Pharmacology, Biochemistry and Behavior 181 (2019) 93–100

Contents lists available at ScienceDirect

Pharmacology, Biochemistry and Behavior

journal homepage: www.elsevier.com/locate/pharmbiochembeh

Cannabidiol (CBD) reduces anxiety-related behavior in mice via an *FMRP*-independent mechanism

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ARTICLE INFO

Keywords:
Cannabidiol (CBD)
Fragile X syndrome
Mouse model
Locomotion
Anxiety
Cognition
Social behaviours

ABSTRACT

Fragile X Syndrome is a neurodevelopmental disorder which affects intellectual, social and physical development due to mutation of the Fragile X mental retardation 1 (*FMR1*) gene. The resultant loss of Fragile X mental retardation protein can be modelled by *Fmr1* gene knockout (KO) in mice. The current study investigated the behavioural effects of cannabidiol (CBD; a non-psychoactive phytocannabinoid) in male *Fmr1* KO mice as a preclinical model for therapeutic discovery. Vehicle or CBD (5 or 20 mg/kg body weight) was administered to adult *Fmr1* KO and wild type-like (WT) mice before they were tested in behavioural tasks including: open field (OF), elevated plus maze (EPM), spontaneous alternation, social preference, and passive avoidance tasks. *Fmr1* KO mice were hyperlocomotive and hyperexplorative and habituated more slowly to a novel environment compared to control animals. Furthermore, *Fmr1* KO mice showed fewer anxiety-related behaviours across tests. Effects of CBD were subtle and limited to the EPM, where CBD decreased the anxiety response of all mice tested. Acute CBD had no impact on locomotion or anxiety-related parameters in the OF. Cognitive performance of *Fmr1* KO mice was equivalent to controls and not affected by CBD treatment. Brain concentrations of CBD were equivalent between genotypes, but in animals sacrificed 90 min post-administration, decreased plasma CBD in *Fmr1* KO mice compared to WT suggested more rapid clearance of CBD by transgenic animals. Overall, acute CBD at the doses chosen did not selectively normalize behavioural abnormalities in *Fmr1* KO mice, but reduced anxiety-like behaviour in both *Fmr1* KO and WT mice.

1. Introduction

Fragile X Syndrome (FXS) is a neurodevelopmental disorder which affects intellectual, social and physical development of both men (1 in 4000) and women (1 in 8000) (Turner et al., 1996). Individuals with FXS experience intellectual disability and symptoms of autism (Thurman et al., 2014; Rogers et al., 2001) as well as altered sensory sensitivity (Kogan et al., 2004; Frankland et al., 2004; Van der Molen et al., 2012), repetitive behaviours (Oakes et al., 2016), social communication deficits (Marschik et al., 2014), and increased anxiety (Thurman et al., 2014). FXS is caused by CGG repeat expansion in the 5′ untranslated region of the Fragile X mental retardation 1 gene (*FMR1*) (Verkerk et al., 1991). Repeat expansion in excess of 200 copies results in epigenetic silencing of *FMR1* and loss of Fragile X mental retardation protein (FMRP). FMRP is an RNA-binding protein (Ashley Jr. et al., 1993) which negatively regulates protein translation and is required for normal neural development, as it binds to transcripts of proteins involved in synaptic function (Darnell et al., 2011). Other molecular signalling pathways are also affected in FXS, such as mTOR signalling (Sharma et al., 2010), a crucial factor for protein synthesis and cellular growth (Laplante and Sabatini, 2012). Treatment options in FXS are currently limited, and have until recently focused on specific domains of symptom relief (predominantly anxiety, attention deficit, and hyperactivity) (Gross et al., 2015; Hagerman and Polussa, 2015). Thus, new treatment alternatives informed by increased understanding of FXS neurobiology are urgently required.

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https://doi.org/10.1016/j.pbb.2019.05.002
Received 21 December 2018; Received in revised form 21 April 2019; Accepted 1 May 2019
Available online 04 May 2019
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As a tool for understanding FXS, a germline *Fmr1* knockout (KO) mouse model was developed (Bakker et al., 1994). This rodent model mimics the complete loss of FMRP which occurs in individuals with the full mutation (> 200 repeats), and can be used for evaluation of novel therapeutic strategies. *Fmr1* KO mice display a range of altered behaviours compared to control mice, some of which are consistent with the clinical picture for FXS or autism (for review see (Bernardet and Crusio, 2006) and (Kazdoba et al., 2014)). *Fmr1* KO mice display less preference for social novelty than wild type-like mice (Qin et al., 2015a), consistent with social deficits in FXS (Marschik et al., 2014) and are consistently hyperactive (Ding et al., 2014; Oddi et al., 2015; Wrenn et al., 2015; Dolan et al., 2013; Uutela et al., 2012). Spatial working memory abnormalities have been described (Bakker et al., 1994; D'Hooge et al., 1997; Sinclair et al., 2017; Bilousova et al., 2009), but not consistently (Peier et al., 2000; Leach et al., 2016). Similarly, studies on the anxiety-related phenotype of this mouse model report both decreased (Ding et al., 2014; Dolan et al., 2013; Uutela et al., 2012; Sinclair et al., 2017; Liu et al., 2011) as well as increased (Bilousova et al., 2009; Sorensen et al., 2015) anxiety-like behaviours compared to control animals. Humans with FXS often exhibit increased anxiety (Thurman et al., 2014; Bailey et al., 2008). *Fmr1* KO mouse model phenotypes can be utilised pre-clinically to evaluate some efficacy parameters of new treatment candidates.

Recently, the endocannabinoid system has become a target of preclinical research into FXS (Busquets-Garcia et al., 2013; Qin et al., 2015b) as FMRP, which is diminished in FXS, facilitates the production of the endocannabinoid 2-arachidonoylglycerol (2-AG) (Jung et al., 2012). In line with this, *Fmr1* KO mice exhibit lower levels of 2-AG than control mice (Jung et al., 2012) and dimished retrograde 2-AG signalling in the hippocampus (Wang et al., 2018). The phosphatidylinositol-3-kinase (PI3K)-protein kinase B (Akt)-mTOR-p70S6 kinase (p70S6K) signalling pathway (Busquets-Garcia et al., 2011; Puighermanal et al., 2012) is a downstream target of the endocannabinoid system and is dysregulated in *Fmr1* KO mice (Sharma et al., 2010). The non-psychoactive phytocannabinoid cannabidiol (CBD) may have benefits for FXS patients based on its capacity to modulate FXS-compromised endocannabinoid signalling. CBD may attenuate the pathophysiology of the disease by indirectly increasing the concentration of the two main endocannabinoids, 2-AG and *N*-arachidonoylethanolamine (AEA, anandamide) (Bisogno et al., 2001; McPartland et al., 2015; Grotenhermen, 2004). In the mouse hippocampus, levels of anandamide but not 2-AG increase after 14 day treatment with 30 mg/kg CBD (Campos et al., 2013). Furthermore, CBD has neuroprotective effects (Hampson et al., 1998; Jones et al., 2012) and can increase adult hippocampal neurogenesis (Wolf et al., 2010). On a behavioural level, CBD has been found to carry anti-anxiety and anti-psychotic-like properties (Leweke et al., 2012; Almeida et al., 2013; Zuardi, 2008) and improve social impairments (Long et al., 2012), suggesting therapeutic potential for FXS. Indeed, CBD is a promising new therapy for Dravet and Lennox-Gastaut syndromes, which cause seizures and developmental delay (Devinsky et al., 2017). Importantly, the ability of CBD to reverse FXS-related behavioural abnormalities of *Fmr1* KO mice has not previously been evaluated.

The current study assessed the ability of CBD treatment to rescue behavioural deficits of male *Fmr1* KO mice. The open field test, elevated plus maze, passive avoidance test, the continuous Y maze, and the social preference test were performed to index locomotion, anxiety-related behaviour, social behaviours and working memory respectively, and to evaluate the potentially therapeutic-like effects of CBD on *Fmr1* KO-related deficits. Plasma and brain concentrations of CBD were also analysed to check for potential differences in CBD pharmacokinetics across genotypes.

2. Materials and methods

2.1. Animals

Fmr1 knockout mice (Bakker et al., 1994) were sourced from the Jackson Laboratory [Bar Harbor, Maine, USA; strain name B6.129P2-*Fmr1*^{tm1Cgr}/J, Stock No. 003025)]. Male *Fmr1* knockout mice (*Fmr1* KO: $n = 36$) and C57BL/6 J controls (WT: $n = 36$) were sent from The Jackson Laboratory to the Animal BioResources (Moss Vale, Australia) post-weaning and group-housed in independently ventilated cages (Airlaw, Smithfield, Australia) for around two weeks of habituation. At 10 weeks of age (± 1 week), test and control mice were transported to Neuroscience Research Australia (NeuRA), where they were group-housed in Polysulfone cages (1144B: Techniplast, Rydalmere, Australia) equipped with nesting material. Mice were kept under a 12:12 h light:dark schedule [light phase: white light (illumination: 124 lx) – dark phase: red light (illumination: < 2 lx)]. Food and water were provided ad libitum. Mice were habituated to the NeuRA facilities for three weeks before behavioural testing commenced. Adult, male A/JArc mice from Animal Resources Centre (Canning Vale, Australia) were used as standard opponent for the social preference test (see more information below). Research and animal care procedures were approved by the University of New South Wales Animal Care and Ethics Committee in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes.

2.2. Acute cannabidiol (CBD) treatment

2.2.1. Drug preparation and administration

Powdered cannabidiol (National Measurement Institute, NSW, Australia) was dissolved in equal amounts of Tween 80 (Sigma-Aldrich Co., St Louis, USA) and 100% ethanol and diluted with 0.9% sodium chloride to the appropriate concentration to a final ratio of 1:1:18 as published previously (Cheng et al., 2014a; Long et al., 2010). Ethanol and Tween 80 comprised 10% of the total volume. A vehicle control treatment was set up similarly without the addition of CBD. *Fmr1* KO and WT mice ($n = 12$ per CBD dose) were administered either vehicle, 5 mg CBD/kg body weight, or 20 mg CBD/kg body weight, one dose before each behavioural test (see below). These doses were chosen based on previous studies which identified benefits of acute or chronic CBD treatment at 5 and 20 mg/kg (Cheng et al., 2014a; Martin-Moreno et al., 2011; Avraham et al., 2011; Schiavon et al., 2014; Rock et al., 2017). Mice received intraperitoneal (i.p.) injection (injection volume of 10 ml/kg body weight) 30 min prior to the start of behavioural testing, with an inter-test interval of at least three days between tests. For the passive avoidance task CBD was administered 30 min before the start of the training session.

2.3. Behavioural phenotyping

Starting at 5 months of age (± 1 week; after 3 weeks of habituation to the facilities), mice were tested in a battery of behavioural tests. All tests were conducted during the first 5 h of the light phase to minimise effects of the circadian rhythm on the performance of test mice. The test order was as follows: open field, elevated plus maze, spontaneous alternation in the Y maze, social preference, and passive avoidance tasks. Equipment and apparatus were cleaned between trials using 70% ethanol except where specified (i.e. open field testing).

2.3.1. Open field (OF)

The OF mimics the natural conflict in mice between the tendency to explore a novel environment and to avoid an exposed open area (DeFries et al., 1966; Denenberg, 1969). Mice were placed into an infrared photobeam controlled open field activity test chamber (MED Associates Inc., USA, Vermont) for 30 min. The arena (43.2 cm × 43.2 cm) was divided into a central and a peripheral zone

(MED Associates Inc. software coordinates for central zone: 3/3, 3/13, 13/3, 13/13). The animal's horizontal activity (i.e. distance travelled), vertical activity (i.e. *rearing*), small motor movements (i.e. movements below the ambulation threshold, e.g. *grooming*), and *resting* behaviour (no infrared photobeam-detectable movements), were recorded automatically for the different zones (software settings for ambulation threshold: box size: 3; ambulatory trigger: 2; resting delay: 1000 ms; resolution: 100 ms). The ratio of central to total distance travelled and time spent in the central zone were taken as measures of anxiety (Karl et al., 2007). Equipment was cleaned between experiments using detergent.

2.3.2. Elevated plus maze (EPM)

The EPM assesses the natural conflict between the tendency of mice to explore a novel environment and avoidance of a brightly lit, elevated and open area (Montgomery and Monkman, 1955). The grey plus maze was "+" shaped [for details of apparatus see (Karl et al., 2008). Mice were placed at the centre of the + (faced towards an enclosed arm) and were allowed to explore the maze for 5 min. The percentage time spent in the open arms and total distance travelled in the open arms were recorded as anxiety measures using Any-Maze™ (Stoelting, Wood Dale, USA) tracking software.

2.3.3. Continuous spontaneous alternation in the Y-maze (SA)

The Y-maze SA test measures the willingness of mice to explore novel environments. Rodents typically prefer to investigate a new arm of a maze rather than returning to one that was previously visited (Hughes, 2004). The Y-maze used in our laboratory consisted of three grey acrylic arms (10 cm × 30 cm × 17 cm) placed at 120° with respect to each other. Around the arms were distal cues. Animals were placed into the centre of the Y-maze and allowed to freely explore the environment for 10 min. Order of entries into the three different arms (A, B, or C) was recorded and successful arm entry triplets (i.e. ABC, ACB, BCA, BAC, CAB, CBA) calculated (maximal number of correct triplets = total number of arm entries – 2) (Long et al., 2010). Percentage of correct arm entries was calculated. AnyMaze™ software was used to track the animal's movement in the maze: an arm entry was scored whenever an animal entered the 2nd half of the arm with > 60% of its body length.

2.3.4. Passive avoidance

In this basic hippocampus-dependent learning test, the avoidance of a naturally less aversive dark compartment after it is paired with an electrical foot shock indicates the retention of this memory (Bovet et al., 1969). In the training session, CBD was administered then 30 min later mice were placed in an illuminated compartment (illumination: 25 lx; 25 × 15 × 25 cm; model H10-11R-TC, Coulbourn Instruments, USA). After 10 s, the door to a dark chamber was opened and the latency to enter was measured manually. Once the mouse had entered the dark chamber (illumination: 1 lx; 25 × 12 × 25 cm), the door was closed and a single foot shock (0.4 mA for 2 s) was delivered. Mice were kept in the dark chamber for another 60 s to facilitate the formation of an association between the dark chamber and the foot shock. In the retention session 24 h later, mice were placed in the light compartment and the latency to enter the dark chamber was measured manually (cut-off time: 300 s) as published previously from our laboratory (Duffy et al., 2010). Latency was compared between training and test sessions-increased entry latency on the second day indicates memory of the aversive stimulus.

2.3.5. Social preference test (SPT)

The SPT was used to assess sociability and social recognition memory (Moy et al., 2004) and performed as described in our earlier studies (Cheng et al., 2013; Cheng et al., 2014b). Test animals were isolated for an hour prior to the start of testing. During the habituation trial, mice were allowed to explore a three-chamber apparatus, consisting of a centre chamber (9 × 18 cm; height: 20 cm) and two outer chambers (16 × 18 cm; height: 20 cm), freely for 5 min. For the following *sociability test* an unfamiliar (male A/J) mouse was placed in a small enclosure in one of the outer chambers, which allowed nose contact between A/J and test mice. The test mouse was returned to the apparatus and allowed to explore all three chambers and the animal enclosures for 10 min. Following the sociability test, test mice were observed in the *social recognition test*. For this, a second, unfamiliar A/J standard mouse was placed in the previously empty chamber so that the test mouse had the choice to explore either the familiar mouse (from the previous trial) or the novel, unfamiliar mouse in the following 10 min. The inter-trial interval (ITI) was 5 min. The chambers and enclosures were cleaned with 70% ethanol in-between trials and fresh corn cob bedding was added to the chambers prior to each test trial. AnyMaze™ software was used to determine the time spent in the different chambers, number of entries and distance travelled by the test mice in each trial. Primary measures of interest were the time spent with a mouse (i.e. in the sociability trial) or a novel mouse (i.e. in the social recognition trial) as a percentage of total time in both chambers.

2.4. Analysis of CBD concentrations in plasma and brain

At least one week after the completion of the behavioural testing (see Section 2.3), mice were treated one more time with vehicle or CBD (5 mg or 20 mg i.p.) and blood and brain tissue were collected. One half of the cohort was sacrificed 30 min post CBD administration ($N = 6$ per genotype and dose) and the other half, 90 min post CBD administration. Analysis and quantification of plasma and brain concentrations of CBD were conducted by XenoBiotic Laboratories, Inc. (New York, USA) using liquid chromatography tandem mass spectrometry (LC-MS/MS) with positive electrospray ionization – multiple reaction monitoring mode to quantify CBD. For plasma sample preparation, 50 µl of plasma sample was mixed with internal standard working solution and water, then loaded to a preconditioned solid phase extraction plate. After washing with water and a mixture of water and methanol, CBD and internal standard were eluted with acetonitrile and reconstituted. For brain sample preparation, each sample was individually weighed and the volume of control mouse plasma was adjusted for each sample to achieve 1:4 ratio of brain:plasma (i.e., 200 mg of brain tissue mixed with 800 µl of plasma). This mixture was then homogenized. Next, 50 µl of the processed mouse brain sample was used for the extraction procedure and extracted the same as mouse plasma samples.

2.5. Statistical analysis

Two-way analysis of variance (ANOVA) was performed to investigate main effects of 'genotype' and 'CBD dose' and possible interactions. Repeated measures (RM) three-way ANOVAs were used to investigate total distance travelled across time, i.e. '5-min block' (OF), 'latency' (training session vs test session; PA), and 'chamber' (SPT) and 'time' (CBD concentrations) as published previously (Cheng et al., 2014b). In line with Rothman and Perneger (Perneger, 1998; Rothman, 1990), the data were not adjusted for multiple comparisons and were interpreted as such in the discussion. Paired *t*-tests were performed to investigate the preference of mice in the SPT test against chance levels (i.e. 50%). Finally, for analysis of CBD levels, missing values (i.e. samples below the limit of detection of 0.5 ng/ml) were assigned a value of 0.49. Three-way ANOVAs were then employed to determine relationships between 'genotype', 'CBD dose' and 'time' (between CBD administration and sample collection) and Pearson's correlations to determine the linear relation between plasma and brain concentrations of CBD. Differences were regarded as significant if $p < .05$. F-values and degrees of freedom are presented for ANOVAs. Data are shown as means ± standard error of means (SEM). Analyses were conducted using SPSS 20.0 for Windows.

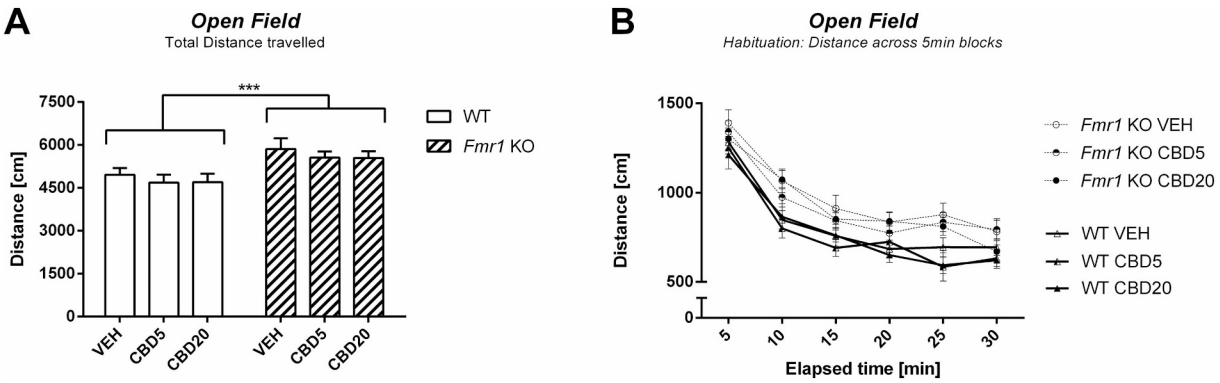

Fig. 1. A–B Overall locomotion and habituation of locomotive response to novelty in the open field (OF): **A**) Total distance travelled [cm] and **B**) distance travelled [cm] across 5-min blocks. Data for control (WT) and *Fmr1* knockout mice (*Fmr1* KO) after acute treatment with vehicle (VEH), 5 mg/kg bodyweight of CBD (CBD5) or 20 mg/kg of CBD (CBD20) are shown as means + SEM. There was a significant main effect of 'genotype' for total distance travelled ($p = .0003$; Fig. 1A) and a significant '5 min block' × 'genotype' interaction ($p = .009$; Fig. 1B). ***$p < .0005$.

3. Results

3.1. Locomotion and exploration

Fmr1 KO mice exhibited a hyperlocomotive and hyperexplorative phenotype in the 30 min OF test (Fig. 1A–B). *Fmr1* KO mice travelled further throughout the test [main effect of 'genotype' on total distance travelled: $F(1,66) = 14.9$, $p < .0001$] (Fig. 1) and exhibited increased vertical activity [$F(1,66) = 28.1$, $p < .0001$] (Table 1) compared to WT mice. These parameters were not influenced by CBD treatment ('genotype' × 'CBD' interactions: p's $> .05$). Mice of both genotypes habituated to the novel OF environment [main effect of '5 min block' on distance travelled: $F(5, 330) = 240.1$, $p < .0001$] (Fig. 1B). However, knockout mice displayed a slower locomoter habituation to the novel environment than control mice, as evidenced by interaction of 'genotype' and '5-min block' [$F(5,330) = 3.1$, $p = .009$; Fig. 1B]. This was not affected by CBD treatment (no 'CBD' × 'genotype' × '5-min block' interaction; $p > .05$).

3.2. Anxiety

The time spent in the central zone of the OF was greater in *Fmr1* KO mice than control mice [$F(1,66) = 58.3$, $p < .0001$] (Fig. 2A). Similarly, the percentage distance travelled in the central zone of the OF was increased in *Fmr1* KO mice [$F(1,66) = 30.7$, $p < .0001$] (Fig. 2B). Acute CBD treatment had no impact on anxiety-related parameters of the OF test (no 'CBD' main effects and no 'genotype' by 'CBD' interactions, all p's $> .05$).

In the EPM, *Fmr1* KO mice spent more time in the open arms [$F(1,63) = 42.3$, $p < .0001$] (Fig. 2C) and also travelled further in the open arms compared to control mice [i.e. as a percentage of total distance: $F(1,63) = 23.9$, $p < .0001$] (Fig. 2D). CBD increased time in the open arms regardless of genotype [main effect: $F(2,63) = 4.3$, $p < .05$, no 'genotype' by 'CBD' interaction, $p > .05$] (Fig. 2C). Collapsed across genotype, animals treated with 20 mg/kg CBD spent longer in the open arm than those treated with vehicle or 5 mg/kg ($p < .005$ and $p < .05$ respectively, Fig. 2C). There was also a strong trend for CBD treatment to increase the percentage of total distance travelled on open arms in all mice [$F(2,63) = 2.9$, $p = .06$, no 'genotype' by 'CBD' interaction, $p > .05$] (Fig. 2D). Collapsed across genotype, animals treated with 20 mg/kg CBD performed a greater percentage of their locomotion in the open arm than those treated with vehicle or 5 mg/kg (both $p < .05$, Fig. 2D).

Table 1
Open field (OF) behaviours of wild type-like control (WT) and *Fmr1* knockout mice (*Fmr1*) after acute treatment with vehicle (VEH), 5 mg/kg bodyweight of CBD (CBD5) or 20 mg/kg of CBD (CBD20). $N = 12$ for each dose and genotype. Data are shown as means ± SEM. There was a main effect of 'genotype' on vertical activity (rearing; $p < .0001$).

	OF Vertical activity [n]	OF Small motor movements [n]
WT VEH	283.7 ± 16.5	2283.5 ± 52.8
Fmr1 VEH	364.8 ± 29.5	2295.9 ± 34.5
WT CBD5	226.8 ± 24.0	2168.4 ± 57.4
Fmr1 CBD5	380.4 ± 27.9	2320.9 ± 45.7
WT CBD20	249.0 ± 21.3	2277.5 ± 62.9
Fmr1 CBD20	326.5 ± 22.8	2226.0 ± 31.5

3.3. Spatial memory

There was no difference between *Fmr1* KO and WT mice in spontaneous alternation (% correct entries) in the Y maze [($F(1,66) = 2.7$, $p > .05$], nor an effect of CBD [($F(2,66) = 0.1$, $p > .05$] (Table 2). Paired *t*-tests showed that only control mice treated with 20 mg CBD displayed levels of spontaneous alternation significantly above the chance level of 50% (Table 2). In the passive avoidance task, there were no baseline differences in the latency to enter the dark compartment during training between genotypes [$F(1,66) = 0.8$, $p > .05$] nor between CBD treatment groups [$F(2,66) = 1.6$, $p > .05$]. Latency to enter the dark compartment increased between training and testing in all mice equally [three-way RM ANOVA for 'latency': $F(1,66) = 18.5$, $p < .0001$; no interaction of 'latency' with 'genotype' or 'CBD' was detected; both $p > .05$], suggesting that all mice had learned equally the association between the foot shock and the dark compartment and CBD treatment did not alter this association (Fig. 3).

3.4. Social behaviours

Mice across all experimental groups demonstrated a significant preference for exploring a mouse over an empty chamber [three-way RM ANOVA for 'chamber': $F(1,66) = 13.8$, $p = .0004$]. There was no difference in sociability (i.e. preference for mouse chamber) between *Fmr1* KO and WT mice ('chamber' × 'genotype' interaction: $p > .05$) and CBD did not influence sociability ('chamber' × 'CBD' interaction: $p > .05$). Paired *t*-test against chance level mouse chamber exploration showed that all groups except control mice treated with CBD developed a trend or significant preference for the chamber containing the mouse (Table 2).

There was also a significant effect of 'chamber' across experimental groups for the social recognition session (i.e. exploring novel and familiar mice) ['chamber': $F(1,66) = 6.2$, $p < .05$]. This phenomenon was not affected by genotype ('chamber' × 'genotype' interaction

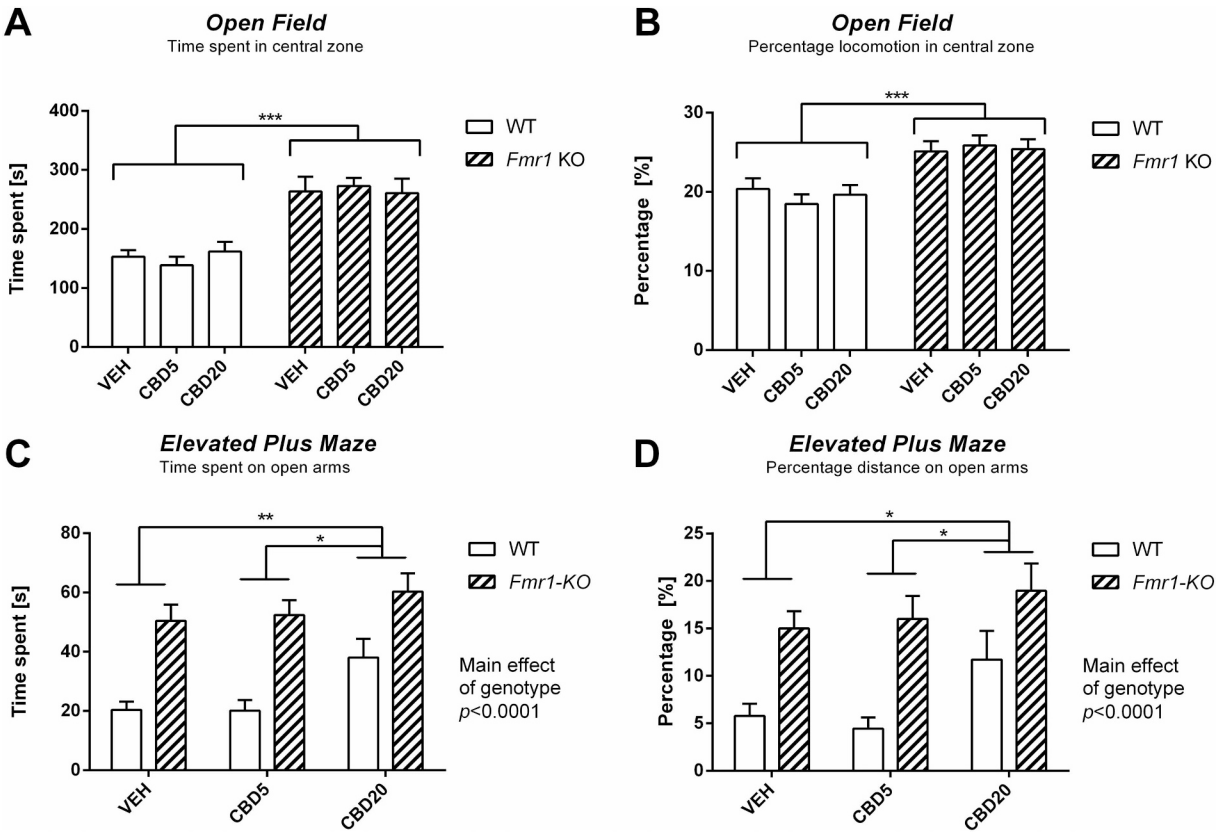

Fig. 2. A–D Anxiety-related behaviours in the open field test (OF) and the elevated plus maze (EPM): **A**) time spent in the central zone of the OF [s], **B**) ratio of total distance travelled in the central zone of the OF, **C)** time spent on open arms [s], and **D**) percentage distance travelled on open arms [%]. Data for control (WT) and *Fmr1* knockout mice (*Fmr1* KO) after acute treatment with vehicle (VEH), 5 mg/kg bodyweight of CBD (CBD5) or 20 mg/kg of CBD (CBD20) are shown as means + SEM. There were significant main effects of 'genotype' for OF centre time and centre distance ratio (both $p < .0001$). In the EPM, time spent as well as distance ratio on open arms was influenced by both 'genotype' (both $p < .0001$) and 'CBD' ($p < .05$ and $p = .06$, respectively). ***$p < .0005$.

Table 2
Y maze (YM), and social preference test (SPT) behaviours of wild type-like control (WT) and *Fmr1* knockout mice (*Fmr1* KO). Data are shown as means ± SEM for WT and *Fmr1* KO mice after acute treatment with vehicle (VEH), 5 mg/kg bodyweight of CBD (CBD5) or 20 mg/kg of CBD (CBD20). $N = 12$ per genotype and dose. For Y maze and SPT testing, paired sample t-test results against chance levels (i.e. 50%) are shown.

	YM Correct entries [%]	SPT Time in mouse chamber [%]	SPT Time with novel mouse [%]
WT VEH	55.7 ± 3.5	57.4 ± 3.5 $p = .06$	44.8 ± 4.3
Fmr1 VEH	55.5 ± 2.6 $p = .07$	56.7 ± 3.4 $p = .07$	49.8 ± 2.7
WT CBD5	54.8 ± 3.2	52.7 ± 3.7	45.3 ± 6.2
Fmr1 CBD5	53.7 ± 2.2	58.1 ± 2.2 $p = .004$	40.2 ± 4.4 $p = .05$
WT CBD20	57.0 ± 2.2 $p = .01$	49.4 ± 5.2	45.4 ± 5.2
Fmr1 CBD20	51.8 ± 3.4	53.6 ± 1.9 $p = .08$	47.0 ± 3.2

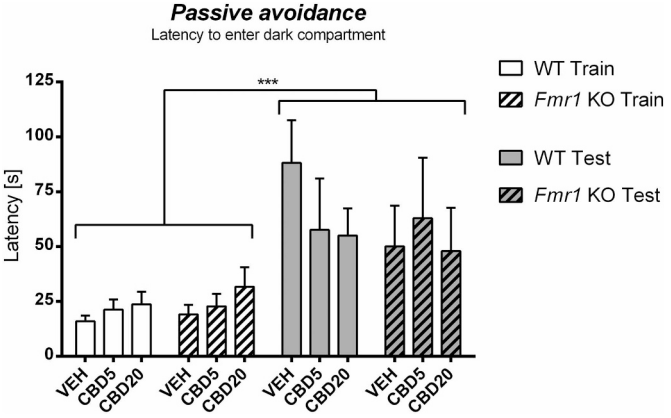

Fig. 3. Fear-associated memory in the passive avoidance test (PA): Latency [s] to enter a dark compartment on training day and again, 24 h later on test day. Data for control (WT) and *Fmr1* knockout mice (*Fmr1* KO) after acute treatment with vehicle (VEH), 5 mg/kg bodyweight of CBD (CBD5) or 20 mg/kg of CBD (CBD20) are shown as means + SEM. *** $p < .0005$.

$p > .05$) nor CBD treatment ('chamber' × 'CBD' interaction $p > .05$). However, detailed paired *t*-test analyses revealed that only *Fmr1* KO mice treated with 5 mg of CBD showed a strong trend for an aversion of the novel mouse (Table 2).

3.5. CBD concentrations

In both *Fmr1* KO and WT mice, CBD concentrations in the brain and plasma were highly correlated (*Fmr1* KO: $r = 0.985$, $p < .001$; WT: $r = 0.980$, $p < .001$). In brain, CBD levels did not differ between genotypes [$F(1,60) = 1.9$, $p > .05$], regardless of CBD dose (vehicle, 5 mg or 20 mg) or time (collection of tissue 30 min or 90 min post CBD administration) (interactions- all *p's* $> .05$, Table 3). Across genotypes, CBD levels differed significantly according to CBD dose [$F(2,60) = 105.2$, $p < .001$] and in brains collected 30 min post CBD administration compared to those collected 90 min post administration ['time': $F(2,60) = 17.0$, $p < .001$]. Across genotypes there was also a

Table 3
Concentration of CBD [ng/ml] in blood plasma and brain of WT and *Fmr1* KO mice, 30 min and 90 min after an acute injection with vehicle, 5 mg of CBD or 20 mg of CBD. $N = 6$ per timepoint, for each dose and genotype. Data are shown as means ± SEM. WT = wild type-like, KO = knockout, CBD = cannabidiol, n.d. = not detectable.

	Brain (30 min)	Brain (90 min)	Plasma (30 min)	Plasma (90 min)
WT VEH	n.d.	n.d.	n.d.	n.d.
Fmr1 VEH	n.d.	n.d.	n.d.	n.d.
WT CBD5	229.3 ± 56.3	95.7 ± 24.6	123.3 ± 22.9	75.5 ± 18.2
Fmr1 CBD5	201.8 ± 47.5	198.2 ± 41.6	114.4 ± 26.3	100.7 ± 16.5
WT CBD20	1580.0 ± 79.4	820.8 ± 122.3	1000.3 ± 64.6	550.5 ± 101.6
Fmr1 CBD20	1209.0 ± 305.2	588.2 ± 147.2	737.4 ± 179.7	301.9 ± 68.3

significant 'CBD' × 'time' interaction ($p < .001$), with CBD levels decreasing more acutely between 30 and 90 min for the 20 mg CBD dose than the 5 mg dose (Table 3).

In plasma, CBD levels did differ subtly between genotypes [$F(1,60) = 4.7$, $p = .03$]. Interestingly, this effect was driven by differences at 20 mg CBD in brains collected 90 min post administration (three-way 'genotype' × 'CBD' × 'time' interaction, $p < .005$) where CBD levels were lower in *Fmr1* KO mice than WT mice (Table 3). As in brain, CBD levels differed significantly according to CBD dose [$F(2,60) = 109.5$, $p < .001$] and time of blood collection [$F(2,60) = 18.0$, $p < .001$] for both genotypes, with a significant 'CBD' × 'time' interaction ($p < .001$; Table 3). Collapsed across genotypes, a strong correlation between plasma and brain concentrations of CBD was observed for both 5 mg CBD ($r = 0.870$, $p < .001$) and 20 mg CBD ($r = 0.956$, $p < .001$).

4. Discussion

Here we present the effects of acute CBD treatment on an established genetic mouse model for FXS. The *Fmr1* KO mice in our study displayed a phenotype which was broadly consistent with the literature. Compared to WT mice, *Fmr1* KO mice were hyperactive, hyperexplorative and displayed fewer anxiety-like behaviours. This is consistent with the majority of studies using these tests (Ding et al., 2014; Oddi et al., 2015; Wrenn et al., 2015; Sinclair et al., 2017; Liu et al., 2011). As in this study, other studies have failed to observe social behaviour deficits (Liu et al., 2011; Hebert et al., 2014) or spatial memory deficits (Peier et al., 2000; Leach et al., 2016). However it is important to note that the *Fmr1* KO behavioural phenotype is variable. There have been differing findings from those reported here for tasks which assess locomotor activity (Sinclair et al., 2017; Veeraragavan et al., 2011a), anxiety (Bilousova et al., 2009; Sorensen et al., 2015), social behaviour (Oddi et al., 2015; Sinclair et al., 2017; Hebert et al., 2014; Gantois et al., 2013) and spatial memory (Bakker et al., 1994; Oddi et al., 2015; D'Hooge et al., 1997; Sinclair et al., 2017; Bilousova et al., 2009). This includes work by a subset of the current authors in a different facility (Sinclair et al., 2017).

In this context, acute CBD had no impact on locomotion or anxiety-related parameters of the OF. However, in the EPM test, 20 mg/kg CBD (but not 5 mg/kg CBD) decreased the anxiety response of all mice tested. CBD treatment did not affect cognitive performance of animals in the spontaneous alternation task and the passive avoidance task.

To assess pharmacokinetics we administered CBD (5 or 20 mg/kg) to *Fmr1* KO and WT mice, then sacrificed them 30 or 90 min post-administration and measured CBD in brain and plasma of each animal. Although CBD levels in brain were equivalent in both genotypes at both timepoints, and in plasma were equivalent at 30 min post-administration, CBD levels were lower in plasma in *Fmr1* KO mice at 90 min post-administration. This suggests that *Fmr1* KO mice may clear CBD more rapidly than WT mice. However, there was no evidence from our behavioural tests that CBD had differential pharmacodynamic effects in *Fmr1* KO and WT mice (all genotype × CBD interactions were non-significant).

Male mice only were used for initial characterisation of effects of CBD, because males with FXS typicaly have greater severity of symptoms and male *Fmr1* KO mice have been more exhaustively characterised in the literature. However, use of homozygous female *Fmr1* KO mice in future experiments would be beneficial.

Individuals affected by FXS have been found to be hyperactive (Sullivan et al., 2006; Bagni et al., 2012). Similarly, *Fmr1* KO tested in our study exhibited a hyper-locomotive and hyper-explorative phenotype in the OF, consistent with previous studies (Ding et al., 2014; Oddi et al., 2015; Wrenn et al., 2015). This OF phenotype was evident in both the vehicle-treated as well as the CBD-treated cohorts. The lack of locomotor effects of CBD in the current study are consistent with previous data from our lab indicating that neither acute nor chronic CBD treatment (ranging from 1 to 50 mg/kg bodyweight) induces sedative-like effects in male C57BL/6J mice (Long et al., 2010) or reverses the hyperactive phenotype of an established genetic mouse model for schizophrenia (Long et al., 2012). However, it is important to note the discrepant findings in this research area, as CBD has been shown to be effective in ameliorating the 'psychotic-like' stimulating effect of acute amphetamine on locomotion (Long et al., 2010). Furthermore, some human studies have observed that high dose CBD can produce sedative-like effects [e.g. at 600 mg: (Zuardi, 2008)].

Analysis of anxiety-related open field and the elevated plus maze behaviours in *Fmr1* deficient mice revealed a pronounced and consistent (i.e. task-independent) decrease in anxiety-like phenotypes in the *Fmr1* KO mice. Previous studies using this *Fmr1* mouse model revealed varying anxiety phenotypes for *Fmr1* KO mice. While some find no genotype effect (Veeraragavan et al., 2011a; Veeraragavan et al., 2011b) others report fewer anxiety-related behaviours (Ding et al., 2014; Dolan et al., 2013; Uutela et al., 2012) in *Fmr1* knockout models as seen in our study. These inconsistencies across research studies appear to be independent of the genetic background of the mouse model in question [reviewed in (Bernardet and Crusio, 2006)] and could be related to the nature of the test protocol (e.g. nature of apparatus, time and duration of testing, level of illumination), housing conditions or other factors. We found that acute CBD (in particular at the dose of 20 mg/kg) decreased the anxiety-like responses of all mice in the EPM. The task-specific characteristics of the anxiolytic-like effect of CBD treatment have been found in other studies including humans (Bergamaschi et al., 2011) as well as mice [e.g. (Long et al., 2010)]. The effect of acute CBD treatment was similar for all mice and the genotype differences were not affected by the treatment, suggesting that FMRP is not required for anxiolytic-like effects of acute CBD. Pharmacological blockade of the cannabinoid receptor 2 (CB_2) (but not the CB_1 receptor) has been effective in normalizing the anxiety behaviour of *Fmr1*-deficient mice (Busquets-Garcia et al., 2013). However, since CBD has low affinity and/or weak indirect action at CB_1 and CB_2 receptors (McPartland et al., 2015) it is more likely that CBD decreases anxiety via another mechanism, such as binding to the 5-HT_{1A} receptor (Rock et al., 2017; Campos and Guimaraes, 2008; Gomes et al., 2011). Given that individuals with FXS experience increased anxiety (Bailey et al.,

2008; Cordeiro et al., 2011) but we (and others) find decreased anxiety in *Fmr1* KO mice, the therapeutic value of CBD's anti-anxiolytic effect in FXS requires clarification in future studies.

Most people with FXS are affected by mild to severe intellectual impairments (Bailey et al., 2008). We did not identify a deficit of fear-associated memory (i.e. passive avoidance) in *Fmr1* KO mice in this study- all mice displayed increased latencies to enter the dark chamber after receiving a foot shock. We also did not observe a difference between *Fmr1* KO mice and controls in spatial working memory (i.e. spontaneous alternation). This result should be interpreted with caution since only WT mice treated with 20 mg/kg CBD showed levels of alternation significantly above chance (50%). Similar to the anxiety phenotype, the literature provides an inconsistent picture of the cognitive phenotype of *Fmr1* KO mice (reviewed in (Kazdoba et al., 2014)) although knockout mice can show impaired spatial memory when tested in the passive avoidance (Bakker et al., 1994; Ding et al., 2014; Qin et al., 2015b; Veeraragavan et al., 2011a; Veeraragavan et al., 2011b), T maze (Oddi et al., 2015; Sinclair et al., 2017) and Y maze (Bilousova et al., 2009) paradigms. In our study, CBD had no impact on spatial working memory (spontaneous alternation) and fear-associated spatial memory (passive avoidance). Other studies in our laboratory have found beneficial effects of CBD in cognitive domains, as deficits in recognition memory of a transgenic mouse model for Alzheimer's disease were rescued and prevented after chronic CBD treatment (Cheng et al., 2014a; Cheng et al., 2014b). In line with the latter finding, two studies explored the effects of modulating the endocannabinoid system on passive avoidance behaviour in *Fmr1* KO mice. Blocking the CB_1 (but not the CB_2) receptor pharmacologically (acutely as well as chronically) using rimonabant ameliorated impairments in recognition memory (Busquets-Garcia et al., 2013). In another study, increasing the endocannabinoid tone using propofol or URB-597, both inhibitors of fatty acid amide hydrolase activity (FAAH: catabolic enzyme for endocannabinoids), post training resulted in an improved passive avoidance performance of *Fmr1* KO mice without any effect on control animals (Qin et al., 2015b). However, the experimental protocol of Qin et al. (2015a, b) was substantially different from our study, as they used habituation trials, two training days, and a post-training administration regime.

Although all mice (regardless of genotype and treatment) developed the preference to explore a mouse over an empty chamber, a significant preference for social novelty was absent in these mice. This lack of social novelty preference in control mice in the vehicle condition was unexpected, as previous studies suggest the test protocol is valid in other mouse models (Cheng et al., 2013). FXS patients are often diagnosed with social withdrawal or social phobias (Bailey et al., 2008), suggesting that *Fmr1*-deficient animals would demonstrate similar reductions in the social preference test. However, findings regarding the social behaviour of *Fmr1*-deficient mice are inconsistent across previous studies with some studies reporting intact preference for social novelty or even increased social interaction in free-running social tests whereas others report deficient social preference (reviewed in (Kazdoba et al., 2014)). Furthermore, studies have found that testing mice in the 3-chamber preference test can result in different results dependent on which parameters have been evaluated (Qin et al., 2015b). CBD did not substantially impact the social behaviours of test mice in this 2-test trial paradigm despite having been found to improve social preference and interaction deficits in mouse models for schizophrenia (Long et al., 2012) and Alzheimer's disease (Cheng et al., 2014a; Cheng et al., 2013). Interestingly, by post-hoc analysis *Fmr1* KO mice displayed significant preference for the familiar mouse when treated with 5 mg/kg CBD but not under other conditions. This may suggest that CBD may have benefit for increasing social affiliation and/or decreasing social anxiety in the context of repeated exposures but future research will have to clarify this in more detail.

The current study investigated the effects of acute CBD across multiple behavioural domains in *Fmr1* KO mice as a preclinical model for therapeutic discovery. Data indicate that *Fmr1*-deficient male mice displayed behaviours consistent with increased activity, reduced anxiety, and preserved social and cognitive performance. CBD administration resulted in a further reduction in anxiety-like behaviour in both *Fmr1*-deficient and WT mice, without concomitant effects on locomotor activity, social or cognitive performance. Data suggest that CBD may have anxiolytic effects, which are not dependent on *Fmr1*, and thus may be considered for use in individuals with FXS. Future studies could evaluate the chronic effects of CBD on FXS-related mouse phenotypes.

Acknowledgements and conflict of interest disclosure

This work was funded by Zynerba Pharmaceuticals, USA. TK was supported by a Career Development Fellowship (Level 2) from the National Health and Medical Research Council (NHMRC: #1045643), and currently receives funding from two NHMRC project grants (#1102012 and #1141789), and the NHMRC dementia research team initiative (#1095215). JZ is supported by an A.M. Wood Scholarship from the Schizophrenia Research Institute. DS was supported by a NHMRC CJ Martin Fellowship (#1072878).

SJS is a consultant to, and receives grant support from, Zynerba Pharmaceuticals Inc., Astellas Pharma Inc. and Bohringer Ingleheim. TS, MB-M and DG are full-time employees of Zynerba Pharmaceuticals Inc. All other authors declare no competing financial interests. We would like to thank Jerry Tanda for critical comments on the manuscript and Adam Bryans for taking care of our test mice at NeuRA.

References

Almeida, V., Levin, R., Peres, F.F., Niigaki, S.T., Calzavara, M.B., Zuardi, A.W., et al., 2013. Cannabidiol exhibits anxiolytic but not antipsychotic property evaluated in the social interaction test. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 41, 30–35.

Ashley Jr., C.T., Wilkinson, K.D., Reines, D., Warren, S.T., 1993. FMR1 protein: conserved RNP family domains and selective RNA binding. Science 262 (5133), 563–566.

Avraham, Y., Grigoriadis, N., Poutahidis, T., Vorobiev, L., Magen, I., Ilan, Y., et al., 2011. Cannabidiol improves brain and liver function in a fulminant hepatic failure-induced model of hepatic encephalopathy in mice. Br. J. Pharmacol. 162 (7), 1650–1658.

Bagni, C., Tassone, F., Neri, G., Hagerman, R., 2012. Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. J. Clin. Invest. 122 (12), 4314–4322.

Bailey, D.B., Raspa, M., Olmsted, M., Holiday, D.B., 2008. Co-occurring conditions associated with FMR1 gene variations: findings from a national parent survey. Am. J. Med. Genet. A 146, 2060–2069.

Bakker, C.E., Verheij, C., Willemsen, R., Vanderhelm, R., Oerlemans, F., Vermey, M., et al., 1994. Fmr1 knockout mice: a model to study fragile X mental retardation. Cell 78 (1), 23–33.

Bergamaschi, M.M., Queiroz, R.H., Chagas, M.H., de Oliveira, D.C., De Martinis, B.S., Kapczinski, F., et al., 2011. Cannabidiol reduces the anxiety induced by simulated public speaking in treatment-naive social phobia patients. Neuropsychopharmacology 36 (6), 1219–1226.

Bernardet, M., Crusio, W.E., 2006. Fmr1 KO mice as a possible model of autistic features. Sci. World J. 6.

Bilousova, T.V., Dansie, L., Ngo, M., Aye, J., Charles, J.R., Ethell, D.W., et al., 2009. Minocycline promotes dendritic spine maturation and improves behavioural performance in the fragile X mouse model. J. Med. Genet. 46 (2), 94–102.

Bisogno, T., Hanus, L., De Petrocellis, L., Tchilibon, S., Ponde, D.E., Brandi, I., et al., 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br. J. Pharmacol. 134 (4), 845–852.

Bovet, D., Bovet-Nitti, F., Oliverio, A., 1969. Genetic aspects of learning and memory in mice. Science 163 (3863), 139–149.

Busquets-Garcia, A., Puighermanal, E., Pastor, A., de la Torre, R., Maldonado, R., Ozaita, A., 2011. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol. Psychiatry 70 (5), 479–486.

Busquets-Garcia, A., Gomis-Gonzalez, M., Guegan, T., Agustin-Pavon, C., Pastor, A., Mato, S., et al., 2013. Targeting the endocannabinoid system in the treatment of fragile X syndrome. Nat. Med. 19 (5), 603–607.

Campos, A.C., Guimaraes, F.S., 2008. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. Psychopharmacology 199 (2), 223–230.

Campos, A.C., Ortega, Z., Palazuelos, J., Fogaca, M.V., Aguiar, D.C., Diaz-Alonso, J., et al., 2013. The anxiolytic effect of cannabidiol on chronically stressed mice depends on hippocampal neurogenesis: involvement of the endocannabinoid system. Int. J. Neuropsychopharmacol. 16 (6), 1407–1419.

Cheng, D., Logge, W., Low, J.K., Garner, B., Karl, T., 2013. Novel behavioural characteristics of the APP(Swe)/PS1DeltaE9 transgenic mouse model of Alzheimer's disease. Behav. Brain Res. 245, 120–127.

Cheng, D., Low, J.K., Logge, W., Garner, B., Karl, T., 2014a. Chronic cannabidiol treatment improves social and object recognition in double transgenic APPswe/PS1E9 mice. Psychopharmacology 231 (15), 3009–3017.

Cheng, D., Low, J.K., Logge, W., Garner, B., Karl, T., 2014b. Novel behavioural characteristics of female APPSwe/PS1DeltaE9 double transgenic mice. Behav. Brain Res. 260, 111–118.
Cordeiro, L., Ballinger, E., Hagerman, R., Hessl, D., 2011. Clinical assessment of DSM-IV anxiety disorders in fragile X syndrome: prevalence and characterization. J. Neurodev. Disord. 3 (1), 57–67.
Darnell, J.C., Van Driesche, S.J., Zhang, C., Hung, K.Y., Mele, A., Fraser, C.E., et al., 2011. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell 146 (2), 247–261.
DeFries, J.C., Hegmann, J.P., Weir, M.W., 1966. Open-field behavior in mice: evidence for a major gene effect mediated by the visual system. Science 154 (756), 1577–1579.
Denenberg, V.H., 1969. Open-field behavior in the rat: what does it mean? Ann. N. Y. Acad. Sci. 159 (3), 852–859.
Devinsky, O., Cross, J.H., Laux, L., Marsh, E., Miller, I., Nabbout, R., et al., 2017. Trial of cannabidiol for drug-resistant seizures in the Dravet syndrome. N. Engl. J. Med. 376 (21), 2011–2020.
D'Hooge, R., Nagels, G., Franck, F., Bakker, C.E., Reyniers, E., Storm, K., et al., 1997. Mildly impaired water maze performance in male Fmr1 knockout mice. Neuroscience 76 (2), 367–376.
Ding, Q., Sethna, F., Wang, H., 2014. Behavioral analysis of male and female Fmr1 knockout mice on C57BL/6 background. Behav. Brain Res. 271, 72–78.
Dolan, B.M., Duron, S.G., Campbell, D.A., Vollrath, B., Shankaranarayana Rao, B.S., Ko, H.Y., et al., 2013. Rescue of fragile X syndrome phenotypes in Fmr1 KO mice by the small-molecule PAK inhibitor FRAX486. Proc. Natl. Acad. Sci. U. S. A. 110 (14), 5671–5676.
Duffy, L., Cappas, E., Lai, D., Boucher, A.A., Karl, T., 2010. Cognition in transmembrane domain neuregulin 1 mutant mice. Neuroscience 170 (3), 800–807.
Frankland, P.W., Wang, Y., Rosner, B., Shimizu, T., Balleine, B.W., Dykens, E.M., et al., 2004. Sensorimotor gating abnormalities in young males with fragile X syndrome and Fmr1-knockout mice. Mol. Psychiatry 9 (4), 417–425.
Gantois, I., Pop, A.S., de Esch, C.E., Buijsen, R.A., Pooters, T., Gomez-Mancilla, B., et al., 2013. Chronic administration of AFQ056/Mavoglurant restores social behaviour in Fmr1 knockout mice. Behav. Brain Res. 239, 72–79.
Gomes, F.V., Resstel, L.B., Guimaraes, F.S., 2011. The anxiolytic-like effects of cannabidiol injected into the bed nucleus of the stria terminalis are mediated by 5-HT1A receptors. Psychopharmacology 213 (2–3), 465–473.
Gross, C., Hoffmann, A., Bassell, G.J., Berry-Kravis, E.M., 2015. Therapeutic strategies in fragile X syndrome: from bench to bedside and Back. Neurotherapeutics 12 (3), 584–608.
Grotenhermen, F., 2004. Pharmacology of cannabinoids. Neuro Endocrinol. Lett. 25 (1–2), 14–23.
Hagerman, R.J., Polussa, J., 2015. Treatment of the psychiatric problems associated with fragile X syndrome. Curr. Opin. Psychiatry 28 (2), 107–112.
Hampson, A.J., Grimaldi, M., Axelrod, J., Wink, D., 1998. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. Proc. Natl. Acad. Sci. U. S. A. 95 (14), 8268–8273.
Hebert, B., Pietropaolo, S., Meme, S., Laudier, B., Laugeray, A., Doisne, N., et al., 2014. Rescue of fragile X syndrome phenotypes in Fmr1 KO mice by a BKCa channel opener molecule. Orphanet J. Rare Dis. 9, 124.
Hughes, R.N., 2004. The value of spontaneous alternation behavior (SAB) as a test of retention in pharmacological investigations of memory. Neurosci. Biobehav. Rev. 28 (5), 497–505.
Jones, N.A., Glyn, S.E., Akiyama, S., Hill, T.D., Hill, A.J., Weston, S.E., et al., 2012. Cannabidiol exerts anti-convulsant effects in animal models of temporal lobe and partial seizures. Seizure 21 (5), 344–352.
Jung, K.M., Sepers, M., Henstridge, C.M., Lassalle, O., Neuhofer, D., Martin, H., et al., 2012. Uncoupling of the endocannabinoid signalling complex in a mouse model of fragile X syndrome. Nat. Commun. 3, 1080.
Karl, T., Duffy, L., Scimone, A., Harvey, R.P., Schofield, P.R., 2007. Altered motor activity, exploration and anxiety in heterozygous neuregulin 1 mutant mice: implications for understanding schizophrenia. Genes Brain Behav. 6 (7), 677–687.
Karl, T., Duffy, L., Herzog, H., 2008. Behavioural profile of a new mouse model for NPY deficiency. Eur. J. Neurosci. 28 (1), 173–180.
Kazdoba, T.M., Leach, P.T., Silverman, J.L., Crawley, J.N., 2014. Modeling fragile X syndrome in the Fmr1 knockout mouse. Intractable Rare Dis. Res. 3 (4), 118–133.
Kogan, C.S., Bertone, A., Cornish, K., Boutet, I., Der Kaloustian, V.M., Andermann, E., et al., 2004. Integrative cortical dysfunction and pervasive motion perception deficit in fragile X syndrome. Neurology 63 (9), 1634–1639.
Laplante, M., Sabatini, D.M., 2012. mTOR signaling in growth control and disease. Cell 149 (2), 274–293.
Leach, P.T., Hayes, J., Pride, M., Silverman, J.L., Crawley, J.N., 2016. Normal performance of Fmr1 mice on a touchscreen delayed nonmatching to position working memory task. eNeuro 3 (1).
Leweke, F.M., Piomelli, D., Pahlisch, F., Muhl, D., Gerth, C.W., Hoyer, C., et al., 2012. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. Transl. Psychiatry 2, e94.
Liu, Z.H., Chuang, D.M., Smith, C.B., 2011. Lithium ameliorates phenotypic deficits in a mouse model of fragile X syndrome. Int. J. Neuropsychopharmacol. 14 (5), 618–630.
Long, L.E., Chesworth, R., Huang, X.F., McGregor, I.S., Arnold, J.C., Karl, T., 2010. A behavioural comparison of acute and chronic Delta9-tetrahydrocannabinol and cannabidiol in C57BL/6JArc mice. Int. J. Neuropsychopharmacol. 13 (7), 861–876.
Long, L.E., Chesworth, R., Huang, X.F., Wong, A., Spiro, A., McGregor, I.S., et al., 2012. Distinct neurobehavioural effects of cannabidiol in transmembrane domain neuregulin 1 mutant mice. PLoS One 7 (4), e34129.
Marschik, P.B., Bartl-Pokorny, K.D., Sigafoos, J., Urlesberger, L., Pokorny, F., Didden, R., et al., 2014. Development of socio-communicative skills in 9- to 12-month-old individuals with fragile X syndrome. Res. Dev. Disabil. 35 (3), 597–602.
Martin-Moreno, A.M., Reigada, D., Ramirez, B.G., Mechoulam, R., Innamorato, N., Cuadrado, A., et al., 2011. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. Mol. Pharmacol. 79 (6), 964–973.
McPartland, J.M., Duncan, M., Di Marzo, V., Pertwee, R.G., 2015. Are cannabidiol and Delta(9)-tetrahydrocannabivarin negative modulators of the endocannabinoid system? A systematic review. Br. J. Pharmacol. 172 (3), 737–753.
Montgomery, K.C., Monkman, J.A., 1955. The relation between fear and exploratory behavior. J. Comp. Physiol. Psychol. 48, 132–136.
Moy, S.S., Nadler, J.J., Perez, A., Barbaro, R.P., Johns, J.M., Magnuson, T.R., et al., 2004. Sociability and preference for social novelty in five inbred strains: an approach to assess autistic-like behavior in mice. Genes Brain Behav. 3 (5), 287–302.
Oakes, A., Thurman, A.J., McDuffie, A., Bullard, L.M., Hagerman, R.J., Abbeduto, L., 2016. Characterising repetitive behaviours in young boys with fragile X syndrome. J. Intellect. Disabil. Res. 60 (1), 54–67.
Oddi, D., Subashi, E., Middei, S., Bellocchio, L., Lemaire-Mayo, V., Guzman, M., et al., 2015. Early social enrichment rescues adult behavioral and brain abnormalities in a mouse model of fragile X syndrome. Neuropsychopharmacology 40 (5), 1113–1122.
Peier, A.M., McIlwain, K.L., Kenneson, A., Warren, S.T., Paylor, R., Nelson, D.L., 2000. (Over)correction of FMR1 deficiency with YAC transgenics: behavioral and physical features. Hum. Mol. Genet. 9 (8), 1145–1159.
Perneger, T.V., 1998. What's wrong with Bonferroni adjustments. BMJ 316 (7139), 1236–1238.
Puighermanal, E., Busquets-Garcia, A., Maldonado, R., Ozaita, A., 2012. Cellular and intracellular mechanisms involved in the cognitive impairment of cannabinoids. Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci. 367 (1607), 3254–3263.
Qin, M., Huang, T., Kader, M., Krych, L., Xia, Z., Burlin, T., et al., 2015a. R-baclofen reverses a social behavior deficit and elevated protein synthesis in a mouse model of fragile X syndrome. Int. J. Neuropsychopharmacol. 18 (9).
Qin, M., Zeidler, Z., Moulton, K., Krych, L., Xia, Z., Smith, C.B., 2015b. Endocannabinoid-mediated improvement on a test of aversive memory in a mouse model of fragile X syndrome. Behav. Brain Res. 291, 164–171.
Rock, E.M., Limebeer, C.L., Petrie, G.N., Williams, L.A., Mechoulam, R., Parker, L.A., 2017. Effect of prior foot shock stress and Delta(9)-tetrahydrocannabinol, cannabidiolic acid, and cannabidiol on anxiety-like responding in the light-dark emergence test in rats. Psychopharmacology 234 (14), 2207–2217.
Rogers, S.J., Wehner, D.E., Hagerman, R., 2001. The behavioral phenotype in fragile X: symptoms of autism in very young children with fragile X syndrome, idiopathic autism, and other developmental disorders. J. Dev. Behav. Pediatr. 22 (6), 409–417.
Rothman, K.J., 1990. No adjustments are needed for multiple comparisons. Epidemiology 1 (1), 43–46.
Schiavon, A.P., Soares, L.M., Bonato, J.M., Milani, H., Guimaraes, F.S., Weffort de Oliveira, R.M., 2014. Protective effects of cannabidiol against hippocampal cell death and cognitive impairment induced by bilateral common carotid artery occlusion in mice. Neurotox. Res. 26 (4), 307–316.
Sharma, A., Hoeffer, C.A., Takayasu, Y., Miyawaki, T., McBride, S.M., Klann, E., et al., 2010. Dysregulation of mTOR signaling in fragile X syndrome. J. Neurosci. 30 (2), 694–702.
Sinclair, D., Featherstone, R., Naschek, M., Nam, J., Du, A., Wright, S., et al., 2017. GABA-B agonist baclofen normalizes neural oscillations and behavioral deficits in the Fmr1 knockout mouse model of Fragile X syndrome. eNeuro 4 (1), e0380.
Sorensen, E.M., Bertelsen, F., Weikop, P., Skovborg, M.M., Banke, T., Drasbek, K.R., et al., 2015. Hyperactivity and lack of social discrimination in the adolescent Fmr1 knockout mouse. Behav. Pharmacol. 26(8 Spec No), 733–740.
Sullivan, K., Hatton, D., Hammer, J., Sideris, J., Hooper, S., Ornstein, P., et al., 2006. ADHD symptoms in children with FXS. Am. J. Med. Genet. A 140 (21), 2275–2288.
Thurman, A.J., McDuffie, A., Hagerman, R., Abbeduto, L., 2014. Psychiatric symptoms in boys with fragile X syndrome: a comparison with nonsyndromic autism spectrum disorder. Res. Dev. Disabil. 35 (5), 1072–1086.
Turner, G., Webb, T., Wake, S., Robinson, H., 1996. Prevalence of fragile X syndrome. Am. J. Med. Genet. 64 (1), 196–197.
Uutela, M., Lindholm, J., Louhivuori, V., Wei, H., Louhivuori, L.M., Pertovaara, A., et al., 2012. Reduction of BDNF expression in Fmr1 knockout mice worsens cognitive deficits but improves hyperactivity and sensorimotor deficits. Genes Brain Behav. 11 (5), 513–523.
Van der Molen, M.J., Van der Molen, M.W., Ridderinkhof, K.R., Hamel, B.C., Curfs, L.M., Ramakers, G.J., 2012. Auditory Change Detection in Fragile X Syndrome Males: A Brain Potential Study. *Clin Neurophysiol*, Vol. 123. 2011 International Federation of Clinical Neurophysiology. Published by Elsevier Ireland Ltd, Netherlands, pp. 1309–1318.
Veeraragavan, S., Bui, N., Perkins, J.R., Yuva-Paylor, L.A., Carpenter, R.L., Paylor, R., 2011a. Modulation of behavioral phenotypes by a muscarinic M1 antagonist in a mouse model of fragile X syndrome. Psychopharmacology 217 (1), 143–151.
Veeraragavan, S., Bui, N., Perkins, J.R., Yuva-Paylor, L.A., Paylor, R., 2011b. The modulation of fragile X behaviors by the muscarinic M4 antagonist, tropicamide. Behav. Neurosci. 125 (5), 783–790.
Verkerk, A.J., Pieretti, M., Sutcliffe, J.S., Fu, Y.H., Kuhl, D.P., Pizzuti, A., et al., 1991. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. Cell 65 (5), 905–914.
Wang, W., Cox, B.M., Jia, Y., Le, A.A., Cox, C.D., Jung, K.M., et al., 2018. Treating a novel plasticity defect rescues episodic memory in Fragile X model mice. Mol. Psychiatry 23 (8), 1798–1806.
Wolf, S.A., Bick-Sander, A., Fabel, K., Leal-Galicia, P., Tauber, S., Ramirez-Rodriguez, G., et al., 2010. Cannabinoid receptor CB1 mediates baseline and activity-induced survival of new neurons in adult hippocampal neurogenesis. Cell Commun. Signal 8, 12.
Wrenn, C.C., Heitzer, A.M., Roth, A.K., Nawrocki, L., Valdovinos, M.G., 2015. Effects of clonidine and methylphenidate on motor activity in Fmr1 knockout mice. Neurosci. Lett. 585, 109–113.
Zuardi, A.W., 2008. Cannabidiol: from an inactive cannabinoid to a drug with wide spectrum of action. Rev. Bras. Psiquiatr. 30 (3), 271–280.