Molecular Psychiatry (2004) 9, 417–425

www.nature.com/mp

ORIGINAL RESEARCH ARTICLE

Sensorimotor gating abnormalities in young males with fragile X syndrome and *Fmr1*-knockout mice

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Fragile X syndrome (FXS) is the most common single gene (*FMR1*) disorder affecting cognitive and behavioral function in humans. This syndrome is characterized by a cluster of abnormalities including lower IQ, attention deficits, impairments in adaptive behavior and increased incidence of autism. Here, we show that young males with FXS have profound deficits in prepulse inhibition (PPI), a basic marker of sensorimotor gating that has been extensively studied in rodents. Importantly, the magnitude of the PPI impairments in the fragile X children predicted the severity of their IQ, attention, adaptive behavior and autistic phenotypes. Additionally, these measures were highly correlated with each other, suggesting that a shared mechanism underlies this complex phenotypic cluster. Studies in *Fmr1*-knockout mice also revealed sensorimotor gating and learning abnormalities. However, PPI and learning were enhanced rather than reduced in the mutants. Therefore, these data show that mutations of the *FMR1* gene impact equivalent processes in both humans and mice. However, since these phenotypic changes are opposite in direction, they also suggest that murine compensatory mechanisms following loss of *FMR1* function differ from those in humans.
Molecular Psychiatry (2004) **9,** 417–425. doi:10.1038/sj.mp.4001432
Published online 10 February 2004

Keywords: mouse model; FMRP; prepulse inhibition; startle

Introduction

Fragile X syndrome (FXS) is the most prevalent form of inherited mental retardation, affecting about 1 in 4000 males.[1] In most instances, FXS is caused by large expansions of a CGG trinucleotide repeat in the promoter region of the *fragile x mental retardation 1* (*FMR1)* gene.[2] The resulting absence, or reduced expression, of the fragile X mental retardation protein (FMRP) is responsible for a broad spectrum of physical, behavioral and cognitive abnormalities.[3–5] FMRP is an RNA-binding protein that regulates local protein synthesis required for synaptic maturation and plasticity.[6] Targeted deletion of the *Fmr1* gene results in the loss of FMRP in mice.[7] These *Fmr1*-knockout (*Fmr1*-KO) mice exhibit physical features of FXS, such as enlarged testes,[7–9] indicating that these mice provide a useful model of the syndrome. Here, we examine the impact of the loss of FMRP on cognitive and behavioral function in parallel studies in mice and humans.

In addition to learning impairments, one of the most common clinical features of FXS is heightened sensitivity to sensory stimulation (or sensory defensiveness).[2,10] Altered sensitivity to sensory stimulation might reflect underlying abnormalities in the maturation of synaptic connections in sensory circuits.[6] To quantify changes in sensory processing, we examined prepulse inhibition (PPI) of acoustic startle in young males with FXS. PPI is a widely used behavioral model of basic sensorimotor processing,[11] where a weak auditory prepulse attenuates subsequent responses to a loud startling noise.

Materials and methods

Human studies

Subjects Young males diagnosed with the full mutation (n=10) were recruited through the local chapters of the Fragile X Syndrome Association and the FRAXA Research Foundation. Control subjects (n=7), matched for chronological age, puberty status, ethnicity and family demographic variables (number of siblings, parental age, income and education), were recruited through advertising at UCLA. Two of the

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Received 07 May 2003; revised 21 August 2003; accepted 10 September 2003

FXS subjects were brothers. One additional FXS subject refused attachment of the recording electrodes, and did not participate in the study. FXS may be accompanied by hyperactivity, inattention, irritability, anxiety, sensory sensitivities, aggression and seizure disorders. These problems are particularly severe during childhood, and consequently many of the subjects (8/10) in the present study were taking multiple neuropsychiatric medications at the time of testing (see Table 2). Subjects had been taking these medications between 5 months and 9 years. It was considered clinically inadvisable to take them off their medication for the purposes of this study. None of the control subjects were on medication at the time of testing. Prior to the experimental session, all subjects were audiometrically screened for normal hearing.

Cognitive and behavioral assessment
Questionnaires (Child Behavioral Checklist, CBCL; Autism Screening Questionnaire, ASQ) and semi-structured interviews (Vineland Adaptive Behavior Scales, VABS) were administered to parents to assess cognitive and behavioral function. Adaptive behavior (communication, socialization and daily living skills) was characterized using the VABS.[12] Attention deficits were characterized using the attention domain of the CBCL.[13] The prevalence of behaviors associated with autism (reciprocal social interaction, language and communication, and repetitive and stereotyped patterns of behaviors) was measured using the ASQ.[14] This questionnaire was administered only to parents of FXS boys. In addition, the Kaufman Brief Intelligence Test (K-BIT[15]) was administered to all children to measure verbal and nonverbal intelligence.

Startle testing

Stimuli General procedures for examination of PPI in children have been previously described in detail.[16] Briefly, acoustic stimuli were presented binaurally through Sony circumaural earphones. Startle stimuli were 105 dB SPL (50 ms duration) white-noise bursts, with 0 ms rise/fall times. Acoustic prepulses were 75 dB SPL (25 ms duration) 1000 Hz tones, with 4 ms rise/fall times. On prepulse/startle trials, the prepulse preceded the startle stimulus by 120 ms (onset to onset). Ambient room noise was 30 dB SPL.

Recording Orbicularis oculi EMGs were recorded bipolarly from the right eye with gold cup electrodes (1.0 cm apart, edge to edge, as close to the margin of the lower lid as possible, and the lateral electrode 0.6 cm medial to the outer canthus). EMGs were AC amplified (Grass 15RX series Physiodata Amplifier System) at a fixed gain (10 000) with filters set at half-amplitude 30–1000 Hz. Vertical eye and lid movements were recorded bipolarly (with filters set at half-amplitude 0.01–30 Hz) with silver-silver chloride electrodes placed above and below one eye (EOG). All data were digitally recorded at 1000 Hz and stored from 15 s before to 10.5 s after startle stimulus onset.

Data processing On each trial, the rectified orbicularis oculi EMG and the vertical EOG were examined. Trials were rejected prior to data analysis if there was EMG or EOG activity (indicating orbicularis oculi contraction or lid movement) in the 20 ms period following presentation of the startle stimulus. FXS subjects had an average of 2.5 (range 0–8) rejected trials per subject; control subjects had an average of 1.3 (range 0–2) rejected trials per subject. For accepted trials, with response onset between 20 and 80 ms, peak amplitude of the rectified EMG was defined as the highest point within a window from response onset to 105 ms following startle stimulus onset, and was measured relative to a 200 ms prestartle stimulus onset baseline. When there was no measurable increase in EMG activity between 20 and 80 ms following the startle stimulus, the startle response was taken to be zero.

Procedures Electrodes were applied and the subjects were asked to sit quietly and watch a silent videotaped movie. Prior to the first block of trials, subjects were presented either with a single startle only trial, or a series of six adaptation trials (startle only trials increasing in intensity from 80 to 105 dB in 5 dB increments). Responses to these adaptation trials were not used in subsequent data analysis. Following this, subjects were presented with two types of trials: those where only the startle stimulus was presented and those where the lower intensity acoustic prepulse preceded the startle stimulus by 120 ms (prepulse/startle trials). The two trial types were presented in eight blocks of four trials each. One FXS subject completed only six blocks; all other subjects completed all eight blocks. Within each block, each type of trial occurred twice in a pseudorandom order. The minimum intertrial interval was 44–63 s. For each subject, EMG and EOG were continuously monitored throughout the test session. Trials were only initiated if (a) these physiological markers were stable during an 8 s pretrial period, and (b) there were no spontaneous blinks, eye movements or other periocular activity during the 200 ms preceding presentation of stimuli. Specific details of these test conditions and procedures have been described elsewhere.[16]

Data analysis Startle response magnitude was taken to be the peak EMG amplitude occurring within a 105 ms window following the onset of the startle stimulus. Using means of all usable trials, percent PPI was calculated for each subject according to the following formula: $\%PPI=[1-(response_{prepulse+startle\,stimulus}/response_{startle\,stimulus\,alone})]\times 100$. PPI differences were initially analyzed with one-way analysis of variance (ANOVA). Subsequent analysis of covariance (ANCOVA) was used to control for (nonsignificant) group differences in baseline startle and

chronological age. Pearson *r* correlations were calculated to examine the relation between PPI and other cognitive and behavioral measures in both control and FXS subjects. In addition, PPI was calculated using two additional estimates of response amplitude: the highest point of a 2 ms running average and log transformation of the peak EMG amplitude.[16] PPI calculations using these measures yielded equivalent results.

Mouse studies

Mice Male *Fmr1* (B6.129-*Fmr1*^{tm1Cgr}) mice were obtained from Jackson Laboratories (Bar Harbor, ME, USA). These mice had been backcrossed five generations into the C57B6/J (B6) background. We first bred these mice with normal B6 females, to obtain female mice heterozygous for the *Fmr1* mutation (*Fmr1*$^{+/-}$). Second, we crossed these *Fmr1*$^{+/-}$ mice with normal B6 males. Only the male offspring (hemizygous *Fmr1*-KO and male WT littermate controls) were used in experiments. Mice were group housed (2–4 mice per cage), and had continuous access to food and water. The colony was maintained on a 12 : 12 h light : dark cycle, and all testing was carried out during the light phase of the cycle. At the commencement of testing, mice were between 8 and 12 weeks of age. All animal care and testing procedures were approved by the Animal Research Committee at UCLA and were in accordance with the NIH Principles of Laboratory Animal Care.

Acoustic startle experiments

Equipment Startle testing was conducted in a MED-ASR-310 startle testing system (MedAssociates, VT, USA). Mice were placed in a Plexiglas cylinder (3.2 cm internal diameter) for testing. Acoustic startle stimuli and prepulse stimuli were delivered via a high-frequency speaker, placed at a distance of 15 cm from the testing cylinder. Background white noise was generated by a standard speaker. The testing cylinder was mounted on a sensor platform. A piezoelectric accelerometer, attached to the base of the sensor platform, detected and transduced all cage movements, and these were digitized and stored by a computer. The startle amplitude was taken to be the maximal response occurring up to 100 ms following presentation of the startle stimulus. The sound levels for background noise and startle/prepulse stimuli were calibrated with a digital sound level meter. The speakers, testing cylinder and sensor platform were housed within a sound-attenuated chamber.

Prepulse inhibition (experiment 1) Prepulse inhibition was initially tested using identical stimulus parameters to the human PPI studies. Following a 5-min acclimation period where no stimuli were delivered, WT (n=11) and *Fmr1*-KO (n=10) mice were presented with a series of six adaptation trials. These consisted of startle stimuli (50 ms duration white-noise bursts, with 0 ms rise/fall time) of increasing intensity (80–105 dB). Next, mice were presented with a series of 32 trials: on half of these trials, the startle stimulus only was presented (105 dB, 50 ms duration, white-noise burst, with 0 ms rise/fall time); on the remaining trials, a prepulse (75 dB, 25 ms duration, 1000 Hz tone, with a 4 ms rise/fall times) preceded the startle stimulus by 120 ms (onset to onset). Background noise levels were maintained at 50 dB throughout testing, and trials were spaced 60 s apart.

Prepulse inhibition (experiment 2) PPI was also examined in a second group of mice using an alternative set of procedures. WT (n=15) and *Fmr1*-KO (n=14) mice were initially given a habituation session to acclimate them to the testing environment. In this session, mice were presented with 80 startle stimuli, delivered at a fixed intertrial interval of 15 s. The startle stimulus was a 40 ms, 120 dB noise burst with a rise/fall time of less than 1 ms. Background noise levels were maintained at 65 dB.

One day following this, prepulse inhibition was tested. Following an acclimation period of 5 min, mice were presented with a total of 20 noise bursts (40 ms duration, 120 dB, $<$1 ms rise/fall time). In the prepulse inhibition phase, mice were presented with a total of 90 trials. Three prepulse intensities were tested: 70, 75 and 80 dB. Prepulses were 20 ms in duration with a rise/fall time of less than 1 ms. For each prepulse intensity, there were three types of trial: prepulse alone, prepulse/startle stimulus and startle stimulus alone. In the prepulse/startle stimulus trial, the onset of the prepulse preceded the onset of the startle stimulus by 100 ms. Background noise levels were maintained at 68 dB throughout testing, and the trials were spaced 15 s apart.

Approximately 1 week later, mice were given a startle threshold test session. Following an acclimation period of 5 min, mice were presented with a total of 99 trials at a fixed intertrial interval of 15 s. There were 11 trial types: no stimulus (NS), and 10 types of trials where startle stimuli at a range of intensities were presented (75–120 dB; 5 dB increments). The startle stimuli were 40 ms noise bursts with a rise/fall time of less than 1 ms. The 11 trial types were presented in a pseudorandom order such that each trial type was presented once within a block of 11 trials. Background noise levels were maintained at 65 dB throughout the test session. Startle threshold was defined as the minimal intensity at which responding was significantly greater than in the NS trials.

Instrumental conditioning

Training Apparatus and general methods have been previously described.[17]. WT (n=7) and *Fmr1*-KO (n=8) mice were first given two magazine training sessions in which food pellets were freely delivered on a random time 60-s schedule into a recessed

magazine with the levers retracted. Mice were then trained to press a lever positioned to the left of the food magazine with the pellet reward delivered such that a 20-s period had to time out before the next lever press was rewarded (ie a fixed time 20-s schedule). This training was continued until 100 rewards were earned. At no time was lever pressing explicitly shaped by the experimenter.

Devaluation After the initial acquisition phase (above), the same groups of mice were given two 30-min sessions of training on the lever with the reward delivered on a random five schedule. During this period, one WT and one *Fmr1*-KO mouse stopped lever pressing and were, therefore, not included in the devaluation study. Following this training, the WT (n=6) and *Fmr1*-KO (n=7) mice were given two brief, 10-min extinction tests. Prior to one test, they were allowed to eat the food pellets for 1 h prior to a test on the lever. Prior to the other test they were allowed to drink 10% sucrose for 1 h. The two tests were conducted in a counterbalanced order with three WT and four *Fmr1*-KO mice being tested after pellet prefeeding first and after sucrose prefeeding second whereas the remainder received the opposite test order.

Omission The procedures for omission training closely followed those reported previously.[18] After training in which responding on each of two levers, one each side of the central magazine, delivered a pellet reward, WT (n=9) and *Fmr1*-KO (n=11) mice were shifted to a situation in which pellets continued to be delivered by lever pressing, but, in addition, 0.01 ml of 10% sucrose was also delivered freely on a fixed time 20-s schedule. Responding on one lever, the omission lever, delayed the delivery of the sucrose by resetting the 20-s timer with each response, whereas responding on the other lever had no effect on sucrose delivery. For five WT and six *Fmr1*-KO mice the left lever was the omission lever, whereas for the remainder it was the right lever. Omission training was conducted in one, 30 min session each day for 4 days.

Data analysis Percent PPI was calculated for each mouse according to the following formula: $\%PPI=[1-(response_{prepulse+startle\ stimulus}/response_{startle\ stimulus\ alone})]\times 100$. ANOVAs were used to analyze phenotypic differences in PPI and instrumental learning. For the PPI studies, an ANCOVA was used to control for group differences in baseline responding. Where appropriate Newman–Keuls *post hoc* comparisons were used.

Results

Prepulse inhibition is disrupted in males with FXS

PPI was examined in young males (aged 8–17 years old) diagnosed with the full mutation. Control subjects were matched for chronological age, puberty status, ethnicity and family demographic variables (number of siblings, parental age, income and education). Stimuli were presented to subjects via headphones, and EMG responses were recorded from the lower eyelid (*orbicularis oculi* muscle). Two types of trials were presented: those in which only the startle stimulus (a 105 dB, 50 ms white noise burst) was presented (startle only trials) and those in which a lower intensity acoustic prepulse (a 75 dB, 25 ms, 1000 Hz tone) preceded the startle stimulus by 120 ms (prepulse/startle trials). Startle responding was substantially reduced in the prepulse/startle trials in control subjects ($43.8\pm10.0\%$ PPI). In contrast, there was almost no PPI in FXS subjects ($1.6\pm8.5\%$) ($F(1,15)=10.3$, $P<0.01$) (Figure 1a and b). Audiometric screening confirmed that FXS subjects could hear the prepulse at intensities much lower than the 75 dB used during testing. These data indicate that sensorimotor gating was almost completely abolished in FXS subjects.

Examination of the startle alone trials showed that there was a nonsignificant trend for increased baseline responding in FXS subjects ($F(1,15)=1.31$, $P=0.27$) (Figure 1c). In addition, FXS subjects were marginally (nonsignificantly) older than the control subjects (Table 1). To eliminate these possible confounds from our analysis, we conducted a second analysis including these factors as covariates (ANCOVA). Importantly, PPI deficits in FXS subjects remained significant after accounting for differences in baseline startle ($F(1,14)=8.6$, $P<0.05$) and chronological age ($F(1,14)=12.7$, $P<0.01$). It should be noted that while PPI appears to be abolished in FXS subjects, it is possible that the temporal characteristics of the PPI are altered in FXS subjects. Several FXS subjects exhibited prepulse facilitation, rather than inhibition. In normal subjects, facilitation occurs at shorter (<30 ms) and longer (>1400 ms) prepulse–startle stimulus intervals.[11] Future studies are required to evaluate this possibility.

Eight out of the 10 FXS subjects were on medications (or combinations of medications) at the time of testing (Table 2). Within each class of drug, PPI levels were distributed throughout the range of levels for our FXS sample (Figure 1d), suggesting that PPI deficits are independent of possible drug effects. While ethical considerations have limited the study of neuropsychiatric medications on PPI in humans, the data that are available suggest that drug effects are not responsible for the PPI deficits in our sample of FXS patients. Five FXS subjects were taking stimulants (or dopamine agonists). Previous studies have shown that stimulants have mixed effects, either reducing (dextroamphetamine,[19] bromocriptine[20]) or having no effect (amphetamine,[20] bromocriptine[21]) on PPI. Four FXS subjects were taking anxiolytics. Anxiolytics have been shown to have no effect (diazepam[22]) or to reduce PPI (midazolam[23]). The latter effect, however, was associated with sedation and severe reduction in baseline startle responding. Neither alpha-adrenergic drugs, such as clonidine,[22] or atypical antipsychotic

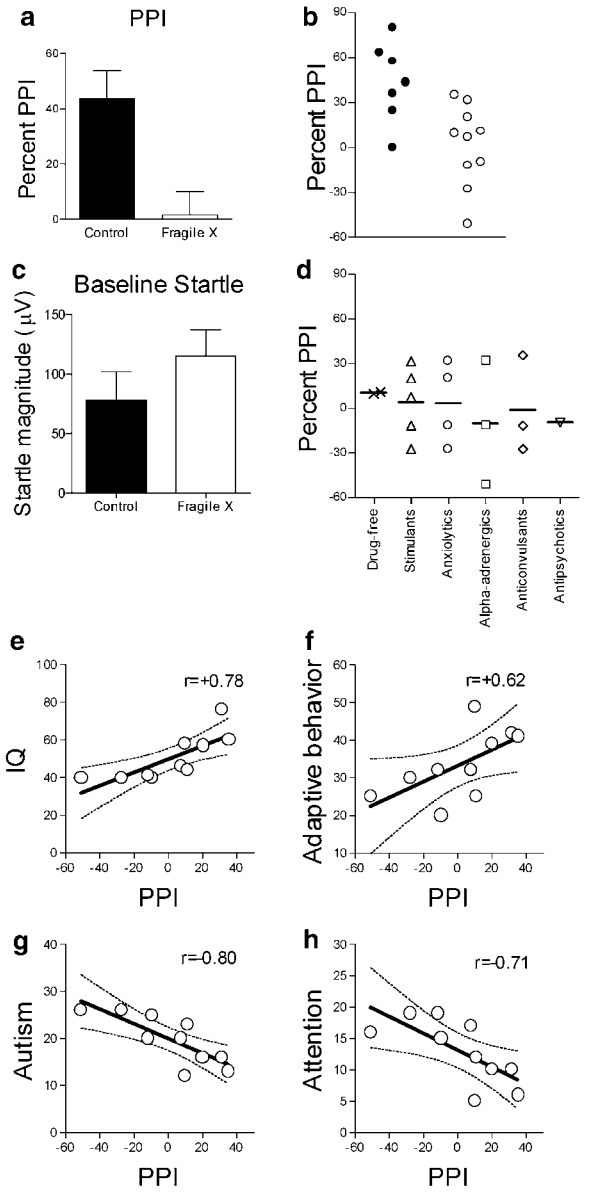

Figure 1 PPI is disrupted in young males with FXS. (a) Mean PPI is shown for control (closed bar; $n=7$) and FXS (open bar; $n=10$) subjects. PPI was significantly reduced in FXS subjects. (b) Scatterplot showing range of PPI scores in control (closed circles) and FXS (open circles) subjects. (c) Mean startle responding on startle only trials for control (closed bar) and FXS (open bar) subjects. There was a nonsignificant trend for higher baseline responding in FXS subjects. (d) Relationship between medication and PPI scores in FXS subjects. For each class of medication, PPI scores were evenly distributed throughout the range of PPI scores for FXS subjects. The horizontal lines correspond to the means for each drug class. (e) Scatterplot of IQ *vs* PPI magnitude for FXS subjects. Lower IQ scores were associated with reduced levels of PPI ($P<0.05$). (f) Scatterplot of adaptive behavior scores *vs* PPI magnitude for FXS subjects. Maladaptive behavior was associated with reduced PPI ($P<0.05$). (g) Scatterplot of autism scores *vs* PPI magnitude for FXS subjects. Subjects scoring higher on the autism scale were likelier to have reduced PPI levels ($P<0.05$). (h) Scatterplot of attention scores *vs* PPI magnitude for FXS subjects. Attention deficits were associated with reduced PPI ($P<0.05$). Data are means ($\pm$SEMs).

Table 1 Chronological age of control ($n=7$) and FXS ($n=10$) subjects, and mean scores for each of the four core cognitive and behavioral measures

	Control	*Fragile X*
Age (years)	11.4 ($\pm$1.2)	13.2 ($\pm$0.7)
IQ	119.9 ($\pm$3.0)	50.2 ($\pm$3.8)
Adaptive behavior	100.9 ($\pm$3.4)	33.5 ($\pm$2.9)
Autism	N/A	19.7 ($\pm$1.7)
Attention	2$\pm$ (1.2)	12.9 ($\pm$1.6)

IQ was measured using the Kaufman Brief Intelligence Test (K-BIT). Adaptive behavior was assessed using the Vineland Adaptive Behavior Scale (VABS). In this scale, lower scores reflect less adaptive behavior. Attention deficits were characterized using the attention domain of the Child Behavioral Checklist (CBCL). Higher scores reflect a higher incidence of attention problems. The prevalence of autistic-like behaviors was measured using the Autism Screening Questionnaire (ASQ). This questionnaire was only administered to parents in FXS subjects. Four (out of 10) of the FXS subjects had scores exceeding the diagnostic cutoff for autism of 22. Data are means ($\pm$SEMs).

drugs, such as risperidone[24] and clozapine,[25] affect PPI in normal adults. Finally, anticonvulants, including carbamazine, have been found to increase, rather than reduce, PPI in normal subjects.[26] At very least, these data suggest it is unlikely that medications can account for the PPI deficits in the FXS patients in this study.

Deficits in PPI predict severity of cognitive and behavioral pathology in FXS

FXS is associated with mental impairment (ranging from learning disabilities to mental retardation), anxiety and emotional problems, autistic-like behaviors, attention deficits and hyperactivity.[2] To characterize the range of cognitive and behavioral deficits in our population of young males with FXS, we measured IQ (K-BIT test[15]), and administered structured interviews and questionnaires to primary caregivers. These were used to evaluate the severity of deficits in attention (Child Behavioral Checklist[13]) and adaptive behavior (Vineland Adaptive Behavior[12]), as well as the severity of the autistic phenotype (Autism Screening Questionnaire[14]). Compared to control children, young males with FXS had lower IQ, and deficits in attention and adaptive behavior. Furthermore, FXS subjects scored high on the autistic scale, with 40% exceeding the diagnostic cutoff for autism (Table 1). The severity of these cognitive and behavioral deficits is typical for young males with FXS,[2] indicating that our sample is representative of the FXS population as a whole.

PPI varied within our FXS population (-50.8 to $+35.6$% PPI) (Figure 1b). Therefore, we next asked whether PPI magnitude was related to the severity of the cognitive and behavioral symptoms. Remarkably, PPI magnitude was highly predictive of the severity of

Table 2 Summary of medications taken by FXS subjects at the time of testing

Subject	*Percent PPI*	*No medications*	*Stimulants*	*Anxiolytics*	*Alpha-adrenergics*	*Anticonvulsants*	*Antipsychotics*
1	35.6					Carbamazepine	
2	31.5		Dextroamphetamine	Venlafaxine	Guanfacine		
3	20.2		Adderall	Clonazepam			
4	11.1	None					
5	9.7	None					
6	7.5		Methylphenidate				
7	−9.6						Risperidone
8	−11.7		Adderall	Lorazepam	Clonidine	Divalproex	
9	−27.4		Adderall	Clonazepam		Lamotrigine	
10	−50.8				Guanfacine		

The number of medications per subject ranged from 0 to 4 and included stimulants, anxiolytics, alpha-adrenergics, anticonvulsants and antipsychotics. All medications are generic names except Adderall, which is the brand name for a mixture of several dextroamphetamine and amphetamine salts. Venflaxine is an antidepressant frequently used to treat anxiety as in the case of subject 2. Risperidone was used to treat severe irritability and aggressive behavior in subject 7.

the cognitive/behavioral phenotype in FXS subjects: each of the four core measures (IQ, attention, adaptive behavior and autism) was highly correlated with PPI (Figure 1e–h). This relationship was only true for FXS subjects. In control subjects, PPI magnitude (range 0.1 to +79.9%) was unrelated to IQ, attention and adaptive behavior scores (data not shown).

Fmr1-KO mice exhibit enhanced PPI

Because of the limitations of studying the implications of these sensorimotor gating deficits in FXS patients, we examined PPI in *Fmr1*-KO mice. PPI is especially suited for translational studies since equivalent procedures and stimuli can be used in human and animal subjects. Furthermore, the brain circuitry and neurochemistry underlying PPI have been extensively characterized in rodents,[27] and there is a large degree of homology between measures of PPI in rodents and humans.[28] In contrast to FXS subjects, *Fmr1*-KO mice showed enhanced PPI using identical stimulus parameters to those in the human studies ($F(1,19)=4.7$, $P<0.05$) (Figure 2a). To confirm the enhanced PPI of the mutant mice, we also tested PPI with three different prepulse intensities (70, 75 and 80 dB). Consistent with previous reports,[9,29] PPI was increased in the *Fmr1*-KO mice at each of these three prepulse intensities ($F(1,27)=40.8$, $P<0.001$) (Figure 2b). While the threshold for startle was similar in *Fmr1*-KO and WT mice, at higher intensities the startle response magnitude was reduced in *Fmr1*-KO mice (Stimulus Intensity × Genotype interaction: $F(9,243)=15.5$, $P<0.001$) (Figure 2c), as previously observed when the *Fmr1* mutation is maintained in a predominantly C57B6/J background.[9] Most importantly, an ANCOVA indicated that increased PPI in *Fmr1*-KO mice was not related to group differences in baseline startle ($F(1,26)=25.9$, $P<0.001$). Although moderated by genetic background, increased PPI and reduced baseline startle has been observed consistently in *Fmr1*-KO mice.[9,29] These results demonstrate that mutations of the *FMR1* gene have profound effects on sensorimotor gating in both humans and mice. However, whereas PPI is reduced (or even absent) in young males with FXS, it is increased in *Fmr1*-KO mice.

Learning is facilitated in Fmr1-KO mice in two tests of instrumental conditioning

In addition to sensorimotor gating problems, FXS is associated with learning impairments. However, previous studies have shown that learning may be normal,[8,30,31] mildly impaired,[7,30–33] or even improved[34] in *Fmr1*-KO mice. However, there are many different types of learning,[35] and the *Fmr1*-KO mutation may affect some forms more than others. To explore this possibility, we tested the *Fmr1*-KO mice in instrumental conditioning. This form of learning has not been extensively explored in these mice, and is especially suited for teasing apart components of complex behaviors.[36] Mice were initially trained to press a lever for food pellet reward. The rate of acquisition of lever pressing was similar in WT and *Fmr1*-KO mice ($F(1,13)<1$, $P>0.05$), indicating that basic motor and motivational processes necessary for instrumental learning are normal in these mice (Figure 2d).

In contrast, *Fmr1*-KO mice showed enhanced performance in outcome devaluation, which is commonly used to assess how well an animal learns the relationship between lever pressing (the action) and the specific food reward (or outcome). During acquisition (above), mice learned to press a lever for a food pellet reward. At the end of this training, both WT (48.7 ± 6.6 presses/min) and *Fmr1*-KO (51.3 ± 7.2 presses/min) mice responded at similar rates. On the test day, mice were first given free access to either food pellets or a sucrose solution until sated. Immediately following this, mice were placed back in the instrumental chamber and allowed to press the

lever. Lever pressing in this extinction session was not reinforced. Prefeeding mice food pellets, but not sucrose, resulted in reduced lever pressing during the extinction test session in both WT ($F(1,11)=15.0$, $P<0.01$) and *Fmr1*-KO ($F(1,11)=53.1$, $P<0.001$) mice. As reduced responding is specific to the outcome that has been devalued, this indicates that mice can encode the relationship between lever pressing and a specific food reward, and not just reward in general. However, *Fmr1*-KO mice were more sensitive to this devaluation procedure, reducing responding (after being prefed pellets) to a greater degree compared to WT littermate controls (Lever × Genotype interaction: $F(1,11)=7.4$, $P<0.05$) (Figure 2e). *Post hoc* comparisons confirmed that the difference in responding for valued *vs* devalued outcome was greater in *Fmr1*-KO than WT mice ($P<0.05$).

Fmr1-KO mice also showed enhanced performance in a complex omission procedure.[18] This task has two components: first, mice were trained to press two levers for food pellet reward. Both WT and *Fmr1*-KO mice showed similar rates of lever pressing at the end of acquisition (data not shown). After earning 100 food pellet rewards on both levers, a new contingency was introduced: although both levers continued to deliver food pellets, pressing on one of the two levers (the omission lever) delayed delivery of an additional (freely delivered) sucrose reward. Therefore, in order to gain access to the sucrose, mice must learn to withhold responses on the omission lever. Both WT ($F(1,18)=7.2$, $P<0.01$) and *Fmr1*-KO ($F(1,18)=19.6$, $P<0.001$) mice reduced responding on the omission lever. However, *Fmr1*-KO mice suppressed responding on the omission lever to a greater degree compared to their WT littermate controls (Lever × Genotype interaction: $F(1,18)=6.6$, $P<0.01$) (Figure 2f); that is, they demonstrated greater ability to selectively withhold pressing the lever that delayed access to the highly valued sucrose reward. *Post hoc* comparisons confirmed that the difference in responding on omission *vs* noncontingent levers was greater in *Fmr1*-KO than WT mice ($P<0.05$).

In both the devaluation and omission procedures, enhanced performance in the *Fmr1*-KO was characterized by more selective responding. Since general changes in motivation (eg, frustration) would be expected to have nonspecific effects on instrumental performance, this suggests that the *Fmr1*-KO were

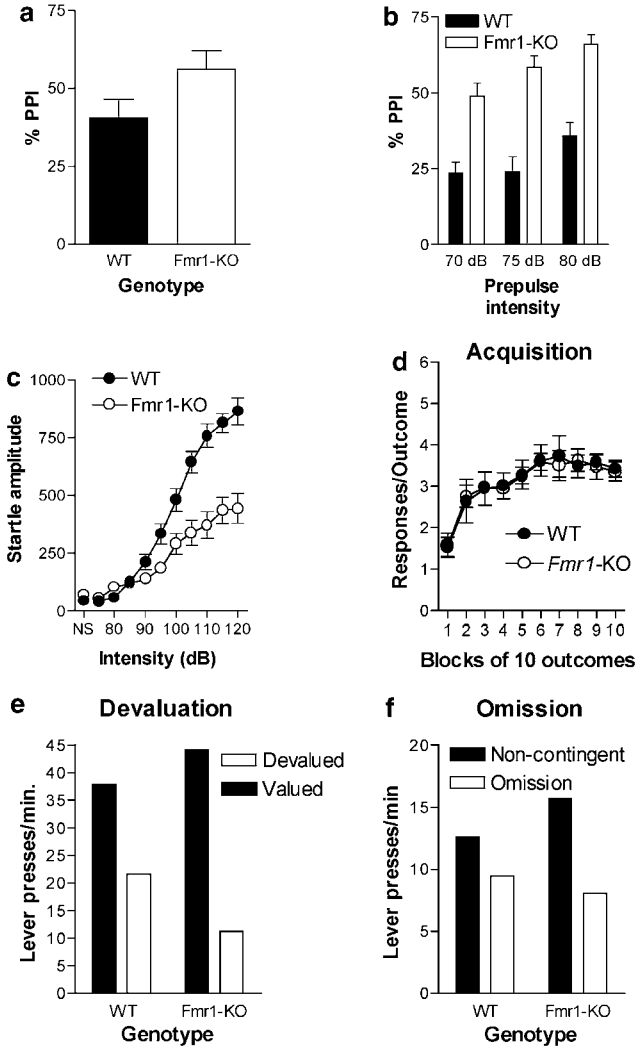

Figure 2 Summary of behavioral experiments in *Fmr*1-KO mice. (a) PPI was enhanced in *Fmr1*-KO ($n=10$) compared to WT ($n=11$) mice. In this experiment, identical stimulus parameters to the human studies were used. (b) In a second set of mice, PPI was examined using prepulses at three different intensities (70–80 dB). PPI was enhanced in *Fmr1*-KO mice (open bars; $n=14$) compared to WT controls (closed bars; $n=15$) at each of the prepulse intensities tested. (c) Acoustic startle threshold curve tested in the same WT (closed circles) and *Fmr1*-KO (open circles) mice. Mean response amplitudes (±SEM) are plotted for trials where no startle stimulus was presented (NS) and for trials where startle stimuli (75–120 dB) were presented. While the threshold for startle was similar in *Fmr1*-KO and WT mice, at higher intensities the magnitude of startle response was reduced in *Fmr1*-KO mice. (d) Acquisition of lever pressing for a food pellet reward is similar in WT (closed circles) and *Fmr1*-KO (open circles) mice. Performance is presented as rate of lever pressing per pellet reward. (e) In the outcome devaluation test, mice were either sated on sucrose (valued group; closed bars) or food pellets (devalued group; open bars). Immediately following this, they were tested on the lever in a nonreinforced extinction session. Both WT and *Fmr1*-KO mice reduced responding when pellets were devalued relative to the sucrose. However, *Fmr1*-KO mice were more sensitive to the devaluation treatment compared to WT controls. (f) In the omission test, responding on the omission lever (open bars), but not the other, noncontingent, lever (closed bars), delays the delivery of an additional sucrose reward. Both WT and *Fmr1*-KO mice reduced responding on the omission lever. However, *Fmr1*-KO mice suppressed responding on the omission lever to a greater degree compared to WT controls. Data are means (±SEMs).

able to learn action–outcome associations more efficiently than WT controls.

Discussion

Our PPI studies indicated that individuals with FXS have dramatic impairments in sensorimotor gating. The severity of these PPI deficits is greater than those typically seen in other disorders, including schizophrenia.[28] Most importantly, deficits in this simple and well-understood phenomenon were highly predictive of cognitive and behavioral pathology in FXS, suggesting that disturbances in sensorimotor gating may reflect a disturbance in a core mechanism that is responsible for normal cognitive and behavioral functioning. Therefore, exploration of these PPI deficits may be especially useful in understanding disease mechanism, and PPI may represent a straightforward and quantitative behavioral measure that can be used to track therapeutic progress of experimental treatments in the future.

This study is the first to examine the impact of a single gene mutation on PPI in humans and mice side by side. PPI is especially suited for these sorts of translational studies since identical stimuli and similar procedures may be used in humans and mice. However, whereas PPI was severely disrupted in individuals with FXS, it was enhanced in the *Fmr1*-KO mice. While these data support a role for FMRP in sensorimotor processing in both humans and mice, the lack of correspondence between mouse and human phenotypes has important implications for the use and interpretation of the *Fmr1*-KO mouse model. Indeed, whereas patients showed severe cognitive deficits, we found enhanced performance in two different learning tasks in *Fmr1*-KO mice. While there is relative sparing of some aspects of cognitive function in FXS, no skills are improved compared to control subjects.[2] Similarly, whereas anxiety problems are prevalent in FXS patients, *Fmr1*-KO mice exhibit reduced anxiety.[8,32] Higher cognitive function is influenced by complex interactions between multiple genes. Therefore, in mouse models of inherited cognitive disorders, even subtle differences in compensatory mechanisms between humans and mice may amplify, blunt or alter the disease phenotype.[37] For example, *FMR1* and the fragile X-related genes, *FXR1* and *FXR2*, encode a family of functionally similar RNA-binding proteins.[38] Although *FXR1* and *FXR2* expression levels are not altered in FXS patients or *Fmr1*-KO mice,[39,40] greater redundancy among these proteins in mice may attenuate the impact of the *FMR1* mutation. In this regard, it is of particular interest that PPI is disrupted in *Fxr2*-KO mice, and learning is more severely affected in these mice compared to *Fmr1*-KO mice.[41] Alternatively, in FXS FMRP can be normally expressed during early embryonic development (before hypermethylation leads to transcriptional silencing of *FMR1*), whereas in *Fmr1*-KO mice the protein is absent throughout development. Therefore, it is possible that transient exposure to FMRP during development may have a more devastating impact on later cognitive function.

Acknowledgements

These studies were supported by FRAXA Research Foundation grants to PWF and AJS, and to EMD and EMO, and by support from the Virginia Friedhofer Charitable Trust to EMO. We thank Sheena Josselyn and Anna Matynia for comments on earlier versions of this manuscript.

References

1 Turner G, Webb T, Wake S, Robinson H. Prevalence of fragile × syndrome. *Am J Med Genet* 1996; **64**: 196–197.
2 Verkerk AJ *et al*. Identification of a gene (FMR-1) containing a CGG repeat coincident with a breakpoint cluster region exhibiting length variation in fragile X syndrome. *Cell* 1991; **65**: 905–914.
3 Hagerman RJ, Hagerman PJ. *Fragile X Syndrome: Diagnosis, Treatmen, and Research*, Vol xii Johns Hopkins University Press: Baltimore, 2002 540pp.
4 Kooy RF, Willemsen R, Oostra BA. Fragile X syndrome at the turn of the century. *Mol Med Today* 2000; **6**: 193–198.
5 O'Donnell WT, Warren ST. A decade of molecular studies of fragile X syndrome. *Annu Rev Neurosci* 2002; **25**: 315–338.
6 Greenough WT *et al*. Synaptic regulation of protein synthesis and the fragile X protein. *Proc Natl Acad Sci (USA)* 2001; **98**: 7101–7106.
7 Bakker CE *et al*. Fmr1 knockout mice—a model to study fragile X mental retardation. *Cell* 1994; **78**: 23–33.
8 Peier AM *et al*. (Over)correction of FMR1 deficiency with YAC transgenics: behavioral and physical features. *Hum Mol Genet* 2000; **9**: 1145–1159.
9 Nielsen DM, Derber WJ, McClellan DA, Crnic LS. Alterations in the auditory startle response in Fmr1 targeted mutant mouse models of fragile X syndrome. *Brain Res* 2002; **927**: 8–17.
10 Miller LJ *et al*. Electrodermal responses to sensory stimuli in individuals with fragile X syndrome: a preliminary report. *Am J Med Genet* 1999; **83**: 268–279.
11 Geyer MA, McIlwain KL, Paylor R. Mouse genetic models for prepulse inhibition: an early review. *Mol Psychiatry* 2002; **7**: 1039–1053.
12 Sparrow SS, Balla D, Cicchetti DV. *Vineland Adaptive Behavior Scales*. American Guidance Service: Circle Pines, MN, 1984.
13 Achenbach TM. *Manual of the Child Behavior Checklist*. University of Vermont: Burlington, VT, 1991.
14 Berument SK, Rutter M, Lord C, Pickles A, Bailey A. Autism screening questionnaire: diagnostic validity. *Br J Psychiatry* 1999; **175**: 444–451.
15 Kaufman AS, Kaufman NL. *Kaufman Brief Intelligence Test*. American Guidance Service: Circle Pines, MN, 1990.
16 Ornitz EM *et al*. Prepulse inhibition of startle and the neurobiology of primary nocturnal enuresis. *Biol Psychiatry* 1999; **45**: 1455–1466.
17 Carvalho OM, Silva AJ, Balleine BW. Evidence of selective learning deficits on tests of Pavlovian and instrumental conditioning on alpha-CaMKII-T286 mutant mice. *Int J Comp Psychol* 2001; **14**: 161–174.
18 Dickinson A, Squire S, Varga Z, Smith JW. Omission learning after instrumental pretraining. *Quart J Exp Psychol Comp Physiol Psychol* 1998; **51B**: 271–286.
19 Hutchison KE, Swift R. Effect of D-amphetamine on prepulse inhibition of the startle reflex in humans. *Psychopharmacology (Berl)* 1999; **143**: 394–400.
20 Swerdlow NR *et al*. Dopamine agonist effects on startle and sensorimotor gating in normal male subjects: time course studies. *Psychopharmacology (Berl)* 2002; **161**: 189–201.

21 Abduljawad KA, Langley RW, Bradshaw CM, Szabadi E. Effects of bromocriptine and haloperidol on prepulse inhibition of the acoustic startle response in man. *J Psychopharmacol* 1998; **12**: 239–245.
22 Abduljawad KA, Langley RW, Bradshaw CM, Szabadi E. Effects of clonidine and diazepam on prepulse inhibition of the acoustic startle response and the N1/P2 auditory evoked potential in man. *J Psychopharmacol* 2001; **15**: 237–242.
23 Schachinger H, Muller BU, Strobel W, Langewitz W, Ritz R. Midazolam effects on prepulse inhibition of the acoustic blink reflex. *Br J Clin Pharmacol* 1999; **47**: 421–426.
24 Mackeprang T, Kristiansen KT, Glenthoj BY. Effects of antipsychotics on prepulse inhibition of the startle response in drug-naive schizophrenic patients. *Biol Psychiatry* 2002; **52**: 863–873.
25 Graham SJ, Langley RW, Bradshaw CM, Szabadi E. Effects of haloperidol and clozapine on prepulse inhibition of the acoustic startle response and the N1/P2 auditory evoked potential in man. *J Psychopharmacol* 2001; **15**: 243–250.
26 Pouretemad HR, Thompson PJ, Fenwick PB. Sensorimotor gating in patients with non-epileptic seizures. *Epilepsy Res* 1998; **31**: 1–12.
27 Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology* 2001; **156**: 117–154.
28 Braff DL, Geyer MA, Swerdlow NR. Human studies of prepulse inhibition of startle: normal subjects, patient groups, and pharmacological studies. *Psychopharmacology (Berl)* 2001; **156**: 234–258.
29 Chen L, Toth M. Fragile X mice develop sensory hyperreactivity to auditory stimuli. *Neuroscience* 2001; **103**: 1043–1050.
30 Van Dam D *et al*. Spatial learning, contextual fear conditioning and conditioned emotional response in Fmr1 knockout mice. *Behav Brain Res* 2000; **117**: 127–136.
31 Dobkin C *et al*. Fmr1 knockout mouse has a distinctive strain-specific learning impairment. *Neuroscience* 2000; **100**: 423–429.
32 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 (USA)* 2002; **99**: 15758–15763.
33 D'Hooge R *et al*. Mildly impaired water maze performance in male Fmr1 knockout mice. *Neuroscience* 1997; **76**: 367–376.
34 Fisch GS, Hao HK, Bakker C, Oostra BA. Learning and memory in the FMR1 knockout mouse. *Am J Med Genet* 1999; **84**: 277–282.
35 Squire LR, Knowlton B, Musen G. The structure and organization of memory. *Annu Rev Psychol* 1993; **44**: 453–495.
36 Balleine BW, Dickinson A. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. *Neuropharmacology* 1998; **37**: 407–419.
37 Watase K, Zoghbi HY. Modelling brain diseases in mice: the challenges of design and analysis. *Nat Rev Genet* 2003; **4**: 296–307.
38 Jin P, Warren ST. New insights into fragile X syndrome: from molecules to neurobehaviors. *Trends Biochem Sci* 2003; **28**: 152–158.
39 Feng Y *et al*. FMRP associates with polyribosomes as an mRNP, and the I304N mutation of severe fragile X syndrome abolishes this association. *Mol Cell* 1997; **1**: 109–118.
40 Tamanini F *et al*. Differential expression of FMR1, FXR1 and FXR2 proteins in human brain and testis. *Hum Mol Genet* 1997; **6**: 1315–1322.
41 Bontekoe CJ *et al*. Knockout mouse model for Fxr2: a model for mental retardation. *Hum Mol Genet* 2002; **11**: 487–498.