HHS Public Access

Author manuscript

Behav Brain Res. Author manuscript; available in PMC 2016 September 15.

Published in final edited form as:

Behav Brain Res. 2015 September 15; 291: 164–171. doi:10.1016/j.bbr.2015.05.003.

Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome

Mei Qin, **Zachary Zeidler**, **Kristen Moulton**, **Leland Krych**, **Zengyan Xia**, and **Carolyn B. Smith***

Section on Neuroadaptation and Protein Metabolism, National Institute of Mental Health, National Institutes of Health, 10 Center Drive, Bldg. 10, Rm. 2D54, Bethesda, MD 20892, USA

Abstract

Silencing the gene *FMR1* in fragile X syndrome (FXS) with consequent loss of its protein product, FMRP, results in intellectual disability, hyperactivity, anxiety, seizure disorders, and autism-like behavior. In a mouse model (*Fmr1* knockout (KO)) of FXS, a deficit in performance on the passive avoidance test of learning and memory is a robust phenotype. We report that drugs acting on the endocannabinoid (eCB) system can improve performance on this test. We present three lines of evidence: (1) Propofol (reported to inhibit fatty acid amide hydrolase (FAAH) activity) administered 30 min after training on the passive avoidance test improved performance in *Fmr1* KO mice but had no effect on wild type (WT). FAAH catalyzes the metabolism of the eCB, anandamide, so its inhibition should result in increased anandamide levels. (2) The effect of propofol was blocked by prior administration of the cannabinoid receptor 1 antagonist AM-251. (3) Treatment with the FAAH inhibitor, URB-597, administered 30 min after training on the passive avoidance test also improved performance in *Fmr1* KO mice but had no effect on WT. Our results indicate that the eCB system is involved in FXS and suggest that the eCB system is a promising target for treatment of FXS.

Keywords

Fragile X syndrome; FAAH; Passive avoidance; Propofol; *Fmr1* KO mice; Memory

1. Introduction

Fragile X syndrome (FXS) is the most common inherited intellectual disability and a recognized monogenic cause of autism. FXS is caused by the absence of fragile X mental retardation protein (FMRP) due to silencing of the *FMR1* gene. FMRP is an RNA-binding protein that associates with mRNA and stalls ribosome translocation along targeted mRNAs [1]. The absence of FMRP results in a loss of translational control that is thought to be at the core of the disease.

Consistent with this loss of translational control, rates of cerebral protein synthesis (rCPS) measured *in vivo* are increased in some regions of the brain in an *Fmr1* knockout (KO)

*Corresponding author. Tel.: +1 3014023120; fax: +1 3014801668. beebe@mail.nih.gov (C.B. Smith).

mouse model of FXS [2]. We attempted to confirm this change in rCPS in the human disease. Surprisingly, our findings indicated that subjects with FXS under propofol sedation had lower rCPS than age-matched, propofol-sedated healthy volunteers [3], despite a lack of an effect of propofol on rCPS in controls [4]. We hypothesized that the decreased rCPS could be due to a selective effect of propofol in subjects with FXS. Further studies in mice showed that propofol had no effect in wild type (WT) mice, but decreased rCPS in *Fmr1* KO mice.

In this study, we further investigated this genotype-selective effect of propofol on a test of aversive memory. We studied the effects of propofol on a robust behavioral phenotype in *Fmr1* KO mice, deficient performance on the passive avoidance test [5–7]. It had been reported that in adult, male Sprague-Dawley rats, propofol treatment after training improved performance on this memory test [8]. We assessed the effects of propofol on the passive avoidance test in WT and *Fmr1* KO mice, and we report that propofol treatment improved performance in *Fmr1* KO, but not in WT mice. Additionally we explored the mechanism by which propofol effects this genotype-specific change in the hope that it might aid in the discovery of new therapeutics. Propofol acts via a wide range of sites, including positive modulation of $GABA_A$ receptors [9] and inhibition of fatty acid amide hydrolase (FAAH) [10], an enzyme that catalyzes the metabolism of the endogenous cannabinoid (eCB), anandamide. We focused our study on the latter site of action because it has been implicated in an effect of propofol on the enhancement of memory consolidation in rats [8]. Moreover, eCB systems have been reported to be altered in *Fmr1* KO mice [11–15]. To see if inhibition of FAAH could have therapeutic relevance in FXS, we tested the effects of a specific inhibitor of FAAH on passive avoidance performance and on tests of other behavioral phenotypes in *Fmr1* KO mice. Results of our studies point to the eCB system as a promising therapeutic target in FXS.

2. Materials and methods

2.1. Animal subjects

Male WT and *Fmr1* KO mice on a C57Bl/6J background were bred and genotyped as previously described [2]. Mice were group-housed in a central facility, and naïve mice (90–120 days old) were used for each test. All procedures were performed between 9 am and 11 am and carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the National Institute of Mental Health Animal Care and Use Committee.

2.2. Drugs

Propofol (2,6-diisopropylphenol) (Diprovan®) was purchased from Astra-Zeneca Pharmaceuticals LP (Wilmington, DE). Intralipid®, a 20% fat emulsion, was obtained from Fresenius Kabi (Uppsala, Sweden) for use as the propofol vehicle (1:1 dilution with normal saline). URB-597 (cyclohexylcarbamic acid 3′-(aminocarbonyl)-[1,1′-biphenyl]-3-yl ester) was purchased from Tocris Bioscience (Ellisville, MO, USA) and JZL-184 (4-nitrophenyl-4-(dibenzo[d][1,3]dioxol-5-yl(hydroxy)methyl)piperidine-1-carboxylate) was purchased from Cayman Chemical (Ann Arbor, MI, USA). Captisol® (sulfobutyl ether β-cyclodextrin) was

purchased from CyDex Pharmaceuticals, Inc. (Lawrence, KS, USA) dissolved in water (400 mg/mL) for use as a vehicle for ganaxolone, URB-597 and JZL-184. Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-a][1,4]benzodiazepine) was purchased from Hospira, Inc. (Lake Forest, IL, USA) and dissolved in normal saline. Ganaxolone (3α, 5α)-3-hydroxy-3-methylpregnan-20-one) was a gift from Marinus Pharmaceuticals, Inc. (Franklin Lakes, NJ, USA). AM 251 (N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide) was purchased from Tocris Bioscience and dissolved in DMSO:Tween80:NS (1:1:8, v:v:v).

2.3. Behavioral tests

2.3.1. Passive avoidance—We used the two-chambered apparatus (14″ × 7″ × 12″), one lighted chamber and one dark chamber separated by a guillotine door (Passive Avoidance Cage, Coulbourn Instruments, USA). The floor of the dark chamber is a grid for delivery of a foot shock. We used two different protocols for this test. In initial studies to test the effects of dose of propofol and the involvement of $GABA_A$ and eCB systems, we used our previously described protocol [5]. Briefly, on Day 1, each mouse underwent habituation to ensure a natural preference for the dark chamber. Mice were placed in the lighted chamber and the guillotine door was opened after 30 s. Mice that failed to enter the dark chamber after 90 s were excluded from the study. On Day 2 the mouse was placed in the lighted chamber with the guillotine door closed. After 30 s the door was raised. Once the mouse entered the dark chamber, the door automatically closed and an electric foot shock (0.3 mA for 1 s) was administered. The mouse was removed from the apparatus after 15 s, returned to its home cage, and 30 min later drugs were administered i.p. On Day 3, 24 h after training, the mouse was again placed in the lighted chamber with the guillotine door closed. After 30 s the door was opened and the time for the mouse to enter the dark chamber (latency) was measured. Mice did not receive a shock during the testing. The maximum time allowed was 300 s. A second protocol designed to enhance learning was instituted for all other studies [16]. This protocol was the same as the first with the introduction of a second training trial on Day 2, 120 s after the first trial. In this second protocol, the mouse was moved to its home cage 15 s after the first shock. After 120 s. the mouse was returned to the lighted compartment of the passive avoidance apparatus and the training with foot shock was repeated. After 15 s the mouse was returned to its home cage and 30 min later drugs were administered i.p. On Day 3, 24 h after training, latency to enter the dark compartment of the apparatus without shock was recorded up to 300 s.

2.3.2. Elevated plus maze—Thirty min after i.p. injection of Captisol vehicle or URB-597 (0.3 mg/kg) mice were tested for general anxiety in an elevated plus maze (EPM) as described previously [17]. Briefly, the mouse was placed in the center of the apparatus facing an open arm. The time spent and the time grooming in the closed and open arms were recorded for 5 min. We defined an arm entry as the mouse having his head and forepaws in the arm.

2.3.3. Test of social behavior—Thirty min after i.p. injection of URB-597 (0.3 mg/kg) or Captisol vehicle, mice were tested in an automated three-chambered social approach apparatus as previously described [6]. The test had three consecutive phases: (A)

Habituation, in which the test mouse was allowed to freely explore the apparatus for 5 min, (B) Sociability, in which an unfamiliar mouse (Stranger-1) was placed inside a wire cup in one of the side chambers and an empty cup was placed in the other chamber, the test mouse freely explored the apparatus for 5 min, and (C) Preference for Social Novelty, in which a second unfamiliar mouse (Stranger-2) was placed inside the previously empty cup in the opposite chamber, and the test mouse was allowed to freely explore the apparatus for 5 min. Each test was video recorded and sniffing behavior was assessed by a rater blinded to the genotype. Total time spent in each chamber and time spent sniffing a stranger mouse or an empty cup were recorded. During habituation, mice that showed a preference for a chamber, i.e., spent three or more min in any one chamber, were eliminated from the study.

2.4. Statistical analysis

Data are expressed as means ± SEM. Data were analyzed by means of repeated measures analysis of variance (RM ANOVA) and, when appropriate, further probed by means of *post-hoc* Bonferroni *t*-tests. The significance level was set at $P \leq 0.05$. We used the SPSS program (IBM, Armonk, NY) and GraphPad (GraphPad Software Inc, La Jolla, CA) for statistical computations.

3. Results

3.1. Optimal dose of propofol

We administered propofol (i.p.) 30 min after training on the passive avoidance test at doses of 0, 100, 150, 200, and 300 mg/kg to WT and *Fmr1* KO mice (Fig. 1). Following doses of either 100 or 150 mg/kg, mice were lightly sedated. At these doses, mice placed in a supine position could return themselves to a prone position and were fully awake 2.5–3 h after injection. Following a dose of 200 mg/kg, mice were deeply sedated and could not return to a prone position when placed in a supine position. At the 200 mg/kg dose mice remained deeply sedated for 3.5–4 h after injection. Following a dose of 300 mg/kg, mice were deeply sedated, hypothermic, and had labored respiration. We discontinued studies at this high dose. Twenty four hours after training we tested mice for latency to enter the dark chamber. Latencies were much shorter in vehicle-injected *Fmr1* KO mice compared to WT as we have shown previously [5,6]. At the lowest dose of propofol tested mean latency of WT mice was considerably reduced. At the 200 mg/kg dose, WT mice behaved similarly to vehicle-injected mice. Performance of *Fmr1* KO mice at this dose of propofol was similar to that of WT, i.e., latency to enter the dark compartment was considerably increased. Based on these results, we chose a dose of 200 mg/kg propofol for further study.

3.2. Effects of $GABA_A$ activation and CB1 receptor blockade

Next we tested whether the propofol-mediated behavioral improvement of *Fmr1* KO mice on the passive avoidance test involves a$GABA_A$-dependent or an eCB-dependent mechanism. We examined whether the behavioral improvement of *Fmr1* KO mice on the passive avoidance test could be mimicked by treatment with drugs activating $GABA_A$ receptors. We tested midazolam, a benzodiazepine, at two doses (Fig. 2A) and ganaxolone, a positive allosteric modulator of $GABA_A$ receptors (Fig. 2B). As in the propofol studies, drugs were administered 30 min after training. Midazolam at both doses tested resulted in sedation and a

loss of the righting reflex. At doses of 5 and 10 mg/kg, midazolam sedation lasted for 1 and 2 h, respectively. Ganaxolone did not produce sedation in our animals. We analyzed these data by means of two-way ANOVA with genotype and drug treatment as between subjects factors. Interactions between genotype and treatment were not statistically significant for either drug. For both drugs the main effect of genotype was statistically significant, indicating that regardless of treatment, latencies were lower in *Fmr1* KO mice. In the case of ganaxolone, we found a statistically significant main effect of treatment, indicating that regardless of genotype, ganaxolone decreased latencies. These data suggest that treatment via activation of the $GABA_A$ system does not improve performance of *Fmr1* KO mice on this memory test and it may even diminish performance in both WT and *Fmr1* KO mice.

To test the involvement of the eCB system we administered the cannabinoid 1 (CB1) receptor antagonist, AM251 (2.5 mg/kg, i.p.), immediately after training and 30 min before propofol administration (Fig. 3). Pre-treatment with a CB1 receptor antagonist had no effect itself on the behavior of WT mice but completely blocked the restorative effect of propofol in *Fmr1* KO mice. These results indicate that the positive effect of propofol on this memory task in *Fmr1* KO mice could occur via eCB receptors.

3.3. Effects of FAAH or MAGL inhibition

In addition to its effects on $GABA_A$ receptor activation, it is reported that propofol inhibits FAAH, the enzyme that metabolizes the eCB, anandamide [10]. Next we tested the effects of the FAAH inhibitor, URB-597, to see if it would mimic the effects of propofol on performance on the passive avoidance task (Fig. 4). We also altered the protocol for the passive avoidance test by adding a second training trial 2 min after the first one. We made this change to ensure sufficient training for *Fmr1* KO mice [16]. This change in protocol also gave us an indication of short-term memory function in *Fmr1* KO mice. In mice trained with the two shock protocol mean latencies to enter the dark compartment were similar on the initial training trial as expected (Fig. 4A). On the second training trial, mean latency in *Fmr1* KO mice was 40% shorter than that of WT mice, but the interaction between training trial and genotype only approached statistical significance (RM ANOVA). With the two shock protocol we replicated our previous finding that propofol treatment 30 min after the final training shock improved performance in the *Fmr1* KO mice (Fig. 4B) indicating a reversal of the long-term memory deficit. Treatment with URB-597 administered 30 min after the second training shock also reversed the long-term memory deficit (Fig. 4C), but treatment with an inhibitor (JZL-184) of monoacylglycerol lipase (MAGL), the enzyme catalyzing the breakdown of the endocannabinoid 2-arachidonoylglycerol (2-AG), did not affect performance (Fig. 4C). Neither treatment with URB-597 nor JZL-184 had a sedating effect on the animals. These results suggest that increasing the concentration of anandamide, but not 2-AG, can reverse the long-term memory deficit in *Fmr1* KO mice.

3.4. Effects of FAAH inhibition on general anxiety and social behavior

Performance on the passive avoidance test is a robust phenotype in *Fmr1* KO mice. We asked if treatment with URB-597 would also reverse other behavioral phenotypes. *Fmr1* KO mice show behavior consistent with reduced general anxiety [6,17]. In the open field, *Fmr1* KO mice move in the center of the field more than WT, and in the EPM, they spend more

time in the open arms than WT. We tested the effects of inhibition of FAAH on behavior in the EPM including % time in the open arms (Fig. 5A) and % time spent grooming (an index of repetitive behavior) (Fig. 5B). Vehicle-injected *Fmr1* KO mice spent greater % time in open arms and % time grooming compared to WT. For the % time in open arms, two way ANOVA results indicate that the interaction between genotype and drug treatment approaches statistical significance. Individual comparisons of WT vs. *Fmr1* KO results under each condition confirm what we have reported previously that, after vehicle injection, *Fmr1* KO mice spend more time in the open arms, but, after treatment with URB-597, both genotypes behaved similarly. For % time spent grooming, the interaction between genotype and drug treatment is not statistically significant but the main effects of both treatment and genotype are statistically significant indicating that *Fmr1* KO mice regardless of treatment spent more time grooming in the EPM and that URB-597 increases % time spent grooming in both genotypes.

We used the three-chambered test to assess the effects of inhibition of FAAH on social behavior in *Fmr1* KO mice (Fig. 6). Mice were injected with either vehicle or URB-597 (0.3 mg/kg, i.p.) 30 min prior to initiation of the test. During the habituation phase of the test, mice in all four groups showed no preference for Chamber-1 or Chamber-2 (Fig. 6A). During the sociability phase of the test, a novel mouse was introduced into the cup in Chamber-1; Chamber-2 contained an empty cup. Mice in all four groups spent more time in Chamber-1 than in Chamber-2 (Fig. 6B). Results of the RM ANOVA (exact results given in the legend to Fig. 6) indicate that the differences between times spent in Chambers 1 and 2 were lower in *Fmr1* KO mice than in WT regardless of treatment. Mice in all four groups spent more time sniffing the novel mouse than the empty cup (Fig. 6D), and differences in time spent sniffing the novel mouse vs. the empty cup were similar for all four groups. During the social novelty phase of the test, a novel mouse was placed in the previously empty cup in Chamber-2. Vehicle-treated WT mice spent more time in Chamber-2 than in Chamber-1, whereas vehicle-treated KO mice spent equal time in the two chambers. After treatment with URB-597, WT mice showed a reversed preference, spending more time in Chamber-1 compared to Chamber-2. The chamber preference of URB-597 treated KO mice was not different from vehicle treated KO; times were similar in both chambers. These differences in genotype-specific response to the URB-597 treatment are reflected in the statistically significant chamber × genotype × treatment interaction (Fig. 6, legend). Times spent sniffing the two stranger mice during the preference for social novelty test (Fig. 6E) also showed a different response to the treatment between the genotypes as reflected in the close to statistically significant chamber × genotype × treatment interaction ($P = .066$). Both vehicle treated WT and KO mice demonstrated a preference for sniffing the novel mouse (Stranger-2), but after URB-597 treatment the preference switched to Stranger-1 for WT mice whereas KO mice showed no preference after URB-597 treatment. Overall our results on social behavior indicate that URB-597 treatment does not reverse the phenotype in *Fmr1* KO mice, but it does have a deleterious effect on social behavior in WT mice.

4. Discussion

ECBs act presynaptically to inhibit further release of glutamate or GABA and consequently to modulate activity at mGluR and GABA synapses. Our findings demonstrate that drugs

that modulate the eCB system can remedy the deficit in *Fmr1* KO mice on a test of long-term memory. We present three lines of evidence: (1) Propofol (reported to inhibit FAAH activity) administered 30 min after training on the passive avoidance test improved performance in *Fmr1* KO mice but had no effect on WT. (2) The effect of propofol was blocked by prior administration of the CB1 receptor antagonist AM-251. (3) Treatment with the FAAH inhibitor, URB-597, administered 30 min after training on the passive avoidance test also improved performance in *Fmr1* KO mice but had no effect on WT. Our results on the EPM test further indicate that treatment with URB-597 may normalize anxiety-like behavior in *Fmr1* KO mice, but URB-597 may also increase repetitive behavior as shown by the increased grooming time in both genotypes. With regard to social behavior, pretreatment with URB-597 did not enhance social interactions in either genotype.

The effectiveness of propofol to reverse the deficit on long-term memory in the *Fmr1* KO mouse was dose-dependent and required a sedating dose of propofol. Other sedatives, i.e., dexmedetomidine (data not shown) and midazolam, however, did not improve performance on this test suggesting that it is not due to sedation *perse*. The propofol dose-dependency and the lack of effectiveness of other sedatives (pentobarbital and midazolam) were also seen in a study of long-term memory in Sprague-Dawley rats [8]. Our design in which we administered propofol 30 min after training is based on a previous study in which later times of administration were examined (90 and 180 min after training) and were shown to be ineffective in improving performance [8]. These results suggest that it is memory consolidation that was positively affected by propofol treatment. In contrast to the previous study in rats [8], we did not find any effect on performance in WT mice. This could be due to a species difference. Another possibility is that in our study we tested animals 24 h after training, whereas in the rat study, animals were tested 48 h after training. It is possible, albeit unlikely, that an effect in WT mice is only seen at later times. Mean latency in our study was increased by 2.3 fold after propofol treatment of *Fmr1* KO mice; a similar effect size was seen in Sprague-Dawley rats.

Inhibition of FAAH by URB-597 also increased latency on the passive avoidance test in Sprague-Dawley rats [18], but only if administered prior to training. If administered immediately after training, increased latencies were seen in both vehicle- and URB-597-injected animals. The vehicle used in the rat study was 20% DMSO. In our preliminary studies, we saw an effect of DMSO vehicle in *Fmr1* KO mice when it was administered 30 min after training (data not shown). We found no such effect in WT mice. For this reason we switched the vehicle to Captisol, a cyclodextrin, which did not affect latency. The reason for the effect of DMSO is not known. DMSO has an anti-inflammatory effect that may itself enhance the training in *Fmr1* KO mice.

In our study, FAAH inhibition with URB-597 reversed the increased time in the open arms of the EPM in *Fmr1* KO mice, i.e., acute URB-597 treatment appeared to increase anxiety in *Fmr1* KO mice. This finding appears to be at variance with the anxiolytic effects of FAAH inhibition in rats subjected to chronic restraint stress [19]. Further studies indicated that stress, through corticotropin-releasing hormone (CRH) receptor 1 activation, increased FAAH activity which in turn reduced the concentration of the eCB, anandamide, in the amygdala [20]. It is possible that the anxiogenic effect of FAAH inhibition in *Fmr1* KO mice

in our present study is a reflection of a defect in coupling between CRH and FAAH activation.

Behavior in vehicle-treated *Fmr1* KO mice in the present study is similar to what has been reported previously for passive avoidance behavior [5–7] and general anxiety-like behavior in the EPM [6,17]. In the test of social behavior we replicate our previous findings in which *Fmr1* KO mice behave like WT during the sociability phase of the test, but in the preference for social novelty phase our results diverge from our previous reports. Along with others we previously found that during the preference for social novelty phase *Fmr1* KO mice do not show the switch in preference for the chamber with the novel mouse nor do they spend more time sniffing the novel mouse that is characteristic of WT mice [6,17,21,22]. In the present study *Fmr1* KO mice, like WT, do spend more time sniffing the novel mouse, but unlike WT mice they spend equal times in the two chambers. We made a change in our procedure in the present study that may have affected the results. In our previous studies, an experimenter remained in the testing room and manually recorded sniffing times. In the present study the experimenter left the room and the sessions were videotaped and analyzed later. We think that analysis of videotapes is likely a more accurate method of recording sniffing times. It is also possible that the presence of the experimenter influenced the sniffing behavior of the *Fmr1* KO mice.

Our study is not the first to implicate the eCB system in the pathophysiology of FXS. eCBs mobilized in response to group 1 mGluR activation act on presynaptic CB1 receptors to depress GABA or glutamate release, and most CB1 receptors are located on inhibitory rather than excitatory cells [23]. In hippocampal slices from *Fmr1* KO mice, application of a group 1 mGluR agonist resulted in larger eCB-mediated responses at GABA synapses suggesting that coupling between mGluR activation and eCB mobilization is enhanced [12]. Behavioral effects of treatment with drugs acting on the eCB system were also assessed in a previous study of *Fmr1* KO mice [14]. Results indicate that either acute or chronic treatment with the CB1 inverse agonist, rimonabant, improved performance on the object recognition test of learning and memory. Additionally, treatment with the CB2 antagonist, AM630, may have mitigated the decreased anxiety phenotype seen in the EPM. Our study does not address the involvement of CB2 receptors, but our passive avoidance test results suggest that enhanced, not diminished, eCB signaling through CB1 receptors improves performance of *Fmr1* KO mice on this memory task. The apparent difference could be due to the different memory tests used. In another study of *Fmr1* KO mice, treatment with JZL-184 also normalized behavior on the EPM and normalized activity in the open field [13]. We do not report effects of JZL-184 on behavior in the EPM, but we did find that it was not effective on the passive avoidance test. In our study, performance on passive avoidance was apparently sensitive to anandamide and not 2-AG levels.

Results of our behavioral study taken together with results of other behavioral and neurophysiological studies [11–15,24] indicate that the eCB system is affected in FXS. Phenotypes may be due to disruptions in Homer–mGluR interactions [24,25] and perhaps also increased expression of diacylglycerol lipase (DAGL), the enzyme that catalyzes the synthesis of 2-AG. DAGL mRNA is one of the 842 targets of FMRP [1]. The endocannabinoid system is thought to be a key modulator of synaptic plasticity [26].

Prevailing evidence suggests that eCBs are involved in cognitive performance, anxiety, nociception, and seizure susceptibility [23]. All of these behaviors are affected in FXS. The role of eCBs as neuromodulators makes the eCB system an attractive pharmacological target. Targeting the synthetic and degradative enzymes of the pathways promises fewer side effects and more subtle nervous system responses than treatment with receptor agonists or antagonists.

Acknowledgments

This work was supported by the Intramural Research Program of the National Institute of Mental Health, National Institutes of Health.

Abbreviations

2-AG	2-arachidonoylglycerol
CB1	cannabinoid receptor-1
eCB	endocannabinoid
FAAH	fatty acid amide hydrolase
FMRP	fragile X mental retardation protein
Fmr1	fragile X mental retardation gene
FXS	fragile X syndrome
MAGL	monoacylglycerol lipase

References

1. Darnell JC, Van Driesche SJ, Zhang C, Hung KY, Mele A, Fraser CE, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell. 2011; 146:247–261. [PubMed: 21784246]
2. Qin M, Kang J, Burlin TV, Jiang C, Smith CB. Post-adolescent changes in regional cerebral protein synthesis: an in vivo study in the Fmr1 null mouse. J Neurosci. 2005; 25:5087–5095. [PubMed: 15901791]
3. Qin M, Schmidt KC, Zametkin AJ, Bishu S, Horowitz LM, Burlin TV, et al. Altered cerebral protein synthesis in fragile X syndrome: studies in human subjects and knockout mice. J Cereb Blood Flow Metab. 2013; 33:499–507. [PubMed: 23299245]
4. Bishu S, Schmidt KC, Burlin TV, Channing MA, Horowitz L, Huang T, et al. Propofol anesthesia does not alter regional rates of cerebral protein synthesis measured with L-[1-^{11}C]leucine and PET in healthy male subjects. J Cereb Blood Flow Metab. 2009; 29:1035–1047. [PubMed: 19223912]
5. Qin M, Kang J, Smith CB. Increased rates of cerebral glucose metabolism in a mouse model of fragile X mental retardation. Proc Natl Acad Sci U S A. 2002; 99:15758–15763. [PubMed: 12427968]
6. Liu ZH, Chuang DM, Smith CB. Lithium ameliorates phenotypic deficits in a mouse model of fragile X syndrome. Int J Neuropsychopharmacol. 2011; 14:618–630. [PubMed: 20497624]
7. Ding Q, Sethna F, Wang H. Behavioral analysis of male and female Fmr1 knockout mice on C57BL/6 background. Behav Brain Res. 2014; 271:72–78. [PubMed: 24886775]

8. Hauer D, Ratano P, Morena M, Scaccianoce S, Briegel I, Palmery M, et al. Propofol enhances memory formation via an interaction with the endocannabinoid system. Anesthesiology. 2011; 114:1380–1388. [PubMed: 21532463]
9. Trapani G, Altomare C, Liso G, Sanna E, Biggio G. Propofol in anesthesia. Mechanism of action, structure-activity relationships, and drug delivery. Curr Med Chem. 2000; 7:249–271. [PubMed: 10637364]
10. Patel S, Wohlfeil ER, Rademacher DJ, Carrier EJ, Perry LJ, Kundu A, et al. The general anesthetic propofol increases brain N-arachidonylethanolamine (anandamide) content and inhibits fatty acid amide hydrolase. Br J Pharmacol. 2003; 139:1005–1013. [PubMed: 12839875]
11. Maccarrone M, Rossi S, Bari M, De Chiara V, Rapino C, Musella A, et al. Abnormal mGlu5 receptor/endocannabinoid coupling in mice lacking FMRP and BC1 RNA. Neuropsychopharmacology. 2010; 35:1500–1509. [PubMed: 20393458]
12. Zhang L, Alger BE. Enhanced endocannabinoid signaling elevates neuronal excitability in fragile X syndrome. J Neurosci. 2010; 30:5724–5729. [PubMed: 20410124]
13. Jung KM, Sepers M, Henstridge CM, Lassalle O, Neuhofer D, Martin H, et al. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat Commun. 2012; 3:1080. [PubMed: 23011134]
14. Busquets-Garcia A, Gomis-González M, Guegan T, Agustín-Pavón C, Pastor A, Mato S, et al. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat Med. 2013; 19:603–607. [PubMed: 23542787]
15. Straiker A, Min KT, Mackie K. Fmr1 deletion enhances and ultimately desensitizes CB(1) signaling in autaptic hippocampal neurons. Neurobiol Dis. 2013; 56:1–5. [PubMed: 23578490]
16. Detrait E, Hanon E, Dardenne B, Lamberty Y. The inhibitory avoidance test optimized for discovery of cognitive enhancers. Behavior Res Methods. 2009; 41:805–811.
17. Liu ZH, Smith CB. Dissociation of social and nonsocial anxiety in a mouse model of fragile X syndrome. Neurosci Lett. 2009; 454:62–66. [PubMed: 19429055]
18. Mazzola C, Medalie J, Scherma M, Panlilio LV, Solinas M, Tanda G, et al. Fatty acid amide hydrolase (FAAH) inhibition enhances memory acquisition through activation of PPAR-alpha nuclear receptors. Learn Mem. 2009; 16:332–337. [PubMed: 19403796]
19. Hill MN, Kumar SA, Filipski SB, Iverson M, Stuhr KL, Keith JM, et al. Disruption of fatty acid amide hydrolase activity prevents the effects of chronic stress on anxiety and amygdalar microstructure. Mol Psychiatry. 2013; 18:1125–1135. [PubMed: 22776900]
20. Gray JM, Vecchiarelli HA, Morena M, Lee TT, Hermanson DJ, Kim AB, et al. Corticotropin-releasing hormone drives anandamide hydrolysis in the amygdala to promote anxiety. J Neurosci. 2015; 35:3879–3892. [PubMed: 25740517]
21. Mines MA, Yuskaitis CJ, King MK, Beurel E, Jope RS. GSK3 influences social preference and anxiety-related behaviors during social interaction in a mouse model of fragile X syndrome and autism. PLoS One. 2010; 5:e9706. [PubMed: 20300527]
22. Heitzer AM, Roth AK, Nawrocki L, Wrenn CC, Valdovinos MG. Brief report: altered social behavior in isolation-reared Fmr1 knockout mice. J Autism Dev Disord. 2013; 43:1452–1458. [PubMed: 23015112]
23. Kano M, Ohno-Shosaku T, Hashimotodani Y, Uchigashima M, Watanabe M. Endocannabinoid-mediated control of synaptic transmission. Physiol Rev. 2009; 89:309–380. [PubMed: 19126760]
24. Tang AH, Alger BE. Homer protein-metabotropic glutamate receptor binding regulates endocannabinoid signaling and affects hyperexcitability in a mouse model of fragile X syndrome. J Neurosci. 2015; 35:3938–3945. [PubMed: 25740522]
25. Ronesi JA, Huber KM. Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. J Neurosci. 2008; 28:543–547. [PubMed: 18184796]
26. Melis M, Greco B, Tonini R. Interplay between synaptic endocannabinoid signaling and metaplasticity in neuronal circuit function and dysfunction. Eur J Neurosci. 2014; 39:1189–1201. [PubMed: 24712998]

HIGHLIGHTS

- Deficit on passive avoidance test of memory is a robust phenotype of *Fmr1* KO mice.
- Propofol treatment 30 min after training reverses passive avoidance deficit.
- Propofol effect blocked by prior treatment with cannabinoid receptor 1 blockade.
- Treatment with FAAH inhibitor, URB-597, reverses passive avoidance deficit.
- URB-597 also normalized anxiety-like behavior, but did not enhance social behavior.

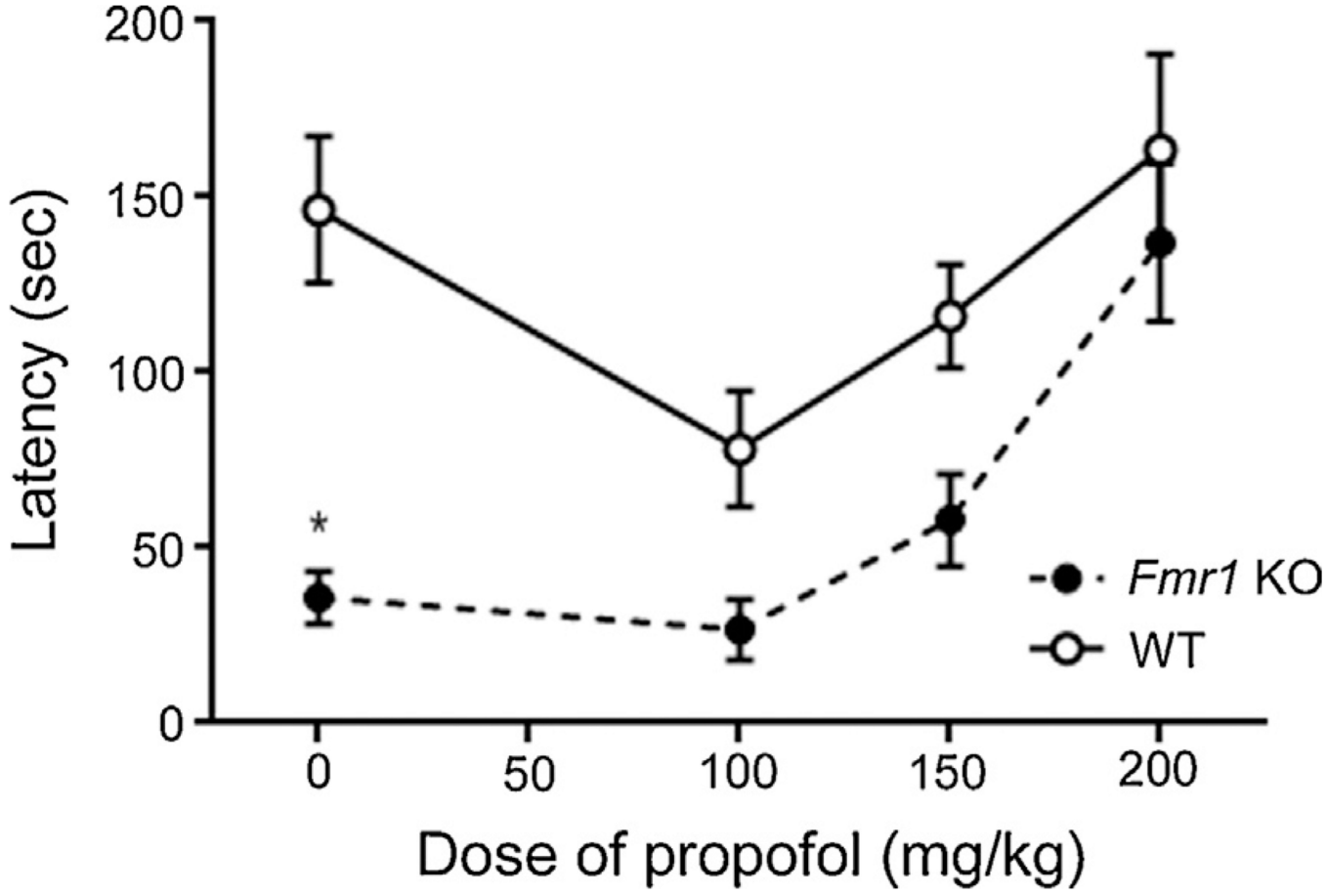

Fig. 1.

Effect of dose of propofol on performance on passive avoidance test in WT and *Fmr1* KO mice. Points represent the means ± SEM for the number of animals indicated below. Doses tested were 0 (Intralipid vehicle) in 9 WT and 8 *Fmr1* KO, 100 mg/kg in 10 WT and 7 *Fmr1* KO, 150 mg/kg in 6 WT and 10 *Fmr1* KO, and 200 mg/kg in 11 WT and 11 *Fmr1* KO mice. Data were analyzed by means of two-way ANOVA with genotype and dose of propofol as between subjects factors. The genotype × dose interaction was not statistically significant ($F_{(3,64)} = 1.664$; $P = .184$). Main effects of genotype ($F_{(1,64)} = 18.90$; $P < .0001$) and dose of propofol ($F_{(3,64)} = 9.290$; $P < .0001$) were both statistically significant. *, Statistically significantly different from similarly treated WT mice by means of Student's *t*-tests, $P \leq .001$.

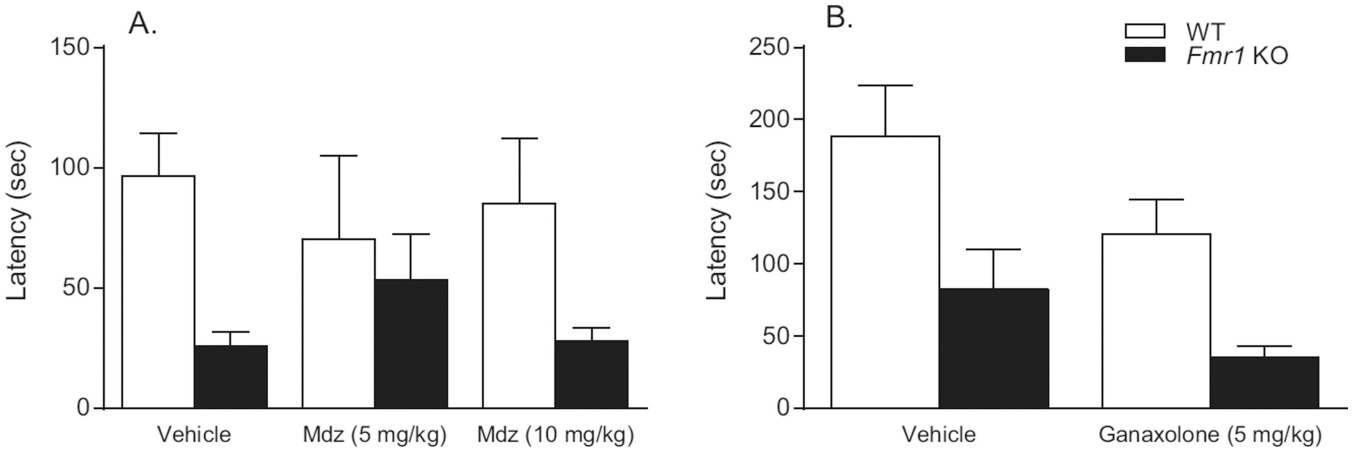

Fig. 2.

Effects of activation of $GABA_A$ receptors on latency to enter the dark compartment 24 h after training on the passive avoidance test. (A) Effect of treatment with normal saline vehicle in WT (12) and *Fmr1* KO (10) mice, midazolam (Mdz), 5 mg/kg in WT (11) and *Fmr1* KO (10), or midazolam, 10 mg/kg in WT (10) and *Fmr1* KO (8) and (B) Captisol vehicle in WT (13) and *Fmr1* KO (14) mice, ganoxolone, 5mg/kg in WT (18) and *Fmr1* KO (20) mice. Drugs were administered 30 min after training. None of these animals was treated with propofol. Bars represent the means ± SEM for the number of animals indicated above in parentheses. Data were analyzed by means of two-way ANOVA with genotype and drug treatment as between subjects factors. For midazolam treatment (A) neither the genotype × dose interaction ($F_{(2,55)} = 0.825$; $P = .444$) nor the main effect of midazolam treatment ($F_{(2,55)} = 0.331$; $P = .968$) was statistically significant. The main effect of genotype ($F_{(1,55)} = 6.973$; $P = .011$) was statistically significant. For ganaxolone treatment (B) the genotype × treatment interaction ($F_{(1,61)} = 0.185$; $P = .669$) was not statistically significant. The main effects of ganaxolone treatment ($F_{(1,61)} = 5.969$; $P = .018$) and genotype ($F_{(1,61)} = 16.73$; $P = .0001$) were statistically significant.

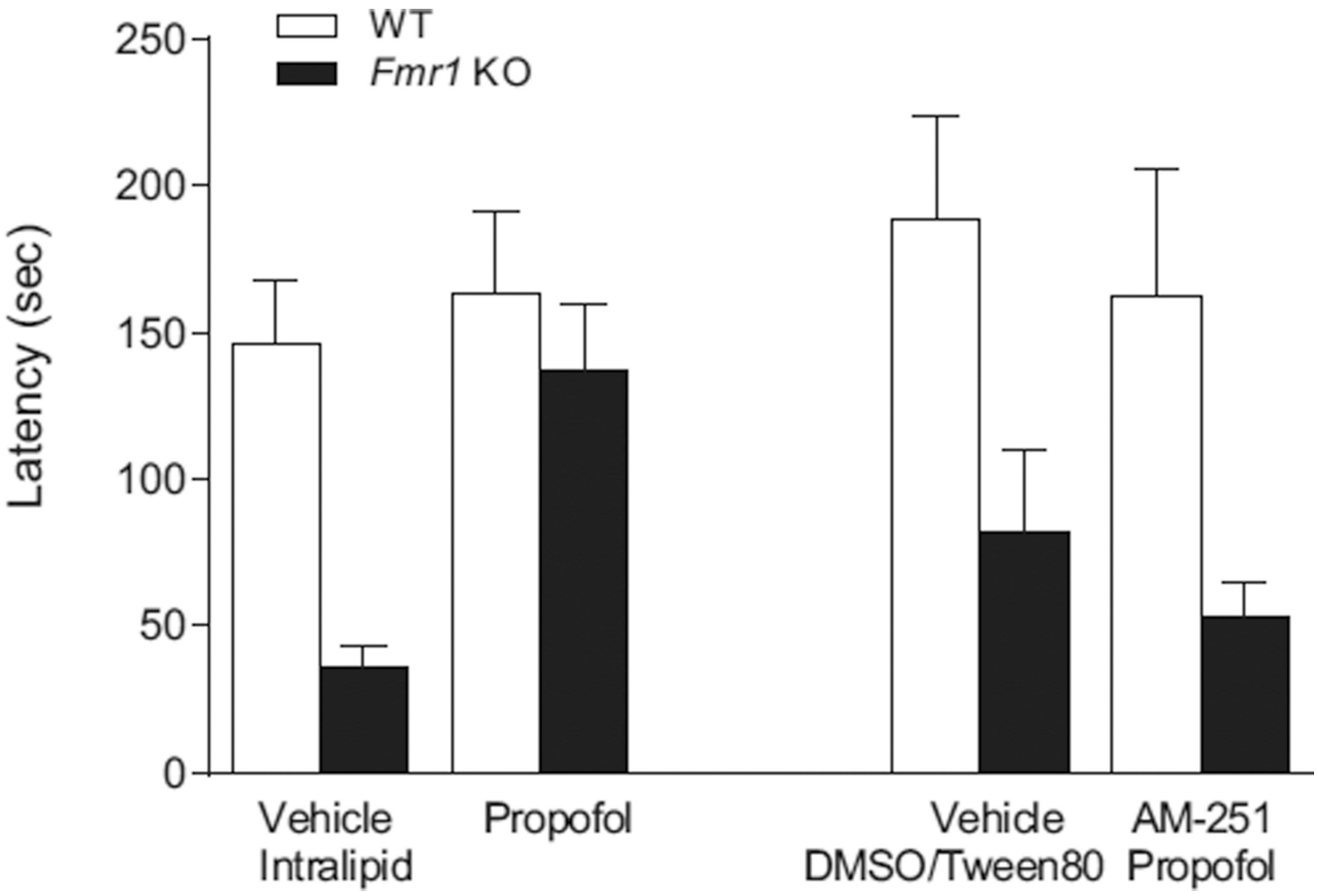

Fig. 3.

Effect of pretreatment with a CB1 receptor antagonist, AM-251, immediately after training and 30 min prior to propofol (200 mg/kg, i.p.) treatment on latency to enter the dark compartment 24 h after training. The effects of propofol (200 mg/kg, i.p.) alone administered 30 min after training (data from the dose–response experiment, Fig. 1) are shown on the left of this fig to illustrate the propofol effect on latency of *Fmr1* KO mice. Pretreatment with AM-251 immediately after training was tested in WT (6) and *Fmr1* KO (11) mice. Control WT (13) and *Fmr1* KO (14) mice were treated with DMSO:Tween 80:NS vehicle (1:1:8, v:v:v), i.p., immediately after training, but were not treated with propofol. Bars represent the means ± SEM for the number of animals indicated above in parentheses. Data were analyzed by means of two-way ANOVA with genotype and AM-251/propofol treatment as between subjects factors. Neither the genotype × treatment interaction ($F_{(1,40)}$ = 0.002; P = .963) nor the main effect of treatment ($F_{(1,40)}$ = 0.740; P = .395) was statistically significant. The main effect of genotype ($F_{(1,40)}$ = 11.12; P = .002) was statistically significant.

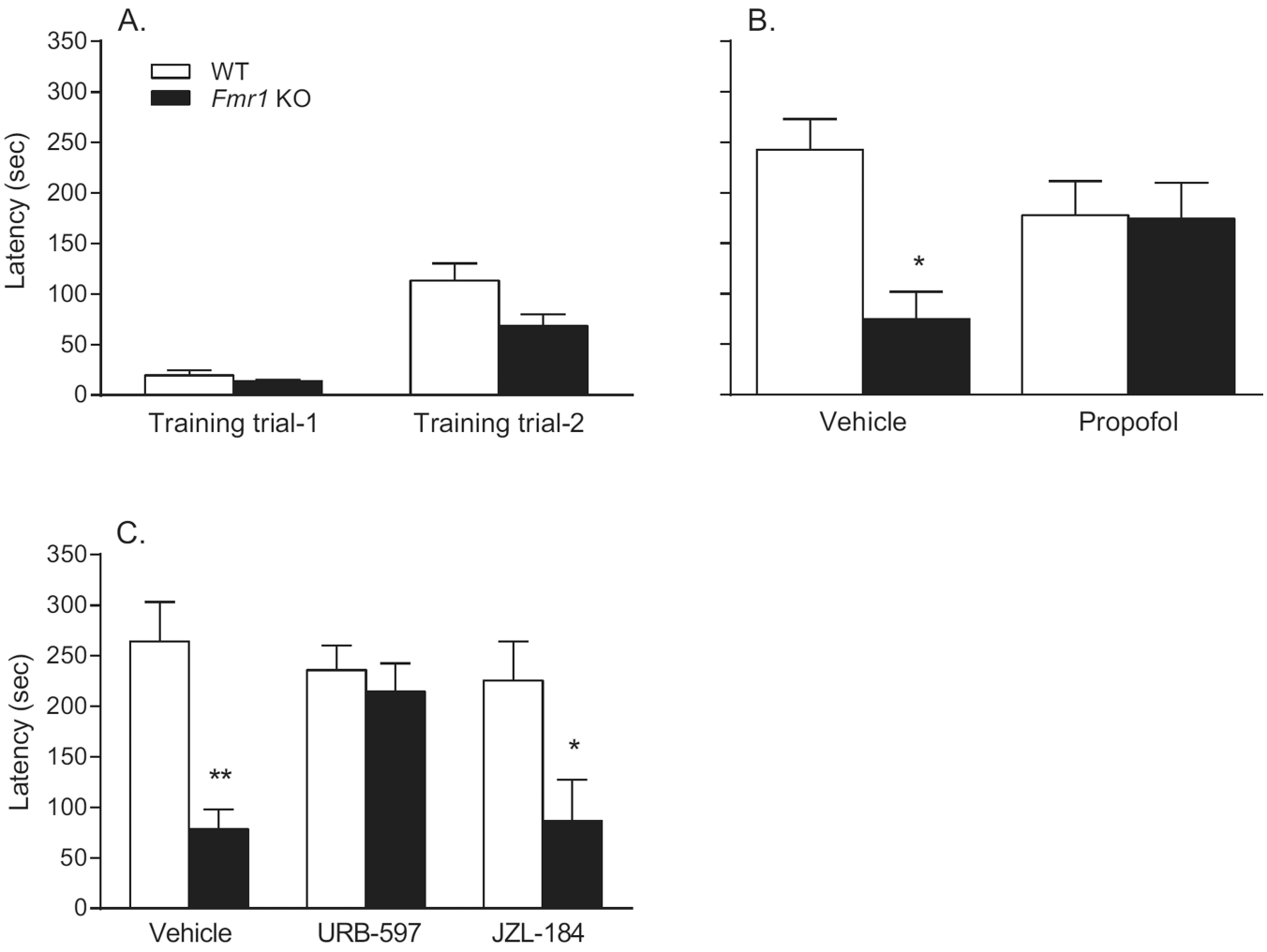

Fig. 4.

Behavior of WT and *Fmr1* KO mice on the passive avoidance protocol with two training shocks: (A) Effects of genotype on short-term memory in WT (60) and *Fmr1* KO (66) mice, (B) Effects of genotype and propofol (200 mg/kg) on long-term memory in vehicle-injected WT (10) and *Fmr1* KO (10) mice and propofol-injected WT (11) and *Fmr1* KO (11) mice, (C) Effects of inhibition of FAAH (URB-597 (0.3 mg/kg)) or MAGL (JZL-184 (16 mg/kg)) on long-term memory in vehicle-injected WT (11) and *Fmr1* KO (9) mice, URB-597-injected WT (18) and *Fmr1* KO (18) mice, and JZL-184-injected WT (10) and *Fmr1* KO (9) mice. Bars represent the means ± SEM for the number of animals indicated above in parentheses. Data on short-term memory (A) were analyzed by means of RM ANOVA with genotype as the between subjects factor and training trial as the within subjects factor. The interaction genotype × training trial ($F_{(1,124)} = 3.161$; $P = .078$) approached statistical significance, and the main effects of training trial ($F_{(1,124)} = 50.317$; $P < .001$) and genotype ($F_{(1,124)} = 5.374$; $P = .022$) achieved statistical significance. Data on the effects of propofol on behavior in passive avoidance test after two-shock training (B) were analyzed by means of two-way ANOVA with genotype and propofol treatment as between subjects factors. The genotype × treatment interaction ($F_{(1,38)} = 6.527$; $P = .015$) and the main effect of genotype ($F_{(1,38)} = 7.055$; $P = .012$) were both statistically significant, but the main effect of propofol

was not ($F_{(1,38)}$ = 0.280; P = .600). Data on the effects of URB-597 and JZL-134 on behavior in passive avoidance test after two-shock training (C) were analyzed by means of two-way ANOVA with genotype and treatment as between subjects factors. The genotype × treatment interaction ($F_{(2,70)}$ = 4.329; P = .017) and the main effect of genotype ($F_{(1,70)}$ = 17.300; P < .000) were both statistically significant, but the main effect of treatment was not ($F_{(2,70)}$ = 2.556; P = .085). In cases in which interactions were statistically significant we probed pairwise comparisons with *t*-tests corrected for multiple comparisons. Statistically significant differences between genotypes with the same treatment are indicated as follows: *, $P \leq .01$; **, $P \leq .001$.

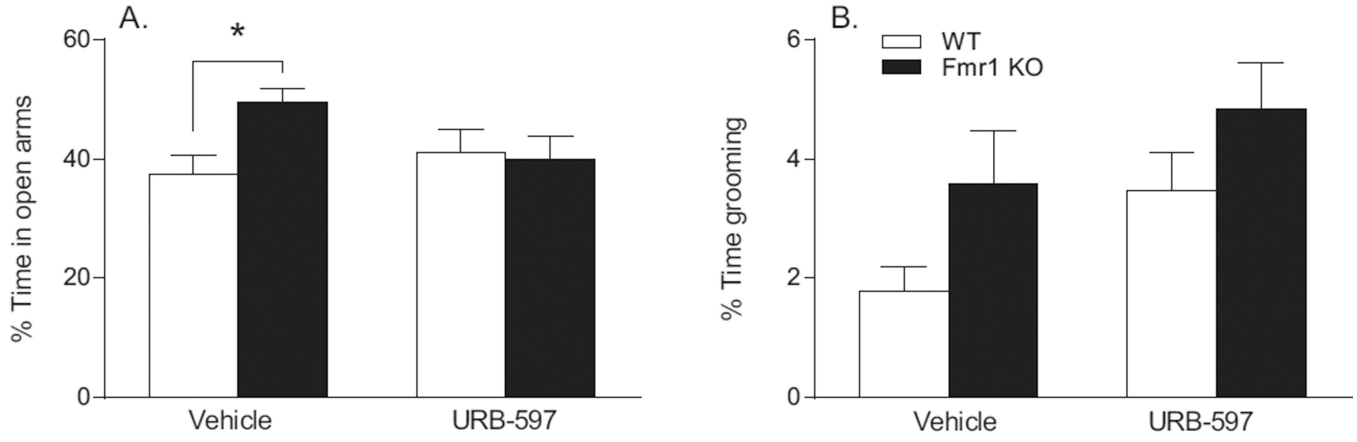

Fig. 5.

Behavior in the EPM after treatment with URB-597 (0.3 mg/kg, i.p.). Animals were injected with either vehicle (WT (12), *Fmr1* KO (12)) or URB-597 (WT (18), *Fmr1* KO (16)) 30 min before testing, and % time in the open arms (A) and % time spent grooming (B) were measured for 5 min. Bars represent the means ± SEM for the number of animals indicated above in parentheses. Data were analyzed by means of two-way ANOVA with genotype and treatment as between subjects factors. For the percent time in the open arms, the genotype × treatment interaction ($F_{(1,54)} = 3.209$; $P = .079$) was not statistically significant, and neither were the main effects of treatment ($F_{(1,54)} = 0.674$; $P = .415$) nor genotype ($F_{(1,54)} = 2.23$; $P = .141$). We probed for individual differences (Bonferroni *t*-tests) and found that the difference between vehicle-treated WT and KO mice was significantly different (*, $P < .05$). For % time spent grooming, the genotype × treatment interaction ($F_{(1,54)} = 0.800$; $P = .778$) was not statistically significant, but both the main effects of treatment ($F_{(1,54)} = 4.140$; $P = .047$) and genotype ($F_{(1,54)} = 4.841$; $P = .032$) were statistically significant.

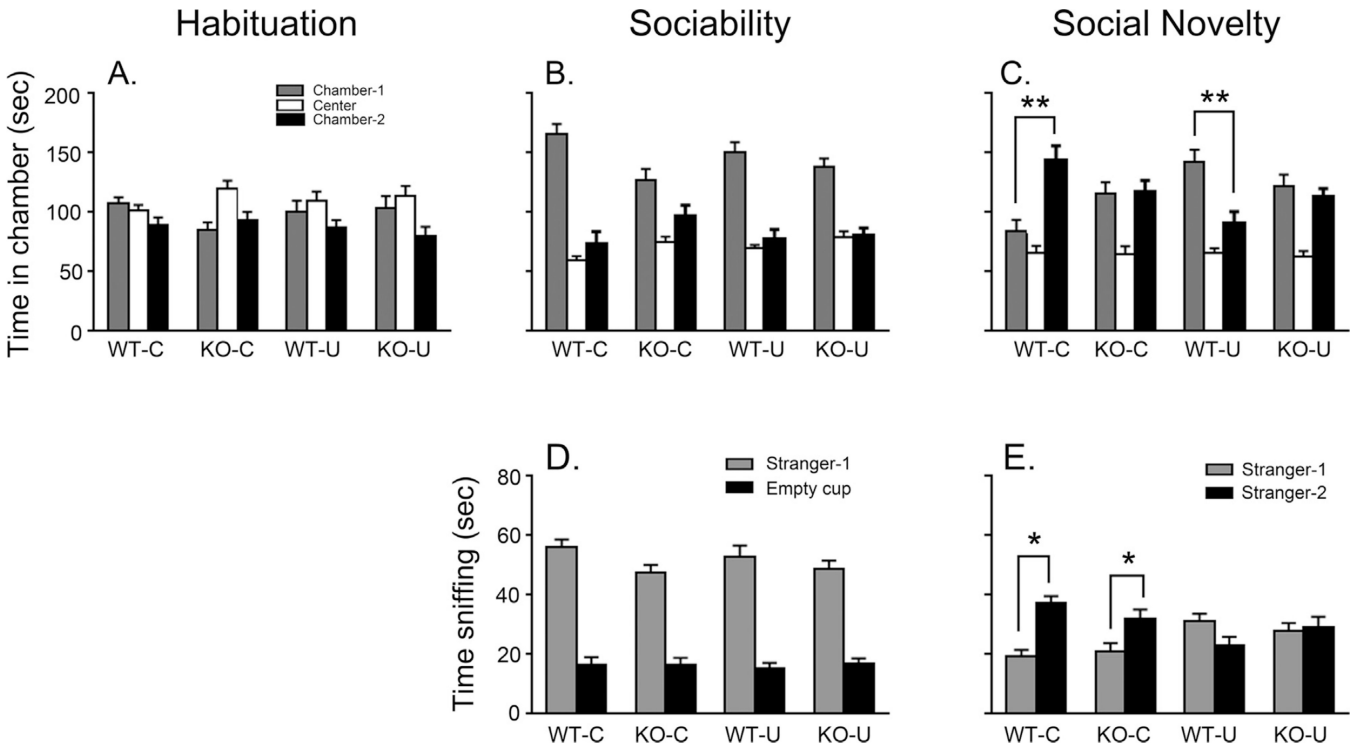

Fig. 6.

Effects of URB-597 treatment on social behavior in vehicle-treated WT (WT-C, 15) and *Fmr1* KO (KO-C, 17) and URB-597 treated WT (WT-U, 15) and *Fmr1* KO (KO-U, 17) mice. Mice were treated with Captisol vehicle or URB-597 (0.3 mg/kg, i.p.) 30 min before testing. The test had three phases, each 5 min in length: Habituation (A) in which animals were permitted to explore all three chambers with no stranger mice present, Sociability (B and D) in which a stranger mouse was placed within the cup in Chamber-1, and Preference for Social Novelty (C and E) in which a second stranger mouse was placed in the cup in Chamber-2. The times spent in each chamber were measured (A–C) and the times spent sniffing the stranger mice or empty cup were recorded (D and E). Bars represent the means ± SEM for the number of animals indicated above in parentheses. Data were analyzed by means of RM ANOVA with genotype and treatment as between subjects factors and chamber or stranger as within subjects factors. For the habituation phase, there were no statistically significant interactions or main effects for time spent in chambers. For time spent in chambers during the sociability phase, the chamber × genotype × treatment interaction ($F_{(1,60)} = 2.015$; $P = .161$) and chamber × treatment ($F_{(1,60)} = 0.074$; $P = .786$) and genotype and treatment interactions ($F_{(1,60)} = 0.386$; $P = .537$) were not statistically significant; the chamber × genotype interaction ($F_{(1,60)} = 5.353$; $P = .024$) was. Of the main effects, only chamber ($F_{(1,60)} = 59.454$; $P < .001$) and genotype ($F_{(1,60)} = 8.748$; $P = .004$) were statistically significant. For time spent sniffing either the stranger mouse or the empty cup during the sociability phase, the chamber × genotype × treatment interaction ($F_{(1,54)} = 1.569$; $P = .216$) and the chamber × treatment ($F_{(1,54)} = 0.016$; $P = .901$), chamber × genotype ($F_{(1,54)} = 2.698$; $P = .106$), and genotype × treatment ($F_{(1,54)} = 1.316$; $P = .256$) interactions were not statistically significant. Of the main effects, only chamber ($F_{(1,54)} = 126.73$; $P < .001$) was statistically significant. For time spent in chambers during the

preference for social novelty phase, the chamber × genotype × treatment interaction ($F_{(1,60)}$ = 7.461; P = .008) was statistically significant. We probed for individual differences (Bonferroni *t*-tests) and found that times in Chamber-1 and Chamber-2 differed significantly (**, P < .01) in WT-C and WT-U mice but not in either KO-C or KO-U mice. For time spent sniffing either Stranger-1 or Stranger-2 during the preference for social novelty phase, the chamber × genotype × treatment interaction ($F_{(1,60)}$ = 3.498; P = .066) approached statistical significance. The chamber × treatment interaction ($F_{(1,60)}$ = 16.265; P < .001) was statistically significant, but chamber × genotype ($F_{(1,60)}$ = 0.075; P = .784) and genotype × treatment ($F_{(1,60)}$ = 1.393; P = .243) interactions were not. Of the main effects, only chamber ($F_{(1,60)}$ = 5.962; P = .018) was statistically significant. We probed for individual differences (Bonferroni *t*-tests) and found that times sniffing Stranger-1 and Stranger-2 differed significantly (*, P < .05) in WT-C and KO-C mice but not in either WT-U or KO-U mice.