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A Mouse Model of Fragile X Syndrome Exhibits Heightened Arousal and/or Emotion Following Errors or Reversal of Contingencies

ABSTRACT: *This study was designed to further assess cognitive and affective functioning in a mouse model of Fragile X syndrome (FXS), the Fmr1^{tm1Cgr} or Fmr1 "knockout" (KO) mouse. Male KO mice and wild-type littermate controls were tested on learning set and reversal learning tasks. The KO mice were not impaired in associative learning, transfer of learning, or reversal learning, based on measures of learning rate. Analyses of videotapes of the reversal learning task revealed that both groups of mice exhibited higher levels of activity and wall-climbing during the initial sessions of the task than during the final sessions, a pattern also seen for trials following an error relative to those following a correct response. Notably, the increase in both behavioral measures seen early in the task was significantly more pronounced for the KO mice than for controls, as was the error-induced increase in activity level. This pattern of effects suggests that the KO mice reacted more strongly than controls to the reversal of contingencies and pronounced drop in reinforcement rate, and to errors in general. This pattern of effects is consistent with the heightened emotional reactivity frequently described for humans with FXS. © 2008 Wiley Periodicals, Inc.* Dev Psychobiol 50: 473–485, 2008.

Keywords: *Fragile X; autism; arousal; emotion; attention; learning set; reversal learning; fmr1 knockout mouse; mouse; fmr1 gene; transfer of learning; Fragile X mental retardation protein (FMRP)*

INTRODUCTION

Fragile X syndrome (FXS) is the most common inherited form of mental retardation and the most common known cause of autism (Hagerman, 2002; Mazzocco, Pennington, & Hagerman, 1993). Expansion of a CGG repeat sequence in the promoter region of the *FMR1* gene (Khandjian, 1999; O'Donnell & Warren, 2002) leads to transcriptional silencing of this gene (Verkerk et al., 1991; reviewed in O'Donnell and Warren, 2002). The resulting deficiency of the encoded protein, called the Fragile X mental retardation protein (FMRP), directly and/or indirectly gives rise to the FXS phenotype. The cognitive dysfunction is not global in nature but rather primarily affects various aspects of executive functioning, such as attention and inhibitory control (Baumgardner, Reiss, Freund, & Abrams, 1995; Cornish, Sudhalter, & Turk, 2004a; Cornish et al., 2004b; Hagerman, 1996; Lachiewicz, Spiridigliozzi, Gullion, Ransford, & Rao, 1994; Largo & Schinzel, 1985; Turk, 1998), with up to 73% of affected individuals meeting the diagnostic criteria for Attention Deficit Hyperactivity Disorder

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(Baumgardner et al., 1995). Other prominent features of FXS include hypersensitivity to sensory stimuli (Baranek & Berkson, 1994; Cohen et al., 1988; Hagerman, 1996; Miller et al., 1999), autistic features (Lachiewicz et al., 1994), heightened emotional reactivity (Borghgraef, Fryns, & van den Berghe, 1990; Hagerman & Sobesky, 1989; Kerby & Dawson, 1994), social anxiety (Cornish et al., 2004; Hagerman, 2002; Hagerman et al., 2002; Reiss & Freund, 1992), and seizure susceptibility (Musumeci, Ferri, Scuderi, Bosco, & Elia, 2001; Musumeci et al., 1999).

The current treatments for FXS focus on providing symptomatic relief, such as methylphenidate for the ADHD symptoms and SSRI's for anxiety; no treatments are clinically available that target the cascade of events leading from loss of FMRP to aberrant brain development. However, recent findings concerning the role of FMRP suggest that it may be possible to develop treatments to intervene in this process and thereby normalize brain development in individuals with FXS. For example, one conceptualization implicates excessive activity at group 1 metabotropic glutamate receptors (mGluRs) as the cause of many, if not all, of the FXS symptoms, including cognitive dysfunction, anxiety, and increased seizure susceptibility (Bear, 2005; Bear, Huber, & Warren, 2004). It follows therefore that treatment with mGluR antagonists, such as MPEP, may dramatically improve outcome in this syndrome. Support for this hypothesis has been provided by two studies, one concerning the *Fmr1* knockout (KO) mouse model of FXS (Yan, Rammal, Tranfaglia, & Bauchwitz, 2005), and one using a Drosophila model of the syndrome (McBride et al., 2005).

One stumbling block to further testing this theory and the potential clinical efficacy of such drugs is that cognitive dysfunction—which is central to the phenotype of FXS—has been very difficult to demonstrate in animal models. Commonly used learning and memory tasks, such as the Morris water maze and the radial arm maze, have either been unable to differentiate the *Fmr1* KO mice from controls (Dobkin et al., 2000; Paradee et al., 1999; Peier et al., 2000; Yan, Asafo-Adjei, Arnold, Brown, & Bauchwitz, 2004), or have revealed very small deficits in the KO mice that are apparent only in some background strains (Bakker et al., 1994; Cianchetti et al., 1991; Hinds et al., 1993; Mineur, Sluyter, de Wit, Oostra, & Crusio, 2002). Results seemingly contradictory with the phenotype of humans with FXS have also been reported. For example, in some learning tasks, *Fmr1* KO mice performed better than their WT littermates (Fisch, Hao, Bakker, & Oostra, 1999; Frankland et al., 2004; Van Dam et al., 2000).

One factor that may contribute to the apparent lack of cognitive dysfunction in the *Fmr1* KO mouse is that the most prominent areas of dysfunction in human FXS have not been assessed, notably including attention, inhibitory control, regulation of arousal or emotion, and resistance to change. The present study was designed to assess these functions in *Fmr1* KO mice. The performance of F1 hybrid *Fmr1* KO mice (a C57BL/6J × FVB/NJ cross) and wild-type (WT) littermate controls was compared on a series of visual attention tasks (described in Moon et al., 2006), an olfactory learning set task, and an olfactory reversal learning task; the latter two are described in the present report. The learning set task was included to tap transfer of learning, an area of dysfunction commonly seen in mental retardation (MR) syndromes (Campione & Brown, 1984; Campione, Brown, Ferrara, Jones, & Steinberg, 1985). Learning set tasks have previously revealed cognitive impairment in animal models of MR syndromes (Strupp, Bunsey, Levitsky, & Hamberger, 1994; Strupp, Himmelstein, Bunsey, Levitsky, & Kesler, 1990; Strupp & Levitsky, 1990), notably including disease models for which basic learning tasks had not revealed dysfunction (reviewed in Strupp & Diamond, 1996; Strupp & Levitsky, 1990). The reversal learning task was hypothesized to reveal dysfunction in the *Fmr1* KO mice for three converging reasons: First, reversal learning taps inhibitory control and adaptability to change, capabilities that are impaired in humans with FXS (Kau, Reider, Payne, Meyer, & Freund, 2000; Rogers, Wehner, & Hagerman, 2001). Second, reversal learning is dependent on the integrity of the prefrontal cortex (Dias, Robbins, & Roberts, 1996; Remijnse, Nielen, Uylings, & Veltman, 2005; Smith, Taylor, Brammer, & Rubia, 2004), a brain region believed to be dysfunctional in FXS (Cornish et al., 2004; Guierreiro et al., 1998; Hagerman, 2002; Menon, Leroux, White, & Reiss, 2004; Tamm, Menon, Johnston, Hessl, & Reiss, 2002). Finally, due to the frustration engendered by the reversal of contingencies and initially high error rate, reversal learning tasks provide an index of emotion regulation, an area of dysfunction in FXS (Borghgraef et al., 1990; Hagerman & Sobesky, 1989; Kerby & Dawson, 1994). The reversal learning task was videotaped to provide richer information on putative genotypic differences in the regulation of arousal than provided by the automated performance measures alone.

MATERIALS AND METHODS

Subjects

Breeding of the mice was conducted at the University of Colorado Health Sciences Center (UCHSC), Denver, CO. Breeder pairs of C57BL/6J-*Fmr1*^{tm1Cgr} (B6.129-*Fmr1*^{tm1Cgr}) (*Fmr1* KO) and wild-type (WT) C57BL/6J mice were purchased from Jackson Laboratory (Bar Harbor, ME). In the KO mice, the

Fmr1 gene had been disrupted by targeting a transgene to exon 5 with homologous recombination (Bakker et al., 1994). Heterozygous breeder females were obtained by breeding C57BL/6J-^{tm1Cgr} mutant female mice with normal inbred C57BL/6J males for 12+ generations. These females were then bred with normal FVB/NJ males (Jackson Laboratory) to produce male KO and WT mice from the same litters. Male offspring (15 WT and 13 *Fmr1* KO) from 15 litters served as subjects in the present experiment. Genotyping was conducted as described in Nielsen, Derber, McClellan, and Crnic (2002).

The strategy of studying the *Fmr1* mutation on an F1 hybrid background was followed for several reasons. First, these mice have normal hearing (unlike C57BL/6J mice) and are not blind or susceptible to seizures (unlike FVB/NJ mice) because these deficits are recessive (Goelz et al., 1998; Johnson, Erway, Cook, Willott, & Zheng, 1997; Pittler & Baehr, 1991; Zheng, Johnson, & Erway, 1999). In addition, this procedure produces *Fmr1* KO and WT mice from the same litters, thereby equating the intrauterine and postnatal environments of the experimental and control groups. Finally, in light of the pronounced strain differences in startle, anxiety, and performance in various learning tasks, it is risky to draw conclusions about the effects of a given mutation from studies of inbred mice, as background strain effects may greatly accentuate or obscure gene effects (Paradee et al., 1999).

At 6–7 months of age, while still at the UCHSC, the mice were tested on a one-trial passive avoidance task, in which they received a single, mild (.2 mA) footshock. The WT and KO mice did not differ in performance (data not shown). The mice were housed in groups, 2–3 per cage, from weaning until the time they were transferred to Cornell.

At 7–8 months of age, the mice were transported to Cornell University for further behavioral testing. At Cornell, the mice were housed singly in polycarbonate cages, with food and water available ad libitum. The mice were housed individually due to previous observations that male mice of this strain, caged in pairs, are prone to fighting when they are reunited after being removed for testing (Crnic, L.S., personal communication). After acclimating to the new environment for several weeks, the mice were placed on a restricted feeding regimen in order to maintain motivation for food reward during the behavioral testing. The daily ration was gradually reduced and then maintained at a level that produced target weights at approximately 80–85% of their prerestriction weight. A target weight of 80–85% (as opposed to 85–90%, our usual target weight) was selected because the mice had been on ad libitum feeding throughout their lives, resulting in somewhat elevated levels of adiposity.

All procedures used in these experiments adhere to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees at UCHSC and Cornell University, both AAALAC accredited institutions.

Apparatus

The mice were tested individually in one of six automated Plexiglas chambers, each controlled by a PC and situated in an insulated, sound-attenuating exterior chamber. The testing chambers were adapted from the ''nine-hole'' operant chambers developed to assess attention in mice (Humby, Laird, Davies, & Wilkinson, 1999). The slightly curved rear wall contained five circular response ports, 1 cm in diameter, located 2 cm above the floor and 5 mm apart. Only three of the five ports were operative for the olfactory learning tasks described in the present report: the far right, far left, and center ports. For these tasks, scented air served as the discriminative cues and was projected from these three ports on each trial. The scented air was produced by passing filtered, compressed air through small bottles of liquid odorant, using solenoid airflow valves and airflow meters. The airflow rate was 1.0 L/min.

On the chamber wall opposite the five response ports was an alcove (15 mm wide, 2 cm above the floor) containing the dipper (ENV0302M, MED Associates, Inc., St. Albans, VT) which dispensed the liquid food reward (liquefied AIN-76A, a sweet, nutritionally complete diet; ''Shake and Pour'', BioServ, Inc., Frenchtown, NJ). Access to the dipper alcove was controlled by a thin metal door, which was activated by a motor located on the outside of the testing chamber.

The mice initiated each trial by making a nosepoke into the dipper alcove port. Then, following a 1 s ''turn-around'' time, the olfactory cues were simultaneously presented from the three response ports. A nosepoke into any of these three ports constituted a response (or choice). Nosepokes into the response ports and the dipper alcove were detected by infrared photodiodes, positioned inside each port, .5 cm from the opening.

Each chamber was fitted with an exhaust system, which transported the air from the chamber directly to the room exhaust ventilator system at a rate of four complete air changes per minute. All automated events (door opening, dipper movement, responses, etc.) within each chamber were timed, controlled, and recorded by custom programs written in QBASIC.

For videotaping, each chamber was equipped with a wide-angle infrared video camera and infrared LED light source attached to the ceiling directly over the center of each testing chamber. The camera allowed full view of the mouse at all times. Each camera was connected to a separate VHS VCR. An array of infrared LEDs, positioned outside the Plexiglas chamber but within viewing range of the camera, provided information about the various events during each trial (e.g., demarcation of the intertrial interval, presentation of the olfactory cue, whether a response was correct or incorrect, access to liquid reward).

Behavioral Testing

At 8 months of age, the mice were administered a four-stage training procedure designed to shape the general response sequence required for completion of each trial in the subsequent tasks. These training stages are described in a prior report (Driscoll et al., 2004). Briefly, the mice learned that the door to the dipper alcove would be raised at the start of each trial and that a nosepoke into the alcove port, followed by a nosepoke into one of the five response ports, would produce the delivery of .01 mL

of the liquid diet in the dipper alcove. These four training phases were mastered in approximately 8–10 daily sessions (in total). The mice were then tested on a series of visual discrimination and attention tasks, lasting approximately 6 months (described in Moon et al., 2006). Briefly, in all of these tasks, one of the five response ports was briefly illuminated on each trial and the mouse was rewarded for making a nosepoke into the illuminated port. These different tasks varied in terms of the duration of the visual cue illumination, the duration of the precue delay(s), and the presence or absence of olfactory distractors on some trials. In each of these tasks, as well as the tasks described in the present report, all mice received one daily test session, 6 days per week.

The tasks described in the present report were initiated when the mice were 20–22 months of age. For each of these three olfactory discrimination tasks, three different odors were presented on each trial, one from each of the three operative ports (middle, far left, and far right). The odor emitted from each port was randomly determined, but balanced for each test session.

The first task, a learning set task, comprised two 3-choice simultaneous olfactory discrimination tasks, administered sequentially, to assess basic associative ability and transfer of learning between versions of the same task (different exemplars). Two different sets of odors were used: (1) rum-pineapple-peach (set A); and (2) lime-vanilla-pear (set B). Half of the mice of each genotype were tested on set A first, half on set B first; then each mouse was tested on the alternate set. In addition, within each of the two sets, the correct odor was pseudo-randomly assigned but counterbalanced across genotypes and order of odor sets. Within set A, the possible correct odors were rum or pineapple; within set B, the possible correct odors were lime or vanilla. For each of the two olfactory discrimination tasks within the Learning Set Task the learning criterion was 80% correct for two of three consecutive test sessions.

Testing on the reversal learning task was initiated for each mouse in the test session immediately following mastery of the learning set task. For this task, the odor that had been correct in the second olfactory discrimination of the learning set task was now designated as incorrect, and one of the previously incorrect odors was now the correct odor (i.e., associated with reward). The learning criterion was the same as for the learning set task.

For all of these tasks, the mouse initiated each trial by making a nosepoke into the dipper alcove, after which the door closed. These nosepokes were required to initiate each trial but did not produce a reinforcement. After a 1-s ''turn around time'' (allowing the mouse to face the response ports), the three different olfactory cues were emitted simultaneously from the three response ports. The scented air was projected continuously for 5 s or until a nosepoke response was made. A nosepoke into the port emitting the correct odor was rewarded by 5 s access to the liquid reinforcer in the dipper alcove. An incorrect response was followed by a 5-s time-out period, signaled by the illumination of a 2-W houselight on the ceiling of the chamber. A time-out was also imposed following a ''nontrial,'' the term given to trials in which the alcove door was raised at trial onset but the mouse did not enter the alcove in the following 60 s; nontrials were very rare (a mean of less than 1%). A 5-s intertrial interval separated adjacent trials. All trials on which the mouse made an initiation poke into the dipper alcove (regardless of the outcome of the trial) were defined as response trials. Each daily test session was terminated after 30 min or 70 response trials, whichever came first.

All testing equipment was thoroughly cleaned and dried following the testing of each mouse, using Odormute (R.C. Steele Co, Brockport, NY), a detergent containing a blend of enzymes and salts designed to eliminate organic odors (including pheromones). The Odormute solution was freshly prepared twice per day to maintain potency.

Videotape Coding

All sessions of the olfactory reversal task were videotaped, and the first two sessions and the last two sessions on this task were coded for various behaviors, described below. The first two sessions, immediately after the change in contingencies, were considered most likely to reveal genotypic differences in regulation of arousal or affect. The final two sessions on the task, after task contingencies had been mastered and reinforcement rate was high, were also scored to ascertain whether group differences in the early sessions, if observed, were specific to arousing conditions, or were uniform throughout the task.

A coder scored each of these sessions for four behaviors: jumping, grooming, exploring, and wall-climbing. For the index of activity level, the chamber was divided into two areas: The half of the chamber containing the response ports was denoted the left area, and the other half of the chamber containing the dipper alcove was denoted the right area. Frequency, duration, and location (right vs. left side of the chamber) of each behavior during each trial was recorded, using a computer program developed for these tasks.

Reliability of the behavioral ratings was determined prior to proceeding with the coding of the reversal learning task. These reliability analyses were based on the coding of session 4 of the learning set task for eight pseudo-randomly selected mice; the eight sessions were balanced by testing chamber and genotype. To determine intrarater reliability, the coder scored each of the eight sessions twice (with time elapsed between recoding of the same session), and the results of the first round of coding were correlated with those of the second. To assess interrater reliability, the same eight sessions were coded by another trained coder, and the results from both individuals were compared. Coding of the reversal learning sessions commenced only after high levels of intra- and interrater reliability were achieved ($r > .9$) for all behavioral measures.

Behavioral testing and coding of the videotapes were conducted by individuals blind to the genotypes of the animals. The two genotypes could not be distinguished by any physical characteristic or motor function.

Statistical Analyses

Statistical analyses were conducted on a Cornell University mainframe computer using the Statistical Analysis System (SAS; SAS Institute, Inc., Carey, NC), version 9.1.

Learning rate measures for the learning set task and the reversal learning task were (1) sum of errors to criterion and

(2) sum of trials to criterion. These measures were analyzed using a mixed models analysis of variance procedure (SAS, PROC MIXED) to account for the repeated observations on each animal.

An additional analysis was conducted for the reversal learning performance data to permit direct comparison with the video coding data, which were available for only the first two sessions and the final two sessions. For each of these four sessions, mean percent correct responses was calculated for each animal for each testing condition, defined by the following variables: Genotype, Previous Trial Outcome (correct or incorrect), and Session number. The analysis was conducted on these means using a generalized linear mixed models procedure for conducting repeated measures analyses of variance with nonnormal data (PROC GLIMMIX in SAS).

PROC GLIMMIX was also used to analyze the video coding data, using the same variables as listed above with the exception that for some analyses (described in the Results Section), the four sessions were grouped into two or three blocks. These analyses, too, were conducted on means calculated for each animal for each session or session-block.

T-tests were used to compare body weight and daily food intake of the two genotypes. For each of these analyses, a mean was calculated for each animal for all testing sessions, and then the means of each group were compared.

RESULTS

Body Weight

The body weights of the groups did not differ ($t_{(27)} = 1.13$, n.s.). Means (SE) for the KO and WT mice were 33.62 (1.1) and 32.12 (.9) g, respectively.

Daily Food Intake

There was no effect of Genotype on mean daily food intake ($t_{(27)} = 1.03$, n.s.). Means (SE) for the KO and WT mice were 3.8 (.07) and 3.7 (.1) g, respectively.

Learning Set Task

Analysis of errors to criterion revealed a significant effect of task (task 1 vs. task 2; $F_{(3,38)} = 4.80$, $p = .006$). The mice learned the second task faster than they learned the first task, reflecting significant transfer of learning (see Fig. 1). There was no effect of the particular sets of olfactory cues or the order in which the two sets were administered. The analysis did not reveal a main effect of Genotype ($F_{(1,38)} = .42, p = .52$). The interaction of Genotype and Task was also not significant ($F_{(1,38)} = .00$, $p = .96$), indicating that the two groups did not differ in transfer of learning. The interaction of Genotype and Correct Olfactory Cue was also not significant ($F_{(3,38)} = .30$; $p = .82$) indicating that the two genotypes did not differ in preference for the different olfactory cues. Analysis of trials to criterion revealed the same results.

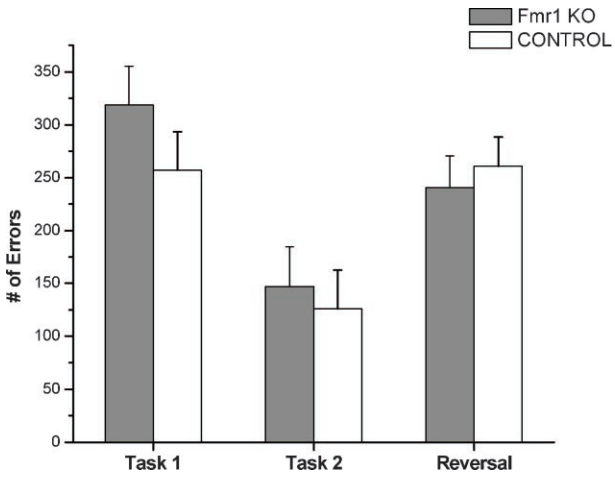

FIGURE 1 Mean (±SE) errors to criterion for the learning set task (tasks 1 and 2) and the reversal learning task. Learning rate did not differ by genotype for any of these tasks.

Reversal Learning Task

An analysis was conducted to compare learning rate on the reversal learning task with that of the second task of the learning set task (i.e., original learning of the odor triplet used in the reversal learning task). The analysis of errors to criterion revealed that the reversal learning task took significantly longer to learn ($F_{(1,38)} = 17.48$, $p = .0002$), indicating that the mice had difficulty changing their behavior after a reversal of the contingencies [means (SE) for the original learning and for the reversal learning were 137.83 (20.24) and 144.85 (20.24), respectively]. This analysis revealed neither a main effect of Genotype ($F_{(1,47)} = .04$, $p = .84$; see Fig. 1), nor an interaction between Genotype and Task ($F_{(1,38)} = .57$, $p = .45$). The analysis of trials to criterion revealed the same results.

For comparison with the videotape data, an additional analysis was conducted on performance (percent correct responses) during the first two sessions and last two sessions of the reversal learning task. This analysis included only the 19 mice for which videotape data were available (described below). This analysis revealed a significant effect of Session ($F_{(3,213)} = 155.11, p < .0001$; see Fig. 2), reflecting the improvement in performance across these four sessions. Again, there was no effect of Genotype ($F_{(1,19.2)} = .11$, $p = .74$), nor an interaction between Genotype and Session ($F_{(3,53.4)} = .23$, $p = .87$).

Coded Videotapes of the Reversal Learning Task

Due to technical difficulties, videotape data were available for only 19 animals (10 WT and 9 KO mice).

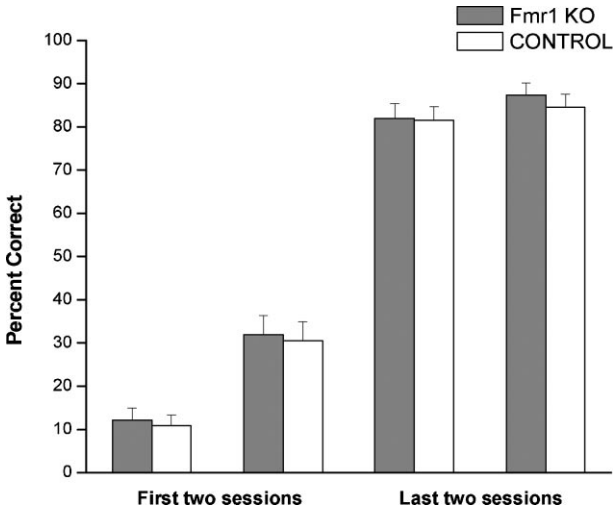

FIGURE 2 Mean (±SE) percent correct for the first two and last two sessions of the reversal learning task. No genotypic differences were seen for any session.

This subgroup of mice did not differ from the full cohort in terms of overall performance, age, body weight, or food intake.

A square root transformation was applied to the data for wall-climbing, jumping, exploring, and grooming to normalize the distributions. For the analyses of these four behaviors, sessions 1 and 2 were collapsed into ''Session-block 1,'' and the final two sessions were collapsed into ''Session-block 2'' because for these dependent measures, sessions 1 and 2 did not differ from each other and sessions 3 and 4 did not differ from each other. In contrast, for the analysis of activity level, only the final two sessions were collapsed, because sessions 1 and 2 differed significantly from each other ($p < .05$).

Activity Level

The dependent measure used to analyze activity level was [(total number of transitions)/(total number of trials)]. One ''transition'' was tallied each time that the mouse moved from the side of the chamber containing the five response ports to the side with the dipper alcove (or vice versa). A significant main effect of Previous Trial Outcome was found ($F_{(1,104)} = 34.01, p < .0001$), indicating that the mice were significantly more active on trials immediately following an error than on trials following a correct response [means (SE) for trials following an error and trials following a correct response were 3.6 (.19) and 2.9 (.19) transitions per trial, respectively]. Furthermore, there was a main effect of Session-block ($F_{(2,129)} = 8.17$, $p = .0005$), reflecting the fact that activity level was highest during the first session and declined thereafter. A significant interaction between Session-block and Previous Trial Outcome ($F_{(2,104)} = 8.34$, $p = .0004$) was also found, indicating that the increase in activity level on trials following an error (relative to trials following a correct response) was more pronounced during the first two sessions than during the final two sessions.

The influence of both Previous Trial Outcome and Session-block on activity level varied by Genotype. The significant interaction between Genotype and Previous Trial Outcome ($F_{(1,110)} = 8.37, p = .004$) reflected the fact that the increase in activity produced by a prior error was more pronounced for the KO mice than for the WT controls. Whereas the two genotypes did not differ in activity level on trials following a correct response ($p = .78$), the KO mice were significantly more active than their WT counterparts on trials following an error ($p = .04$; see Fig. 3). A marginally significant interaction of Session-block and Genotype was also found ($F_{(2,29.1)} = 2.97$, $p = .06$). The elevation in activity level seen early in the reversal learning task was more prolonged for the KO mice than for the WT controls, with group differences being significant during the second session ($p = .05$).

One additional analysis was conducted to exclude the possibility that the increase in activity level seen early in the reversal as well as on trials following an error reflected the fact that following a correct response, the mouse consumed the reinforcer which may have decreased the opportunity to engage in other behaviors. This additional analysis, which focused solely on trials following an error, revealed main effects of Genotype [$F_{(1,16.6)} = 5.02$,

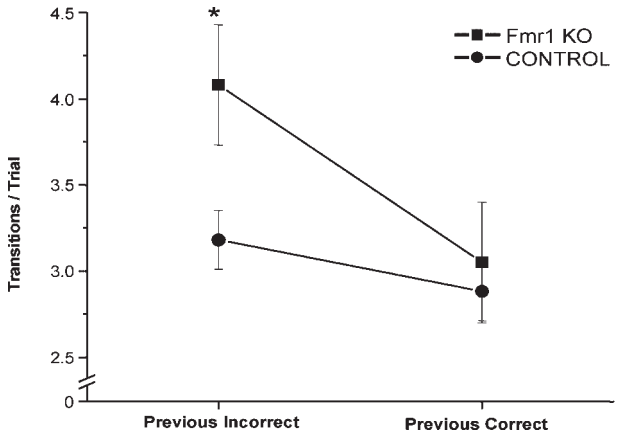

FIGURE 3 Mean (±SE) number of left-right transitions per trial for the reversal learning task as a function of the outcome of the previous trial (correct or incorrect). The significant interaction between Genotype and Previous Trial Outcome ($F_{(1,110)} = 8.37$, $p = .004$) reflected the fact that the increase in activity produced by a prior error was more pronounced for the KO mice than for the WT controls. The KO mice were significantly more active than their WT counterparts on trials following an error ($p = .04$).

$p = .03$] and Session-block [$F_{(2,48)} = 7.56$, $p = .001$], as well as a significant interaction of Genotype and Session-block [$F(2, 48) = 3.54$, $p = .03$]. As seen in Figure 4, for both groups, activity level was greater early in the reversal than during the final (criterial) sessions, even when considering solely the trials that followed an error, demonstrating that this effect in the prior analysis (including all trials) was not an artifact of a competing response on trials following a correct response. Moreover, the significant interaction of Genotype and Session-block confirmed the initial analysis that this effect (indicative of altered arousal and/or emotion) was more pronounced for the KO mice than for the WT controls.

Wall-Climbing

The dependent measure used to analyze wall-climbing was [(total duration of wall-climbing)/(total number of trials)]. The analysis revealed significant main effects of Session-block ($F_{(1,17.9)} = 34.19$, $p < .0001$) and Previous Trial Outcome ($F_{(1,17.9)} = 24.98$; $p < .0001$), indicating that the mice wall-climbed significantly more during the first Session-block than during the final Session-block, and on trials following an error relative to trials following a correct response. In addition, a significant interaction of Genotype and Session-block was found ($F_{(1,17.9)} = 4.69$; $p = .04$; see Fig. 5), demonstrating that the increase in wall-climbing during Session-block 1 was significantly more pronounced for the KO mice than for controls. During Session-block 1, the KO mice wall-climbed significantly more than controls ($p = .04$), whereas genotypic differences were not seen during Session-block 2 ($p = .32$).

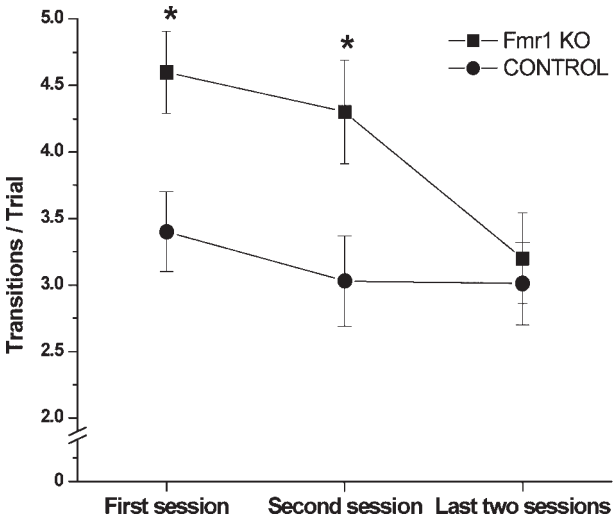

FIGURE 4 Mean (±SE) number of left-right transitions per trial for trials following an error in the reversal learning task. The increase in activity level seen early in the task was more pronounced for the KO mice than for the WT controls, with group differences being significant during the first ($p = .03$) and second ($p = .01$) sessions.

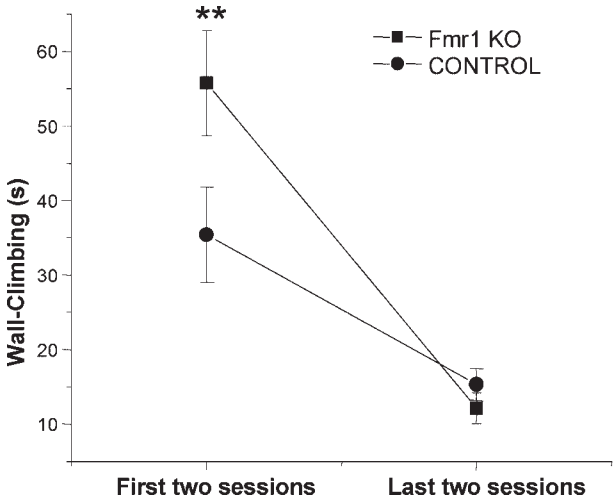

FIGURE 5 Mean (±SE) duration of wall-climbing across the two Session-blocks of the reversal learning task. The heightened wall-climbing during Session-block 1 was significantly more pronounced for the KO mice than for controls. $^{**}p = .007$.

Exploring

Exploring was coded at times that the mouse was moving around but not wall-climbing, jumping, or grooming. The dependent measure used to analyze exploratory behavior was [(total duration of exploring)/(total number of trials)]. The analysis revealed significant main effects of Session-block ($F_{(1,54)} = 7.28$, $p = .009$) and Previous Trial Outcome ($F_{(1,54)} = 8.89$, $p = .004$). Exploratory behavior was greatest during the first Session-block and on trials that followed an error. There was no main effect of Genotype ($F_{(1,17.4)} = 2.59$, $p = .13$; means (±SE) for the KO mice and the WT mice are 3.3 s (.06) and 3.7 s (.06), respectively). Interactions involving Genotype were also not significant.

Grooming

The dependent measure used to analyze grooming behavior was [(total duration of grooming)/(total number of trials)]. There was a significant main effect of Previous Trial Outcome ($F_{(1,60.6)} = 9.85$, $p = .003$), indicating that the mice groomed more on trials that followed an error than on those that followed a correct response (means for trials following an error and trials following a correct response were 1.26 and .93 s per trial, respectively). Grooming was not affected by Genotype ($F_{(1,26.5)} = 1.41$, $p = .25$) or Session-block ($F_{(1,60.6)} = .18$, $p = .67$), nor

were there any significant interactions involving Genotype.

Jumping

The dependent measure used to analyze jumping behavior was [(sum of instances of jumping)/(total number of trials)]. The analysis revealed an effect of Session-block ($F_{(1,13.7)} = 6.48$, $p = .02$), (means (±SE) for session-blocks 1 and 2, respectively, were 1.2 (.07) and .8 (.07)), reflecting the fact that jumping was slightly but significantly more frequent in session-block 1 than in session-block 2. However, jumping behavior did not vary by Genotype ($F_{(1,2.33)} = .00$, $p = .99$), nor was it influenced by any interactions involving Genotype. Jumping incidence also did not vary as a function of Previous Trial Outcome ($F_{(1,13.7)} = .73$, $p = .40$).

DISCUSSION

The present study assessed associative learning, transfer of learning, and reversal learning in male *Fmr1* KO mice and WT littermate controls. Whereas measures of learning rate did not reveal genotypic differences for any of these aspects of learning, analysis of videotapes provided evidence for heightened arousal and/or emotion in the mutant mice (relative to WT controls) when the contingencies were reversed in the reversal learning task, and on trials that followed an error.

The *Fmr1* KO mice did not differ from WT littermate controls in the rate at which they mastered the initial olfactory discrimination task, indicating that basic associative learning was intact in these mutant mice. Consistent with this conclusion, two different cohorts of *Fmr1* KO mice (including the present cohort) did not differ from WT littermate controls in the rate at which they mastered a more difficult 5-choice brightness discrimination task (Moon et al., 2006; Moon, J., et al., unpublished work). These two cohorts were about five months of age, at the time of testing on this brightness discrimination task, arguing against the possibility that learning differences were obscured in the present study by the relatively old age of the mice at the time of testing. The conclusion that *Fmr1* KO mice are not impaired in associative learning is consistent with their normal learning rate in many different types of tasks in previous studies (e.g., Bakker et al., 1994; Mineur et al., 2002; Yan et al., 2004).

Both groups of mice in the present study learned the second olfactory discrimination task more quickly than the first, demonstrating significant transfer of learning across different exemplars of the same basic task. Moreover, the magnitude of this improvement from the first to the second task was comparable for the two genotypes, indicating that learning set formation was also unimpaired in the mutant mice. This finding further defines the cognitive phenotype of this mouse model of Fragile X and identifies a distinction between this animal model and models of other MR syndromes. For example, rats exposed to hyperphenylalaninemia during either gestation or early postnatal life (models of maternal or classic PKU, respectively) do not exhibit impaired associative learning but are deficient, relative to controls, in the ability to transfer learning across similar tasks (Strupp et al., 1994; Strupp et al., 1990), as commonly seen in mentally retarded humans [e.g., (Campione et al., 1985); reviewed in Strupp et al. (1994)]. There is growing evidence that different MR syndromes, while all characterized by low IQ, exhibit very different cognitive profiles in terms of spared and impaired functions (e.g., Bellugi, Lichtenberger, Jones, Lai, & St. George, 2000; Cornish et al., 2004a; Greydanus & Pratt, 2005; Wang & Bellugi, 1994).

In the present study, the *Fmr1* KO mice also performed comparably to WT controls on the reversal learning task. This finding was unexpected because several lines of evidence, reviewed in the Introduction Section of this article, led to the expectation that this type of learning would be impaired in this mouse model. In particular, the absence of genotypic differences in the duration of perseverative responding to the previously correct cue—an inference based on analyses of overall learning rate (trials to criterion; Fig. 1) and percent correct responses during sessions 1 and 2 (see Fig. 2)—appears inconsistent with some prior findings concerning FXS in humans. Specifically, males with FXS committed a higher number of perseverative errors in the Wisconsin Card Sorting Task (WCST) than controls (Cornish, Munir, & Cross, 2001; Mazzocco et al., 1993), and repeatedly responded to target stimuli in a visual search task, under conditions in which only the first response was ''correct'' (Wilding, Cornish, & Munir, 2002). There are several possible reasons for these apparently disparate findings. First, the tasks are quite different, despite the fact that all can be impaired by perseverative responding. For example, the WCST task assesses shifting between sets of predictive cues (i.e., extra-dimensional shifting), whereas reversal learning tasks tap the ability to reverse a previously learned contingency within a single dimension. Increased perseverative errors in the WCST can reflect impaired selective attention, rather than inflexibility or deficient inhibitory control; as such, the findings are not necessarily contradictory. Another factor that may have been instrumental in these disparate outcomes is the differing consequences of perseverative responding in these tasks. In the WCST, the only consequence of committing an error was the feedback that the response was incorrect, whereas in the

visual search task, there was no adverse consequence of repeated responding to the target cue. In contrast, in the reversal learning task used in the present study, perseverative responding to the previously correct cue leads to strings of nonrewarded trials, which may severely curtail this type of responding in hungry animals rewarded for correct responses with food. These task characteristics may have precluded the detection of deficient inhibitory control in the *Fmr1* KO mice, notwithstanding the existence of dysfunction in this area in these mice, as demonstrated in a series of attention tasks (described below).

Although no prior studies of *Fmr1* KO mice have assessed reversal learning within the context of a discrimination task (as in the present study), several previous studies have compared *Fmr1* KO mice and WT controls in maze tasks where the location of the escape location was moved following initial learning, termed a ''reversal.'' Some of these studies reported that the KO mice were impaired in reversal learning (Bakker et al., 1994; D'Hooge et al., 1997; Kooy et al., 1996; Paradee et al., 1999; Van Dam et al., 2000), whereas others found no differences between the *Fmr1* KO mice and controls (Paradee et al., 1999; Yan et al., 2004). However, these apparently discrepant results can be reconciled by a consideration of the background strains. Those instances in which reversal learning deficits were reported for the *Fmr1* mutant mice seem to be due to alleles of the 129 strain segregating with the *Fmr1-tm1Cgr* mutation and/or the presence of modifying genes of the 129 strain influencing the *Fmr1* KO phenotype (for additional discussion, see Paradee et al., 1999; Yan et al., 2004). Reversal learning was uniformly unimpaired in *Fmr1* KO mice when the mutation was studied on highly backcrossed C57BL/6J or F1 hybrid backgrounds that excluded most 129/ReJ alleles, consistent with the present findings.

Despite the absence of genotypic differences in learning rate in the reversal learning task in this study, the videotape data provided evidence that the arousal and/or emotion created by the reversal of contingencies was significantly more pronounced and/or prolonged in the *Fmr1* KO mice than in WT controls. Both groups exhibited higher levels of activity and wall-climbing during the initial sessions of the task than during the final sessions, indicating that the initial high rate of these behaviors reflected heightened arousal and/or frustration created by the reversal of contingencies and dramatic drop in reinforcement rate. This inference is supported by the finding that the incidence of these behaviors was significantly higher on trials following an error than on trials following a correct response. Notably, the increase in both behaviors seen early in the task was significantly more pronounced for the KO mice than for controls, as was the error-induced increase in activity level. This pattern of effects suggests that the arousal and/or emotion created by the reversal of contingencies and by errors in general was more pronounced and/or prolonged in the KO mice than in controls. A similar pattern was seen for this same cohort of mice when they transitioned between two visual attention tasks, the latter of which included, for the first time, unpredictable olfactory distractors (Moon et al., 2006). During the first few sessions on this new task, wall-climbing increased in both groups on the trials with distractors, but only the KO mice exhibited this increase in wall-climbing on the trials without distractors; this pattern indicates that the arousal and/or emotion produced by the unpredictable presentation of the potent olfactory distractors led to a more generalized disruption in performance for the KO mice than for controls. Additionally, a recent study in our lab revealed that *Fmr1* KO mice were also more aroused than WT controls when first confronted with a novel conspecific (McNaughton et al., 2008). Notably, this latter evidence for heightened arousal and/or emotion of *Fmr1* KO mice was seen under conditions of group housing and free access to food, arguing against the possibility that this aspect of the phenotype of the KO mice is specific to situations where the mice are housed alone and/or maintained on a restricted feeding regimen. These findings correspond well with the profile described by Cornish et al. (2004a) for FXS: ''. . . males with FXS react more strongly than those without FXS to many forms of environmental and social stimuli and the hyperarousal that results can take an unusually long time to abate. As a result, individuals with FXS are prone to long periods of sustained hyperarousal . . .''

The observed pattern of results for activity level in the Reversal Learning Task provides new insight into the nature of the hyperactivity reported previously for these mice (in some studies) and, by extension, perhaps in humans with FXS. In the present study, an effect of genotype on activity level was seen only during times of heightened arousal, and thus should be viewed as an indirect effect of this primary alteration in arousal or affect. This pattern of findings may help explain why some prior studies of *Fmr1* KO mice have found effects of the mutation on activity level (Bakker et al., 1994; Peier et al., 2000), whereas others have not (Nielsen et al., 2002).

Whether or not this heightened arousal and/or emotional reactivity of the KO mice affects choice accuracy appears to depend on task characteristics. It did not impair accuracy in the olfactory reversal learning task described in the present report whereas it appears to be at least partially responsible for the impaired performance of these same mice in a series of visual attention tasks (Moon et al., 2006). In these attention tasks, the *Fmr1* KO mice performed less well than their WT littermate controls

under very specific testing conditions, including: (1) trials immediately following an error; and (2) the first few sessions of a new task when the characteristics of the visual cue had changed slightly (e.g., cue duration became variable or when potent olfactory distractors were presented unpredictably). The increased arousal created by each of these conditions disrupted attention and inhibitory control in both groups, but to a greater extent in the *Fmr1* KO mice. The finding that conditions which increase arousal uncovered genotypic differences in performance in these attention tasks whereas it did not do so in the present reversal learning task likely reflects differences in task demands. In these attention tasks, the visual cue was brief and presented after a delay on some trials, thus requiring both inhibitory control and sustained attention. In contrast, in the reversal learning task, the discriminative olfactory cues were presented immediately after trial initiation and were continuously available until a response was made. The pattern of findings in this series of attention tasks indicates that heightened arousal of the KO mice disrupts attention and inhibitory control, which then lowers performance in tasks which tap these functions. An interesting parallel was noted in a study involving humans with FXS: Deficits in inhibitory control were more pronounced for tasks that required higher attentional capacity (Munir, Cornish, & Wilding, 2000).

CONCLUSION

In sum, although male *Fmr1* mutant mice did not differ from WT littermate controls in the rate of learning any of the tasks in the present study, their behavioral response to the reversal was more pronounced than that of controls, as was their reaction to committing an error. This pattern of effects indicates that the arousal and/or emotion created by these conditions was more pronounced and/or prolonged for the KO mice than for WT controls. These videotape data support the interpretation provided for the impaired performance of these same mice in a series of visual attention tasks (Moon et al., 2006). In these attention tasks, impaired performance of the KO mice was seen primarily under two conditions: (1) immediately following a change in task contingencies, and (2) on trials immediately following an error. This pattern had suggested a primary alteration in arousal and/or affect, an inference directly supported by the present findings. These findings support the validity of this mouse model of FXS, as heightened arousal and emotion are believed to underlie many of the behavioral symptoms of FXS (Cornish et al., 2004a). This study lays the ground work for future studies designed to examine possible pathways leading to impaired brain development in FXS and test potential therapies.

NOTES

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