
	Lovastatin treatment	Kolmogorov-Smirnov Test	0.0862	0.1318	0.1163
		Cramer-von Mises Test	0.1848	0.5486	0.3970
		Anderson-Darling Test	1.1048	3.1253	2.2802
		Akaike's Information Criterion	-34.4437	-3.3459	-16.3839
		Bayesian Information Criterion	-28.7086	2.3892	-10.6488
OPR	Development	Kolmogorov-Smirnov Test	0.0735	0.0864	0.0827
		Cramer-von Mises Test	0.0748	0.0892	0.0791
		Anderson-Darling Test	0.4697	0.6073	0.5291
		Akaike's Information Criterion	-24.9644	-22.8549	-24.0745
		Bayesian Information Criterion	-20.4111	-18.3016	-19.5212
	Lovastatin treatment	Kolmogorov-Smirnov Test	0.0690	0.1005	0.0906
		Cramer-von Mises Test	0.0890	0.2892	0.2084
		Anderson-Darling Test	0.5322	1.7488	1.2566
		Akaike's Information Criterion	-39.9249	-23.9173	-30.5527
		Bayesian Information Criterion	-34.2208	-18.2132	-24.8487
OPCR	Development	Kolmogorov-Smirnov Test	0.0710	0.1116	0.0979
		Cramer-von Mises Test	0.0380	0.1524	0.1034
		Anderson-Darling Test	0.3129	1.0685	0.7513
		Akaike's Information Criterion	-28.3373	-17.9092	-22.3206
		Bayesian Information Criterion	-23.7840	-13.3558	-17.7673
	Lovastatin treatment	Kolmogorov-Smirnov Test	0.0640	0.1269	0.1046
		Cramer-von Mises Test	0.1186	0.5863	0.3700
		Anderson-Darling Test	0.7332	3.5438	2.2281
		Akaike's Information Criterion	4.3219	52.0366	30.4778
		Bayesian Information Criterion	10.0415	57.7562	36.1974

Table S8. Linear mixed effects modelling results of WT and *Fmr1* KO object exploration tasks throughout development

		Interaction	Terms	
		Age x Genotype	Age	Genotype
NOR	Df	2	2	1
	AIC	-62.004	-63.673	-60.718
	LRT	1.042	2.330	3.285
	Pr(Chi)	0.594	0.312	0.070
OCR	Df	2	2	1
	AIC	-38.104	-38.392	-38.541
	LRT	1.299	2 3.7118	1.563
	Pr(Chi)	0.522	0.156	0.211
OPR	Df	2		
	AIC	-62.240		
	LRT	22.139		
	Pr(Chi)	<0.001		
OPCR	Df	2		
	AIC	-38.316		
	LRT	12.049		
	Pr(Chi)	0.002		

OPR			
Fixed effects for best fitting model			
	Estimate	Std. Error	t value
(Intercept)	0.0393	0.0344	1.1430
Week 7-9	0.3224	0.0475	6.7950
Week >10	0.2450	0.0497	4.9340
genotype(KO)	-0.0452	0.0497	-0.9110
Week 7-9 x genotype(KO)	-0.2337	0.0685	-3.4120
Week >10 x genotype(KO)	0.1261	0.0709	1.7790
OPCR			
Fixed effects for best fitting model			
	Estimate	Std. Error	t value
(Intercept)	0.0474	0.0435	1.0880
Week 7-9	0.2070	0.0616	3.3630
Week >10	0.2859	0.0643	4.4470
genotype(KO)	0.0336	0.0628	0.5350
Week 7-9 x genotype(KO)	-0.2405	0.0888	-2.7070
Week >10 x genotype(KO)	-0.3134	0.0918	-3.4150

Table S9. Linear mixed effects modelling results of WT and *Fmr1* KO object exploration tasks throughout development with or without lovastatin treatment

		Interactions				Terms		
		Age x Diet x Genotype	Diet x Genotype	Age x Genotype	Age x Diet	Age	Genotype	Diet
NOR	Df	2	1	2	2	2	1	1
	AIC	-48.519	-49.886	-52.083	-51.834	-57.158	-55.721	-58.199
	LRT	1.845	0.634	0.437	0.685	3.676	3.113	0.635
	Pr(Chi)	0.398	0.426	0.804	0.710	0.159	0.078	0.426
OCR	Df	2	1	2	2	2	1	1
	AIC	-21.200	-22.750	-23.297	-23.811	-29.456	-28.862	-28.943
	LRT	0.022	0.450	1.903	1.389	1.938	0.532	0.451
	Pr(Chi)	0.989	0.502	0.386	0.499	0.380	0.466	0.502
OPR	Df	2						
	AIC	-46.367						
	LRT	6.164						
	Pr(Chi)	0.046						
OPCR	Df	2	1	2	2			
	AIC	-17.652	-4.877	-19.246	-20.541			
	LRT	0.014	14.774	2.406	1.111			
	Pr(Chi)	0.993	<0.001	0.300	0.574			

OPR			
Fixed effects for best fitting model			
	Estimate	Std. Error	t value
(Intercept)	0.3617	0.0498	7.2690
Week 14	-0.1578	0.0755	-2.0910
Week 23	-0.0174	0.0735	-0.2370
Diet(lova)	0.0343	0.0718	0.4780
Genotype(KO)	-0.2789	0.0718	-3.8830
Week 14 x Genotype(KO)	0.4361	0.1063	4.1010
Week 23 x Genotype(KO)	0.304134	0.104952	2.898
Week 14 x Diet(lova)	-0.000329	0.106338	-0.003
Week 23 x Diet(lova)	-0.18283	0.106338	-1.719
Diet(lova) x Genotype(KO)	0.217637	0.102602	2.121
Week 14 x Diet(lova) x Genotype(KO)	-0.359095	0.15371	-2.336
Week 23 x Diet(lova) x Genotype(KO)	-0.030717	0.15557	-0.197

OPCR			
Fixed effects for best fitting model			
	Estimate	Std. Error	t value
(Intercept)	0.2850	0.0428	6.6570
Week 14	0.0391	0.0443	0.8820
Week 23	0.0258	0.0449	0.5740
Diet(lova)	0.0071	0.0510	0.1390
Genotype(KO)	-0.2547	0.0507	-5.0260
Diet(lova) x Genotype(KO)	0.30296	0.07388	4.101