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Attentional Dysfunction, Impulsivity, and Resistance to Change in a Mouse Model of Fragile X Syndrome

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On a series of attention tasks, male mice with a mutation targeted to the fragile X mental retardation 1 (*Fmr1*) gene (*Fmr1* knockout [KO] mice) committed a higher rate of premature responses than wild-type littermates, with the largest differences seen when task contingencies changed. This finding indicates impaired inhibitory control, particularly during times of stress or arousal. The KO mice also committed a higher rate of inaccurate responses than controls, particularly during the final third of each daily test session, indicating impaired sustained attention. In the selective attention task, the unpredictable presentation of potent olfactory distractors produced a generalized disruption in the performance of the KO mice, whereas for controls, the disruption produced by the distractors was temporally limited. Finally, the attentional disruption seen following an error was more pronounced for the KO mice than for controls, further implicating impaired regulation of arousal and/or negative affect. The present study provides the first evidence that the *Fmr1* KO mouse is impaired in inhibitory control, attention, and arousal regulation, hallmark areas of dysfunction in fragile X syndrome. The resistance to change also seen in these mice provides a behavioral index for studying the autistic features of this disorder.

Keywords: fragile X syndrome, *Fmr1* mouse, attention, autism, error monitoring

Fragile X syndrome (FXS), the most common inherited form of mental retardation (Crawford et al., 1999), is caused by expansion of a CGG repeat sequence in the promoter region of the fragile X mental retardation 1 (*FMR1*) gene (Khandjian, 1999; O'Donnell & Warren, 2002), which leads to transcriptional silencing of this gene (Oberle et al., 1991; Verkerk et al., 1991; reviewed in O'Donnell & Warren, 2002). 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; Hagerman, 1996; Lachiewicz, Spiridgliozzi, Guillion, Ransford, & Rao, 1994; Largo & Schinzel, 1985; Turk, 1998), with up to 73% of affected individuals meeting the diagnostic criteria for attention-deficit/hyperactivity disorder (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), seizure susceptibility (Musumeci et al., 1999; Musumeci, Ferri, Scuderi, Bosco, & Elia, 2001), emotional difficulties (Borghgraef, Fryns, & van den Berghe, 1990; Hagerman & Sobesky, 1989; Kerby & Dawson, 1994), and autistic features (e.g., Hagerman, 1996; Lachiewicz, Spiridgliozzi, Guillion, Ransford, & Rao, 1994).

Although there are currently no interventions that can prevent the brain damage in FXS, recent research on FMRP suggests that certain pharmacological interventions, such as metabotropic glutamate receptor antagonists, might dramatically improve brain development and function in affected individuals (e.g., Bear, 2005; Bear, Huber, & Warren, 2004; McBride et al., 2005; Yan, Rammal, Tranfaglia, & Bauchwitz, 2005). One stumbling block in testing these treatments in the mouse model of FXS (referred to as *Fmr1*^{tm1Cgr} or *Fmr1* knockout [KO] mice) is that the behavioral differences between the KO mice and wild-type (WT) controls on learning and memory tests have been subtle and strain specific or nonexistent (Bakker et al., 1994; D'Hooge et al., 1997; Kooy et al., 1996; Mineur, Sluyter, de Wit, Oostra, & Crusio, 2002; Yan, Asafo-Adjei, Arnold, Brown, & Bauchwitz, 2004), at odds with the profound cognitive and behavioral problems that characterize humans with FXS. Commonly used learning/memory tasks, such

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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 et al., 2004) or have revealed very small deficits in the KO mice that are apparent only in some background strains (Bakker et al., 2000; Cianchetti et al., 1991; Hinds et al., 1993; Mineur et al., 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). The available data also indicate discrepancies between *Fmr1* KO mice and FXS in prepulse inhibition (PPI), a marker of sensorimotor gating: In a recent study, boys with FXS exhibited reduced PPI relative to controls, whereas *Fmr1* KO mice exhibited greater PPI than their WT littermates (Frankland et al., 2004). These findings, collectively, have raised questions about the validity and utility of this mouse model (e.g., Yan et al., 2004).

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 studied in the mouse model; these include impaired attention, inhibitory control, and regulation of arousal or emotion. The present study was designed to test this hypothesis. The performance of F1 hybrid *Fmr1* KO mice (a C57BL/6J × FVB/NJ cross) and WT littermate controls was assessed on a series of tasks designed to assess inhibitory control and various aspects of attention (sustained, selective, and divided attention). These tasks, modified versions of the five-choice serial reaction time task (Humby, Laird, Davies, & Wilkinson, 1999), are similar to ones used to assess various aspects of attention in human subjects, such as Leonard's (1959) five-choice serial reaction time task and the Continuous Performance Test (reviewed in Robbins, 2002). Regulation of arousal and/or emotion was evaluated in these tasks by examining the reaction of the mice to the unexpected presentation of potent olfactory distractors (in the distraction task), as well as their reaction to committing an error on the previous trial. Reactivity to errors taps both error monitoring (an aspect of executive functioning; Luu, Collins, & Tucker, 2000) and emotion regulation (Elliott et al., 1996; Luu et al., 2000), two domains affected in FXS.

Method and Materials

Subjects

Breeding of the mice was conducted at the University of Colorado Health Sciences Center (UCHSC). Breeder pairs of C57BL/6J-*Fmr1*^{tm1Cgr} (B6.129-*Fmr1*^{tm1Cgr}; i.e., *Fmr1* KO) and 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). The heterozygous breeder females were obtained by breeding C57BL/6J-^{tm1Cgr} mutant female mice with normal inbred C57BL/6J males. These females were then bred with normal FVB/NJ males (also from Jackson Laboratory) to produce male KO and WT mice from the same litters. Male offspring (21 WT and 20 *Fmr1* KO) from these litters served as subjects in the present experiment. Genotyping was conducted as described by 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, Mahler, & Harry, 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 (0.2-mA) footshock. The WT and KO mice did not differ in performance at the 24-hr retention test.

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 because of previous observations that male mice of this strain, caged in pairs, are prone to fighting when reunited after being removed for testing (Crnic, 2004). After acclimating to the new environment for 2 weeks, the mice were placed on a restricted feeding regimen 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% was selected because the mice were somewhat overweight prior to introduction of the food restriction regimen. All procedures used in these experiments adhere to the National Institutes of Health (1986) *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 of which are Association for Assesment and Accreditation of Laboratory Animal Care 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 enclosure. The testing chambers were adapted from the nine-hole operant chambers recently developed to assess attention in mice (Humby et al., 1999). The slightly curved rear wall contained five circular response ports, 1 cm in diameter, located 2 cm above the floor and 5mm apart. A nosepoke into any of these ports constituted a response (or choice). Responses to the ports were detected by infrared photodiodes, positioned inside each port, 0.5 cm from the opening. The discriminative visual cues were provided by illumination of green 4-mA light-emitting diodes (LEDs), one embedded on the back surface of each port. Each port also contained a fitting through which scented air could be dispensed. This scented air served as a distractor in the distraction task. 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. As with the ports, head entries into the alcove were monitored by infrared photodiodes. A nosepoke into this alcove port was required to initiate each trial. 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 (Microsoft Corporation, Redmond, WA).

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 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., location of the visual cue, demarcation of the intertrial interval, presentation of the distractor, whether a response was correct or incorrect, access to liquid reward).

Behavioral Testing

The training tasks began when the mice were 8 months of age. Testing on the four attention tasks was initiated when the mice were 10 months of age. Behavioral testing and coding of the videotapes were conducted by individuals blind to the genotype of the mice.

Training Tasks

The mice first completed 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 have been 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 dipper port, followed by a nosepoke into one of the five response ports, would produce the delivery of 0.04 ml of the liquid diet in the dipper alcove. These four training phases were mastered in approximately 8–10 sessions.

Each mouse was then trained on a five-choice visual discrimination task. In this task, one of the five port LEDs was illuminated on each trial; the mouse was rewarded for making a nose-poke into the illuminated port. After reaching the learning criterion (M = 11 sessions), each mouse progressed through four subsequent visual discrimination tasks, all of which were identical in concept but with progressively shorter cue durations. The cue durations were 5.0, 2.0, 1.4, and 1.0 s; the mice received these durations for 3, 10, 10, and 5 sessions, respectively. These tasks were designed to establish stable performance and prepare the mice for the subsequent attention tasks. For additional details on this task series, see Driscoll et al. (2004).

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 an enzyme that removes olfactory cues (including pheromones).

Attention Task 1: Learning to Wait for the Cue

The mice were then tested on four visual attention tasks that were all identical in terms of the basic rules and procedures but that entailed different cue durations, delays prior to cue onset, and/or presentation of olfactory distractors (see Table 1). In the first of these four tasks, a variable delay was imposed prior to cue onset. This task was designed to tap inhibitory control and prepare the mice for the subsequent sustained attention and distraction tasks. The metal door at the dipper alcove was raised at the onset of each trial. A nosepoke into the alcove initiated each trial. After a variable delay (0, 2, or 4 s), one of the five LEDs in the response ports was illuminated for 1 s. These variable precue delays were added to a constant turn-around time of 1 s, an interval provided on all trials to allow the mouse time to turn around and face the response ports following trial initiation in the dipper alcove. A nosepoke into the illuminated port was the correct response and was rewarded with 5 s of access to the dipper alcove. The three precue delays were presented randomly, but the number of presentations of each combination of precue delay and response port (1–5) were balanced across each session.

Several types of errors were possible. A premature response was recorded if the mouse responded to any of the response ports before onset of the visual cue. A response to an incorrect port following cue presentation was tallied as an inaccurate response. An omission error was scored if the mouse initiated the trial but did not respond to any of the five response ports within 5 s of cue onset, indicative of missing the visual cue. Following any of these types of errors, a 5-s time-out period was imposed. These time-out periods were signaled by the illumination of a 2-W houselight on the ceiling of the chamber. A time-out was also imposed following a *nontrial*, that is, 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, however. 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 session was terminated after 30 min or 70 response trials, whichever came first. The mice were tested on this task for eight sessions.

Table 1
Cue Parameters for the Four Visual Attention Tasks, in the Order in Which They Were Administered

Task	Cue duration	Precue delay	Distractor
Attention Task 1	1.0[a]	0, 2, 4[b]	no
Sustained attention task	0.8, 1.0, 1.4[b]	0, 2, 4[b]	no
Baseline task	1.0[a]	2, 3[b]	no
Distraction task	1.0[a]	2, 3[b]	yes[c]

Note. Cue duration and precue delay times are in seconds. Distractor data indicate whether olfactory distractors were presented to the mice.
[a] Constant across trials. [b] Variable across trials. [c] Distractors were present on one third of the trials per session.

Sustained Attention Task

The sustained attention task was a variation of the preceding task in which both the precue delay and the cue duration varied randomly across trials (see Table 1). Each combination of correct response port (1–5), precue delay (0, 2, and 4 s), and cue duration (0.8, 1.0, and 1.4 s) was presented an approximately equal number of times in each session. The mice were tested for 20 sessions on this task. The design of this task was based on a similar task that we developed for assessing sustained attention in rats (e.g., Gendle et al., 2003; Morgan et al., 2001, 2002; Stangle, Smith, Beaudin, Strawderman, Levitsky, & Strupp, in press).

Distraction Task

The mice were then tested on a variation of the preceding visual attention tasks that included the unpredictable presentation of potent olfactory distractors. This task was designed to tap selective attention and reactivity to salient stimuli. The design of this task was based on a selective attention task for rats (e.g., Gendle et al., 2004; Stangle et al., in press). Immediately prior to the distraction task, the mice were tested on a baseline task that was identical to the distraction task in terms of the visual cue parameters (precue delays and stimulus duration) but did not include olfactory distractors (see Table 1).

The disruption produced by the unpredictable presentation of the olfactory distractors was assessed in two ways. First, within the distraction task, performance on the trials with distractors (distraction trials) was compared with performance on the trials without distractors (nondistraction trials). In addition, performance on the nondistraction trials of the distraction task was compared to performance on the baseline task. This comparison provided an index of the extent to which the unpredictable presentation of the olfactory distractors produced a generalized disruption of performance that extended to the nondistraction trials.

For both the baseline and distraction tasks, cue duration was constant across trials (1 s), but the precue delay varied randomly between 2 or 3 s (in addition to the 1 s turn-around time). For the distraction task, one of

nine different olfactory stimuli was pseudorandomly presented on one third of the trials from one of the five response ports either 1 or 2 s before the visual cue. The nine scents used for these distractors were lemon, hazelnut, apricot, butter, anise, raspberry, maple, coconut, and almond. The liquid odorants were made by diluting artificial flavorings (McCormick, Inc., Hunt Valley, MD) with propylene glycol. Scented air was produced by passing filtered, compressed air through small bottles of these scented liquids, using solenoid airflow valves, as described above. All parameters of visual cue and distractor presentation were balanced for each testing session; these included the location of the visual cue, duration of the precue delay (2 or 3 s), the timing of the distractor relative to the visual cue (1 or 2 s prior to cue onset), and the response port from which the distractor was emitted.

Diet and Control of Motivation

As noted above, the mice were maintained at 80%–85% of their ad libitum weights throughout the study to maintain motivation for the food rewards during testing and to ensure an approximately equal number of trials for all mice. On each testing day (6 days per week), the number of calories obtained during testing was subtracted from the total caloric allotment, and the remainder was fed as chow (ProLab 1000, Purina, Inc., Richmond, IN) in the home cage directly after testing. On nontesting days, each mouse was given 0.4 ml of the liquid diet plus the remainder of the ration in chow in its home case. The goal was to provide the maximal daily caloric intake that would still maintain adequate motivation for 60–70 trials during each daily test session.

Videotape Coding

All sessions of the baseline and distraction tasks were recorded. A coder, blind to the genotype of the mice, scored four test sessions for each mouse: the last two sessions of the baseline task and the first two sessions of the distraction task. The frequency and duration of four behaviors were quantified: wall climbing, grooming, jumping, and exploring (defined below). Also coded was the location of the behavior (the side of the chamber containing the response ports vs. the side containing the dipper).

Reliability of the behavioral ratings was determined prior to proceeding with the coding. For these reliability analyses, eight sessions of Session 2 of the distraction task were pseudorandomly selected (the eight sessions were balanced by box and treatment). To determine the 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 coder, and the results from both coders were correlated. Coding of the remaining 156 sessions commenced only after high levels of inter- and intrarater reliability were achieved ($r > .9$ for all behavioral measures).

Statistical Analyses

The data were analyzed with a generalized linear mixed model, which correctly handles nonnormal data and the repeated measures for each mouse (Wolfinger & O'Connell, 1993). All statistical analyses were conducted with SAS 9.1 for Windows 2000.

The following performance measures were analyzed: percentage of inaccurate responses, percentage of premature responses, percentage of omission errors, and percentage of nontrials. For each of these dependent measures, means were calculated for each mouse for each testing condition, defined by the following variables (as appropriate for the task characteristics): precue delay, stimulus duration, distraction condition (distraction vs. nondistraction trials), session block (blocks of testing sessions; defined below), trial block (blocks of trials within each test session; defined below), and outcome of the previous trial (correct or error). The analyses were conducted on these means. The models used for these analyses included the aforementioned variables plus genotype (*Fmr1* KO and WT) and all relevant higher order interactions. However, simpler models were used in cases in which the outcome was rare for that task, to obtain more observations for each mean.

Nonparametric techniques were used to analyze the dependent measures for the videotape data because of nonnormality of the distributions. Specifically, Wilcoxon rank-sum tests were used to analyze between-conditions differences and Genotype × Condition differences for the dependent measures. Dependent measures were analyzed as a percentage of time spent for a given behavior (i.e., time spent on a given behavior divided by time spent on all behaviors multiplied by 100). Some analyses were conducted on difference scores, which were created by subtracting each mouse's mean percentage of time spent for a given dependent measure during one condition from the mean percentage of time spent during another condition.

We used *t* tests to compare body weight and daily food intake of the two genotypes. For each of these analyses, a mean was calculated for each mouse for the first and last testing sessions, and then the group means were calculated and compared.

Results

Body Weight and Daily Food Intake

The body weights of the groups did not differ, $t(39) = -1.28$, *ns*. There was no effect of genotype on mean daily food intake, $t(39) = -1.58$, *ns*.

Nontrials and Dipper Latency

The KO and WT mice did not differ in the rate of nontrials for any of the tasks (all $ps > .3$), nor for the latency to retrieve the liquid reinforcer following a correct response (all $ps > .9$). These findings indicate that motivation to solve the tasks was comparable for the two groups.

Performance on the Attention Tasks

In all of the tasks, performance was significantly affected by the precue delay (better performance at shorter delays) and the outcome of the prior trial (impaired performance on trials following an error relative to trials following a correct response). Finally, in those tasks in which cue duration and distraction condition varied across trials, these factors also produced consistent and significant effects for all outcome variables (i.e., better performance for trials with longer cue durations and without distractors). However, to streamline the presentation of results, these effects are discussed only within the context of describing the genotypic differences.

Attention Task 1 (First Task With Precue Delays)

Premature responses (responses made prior to cue onset). The analysis of percentage of premature responses revealed a main effect of genotype, $F(1, 53) = 5.02$, $p = .03$. As depicted in Figure 1, the KO mice committed 30% more premature responses than controls, indicative of impaired inhibitory control.

Omission errors and inaccurate responses. For attention task 1, there were no significant differences between the *Fmr1* KO mice and the WT controls for percentage of omission errors or percentage of inaccurate responses.

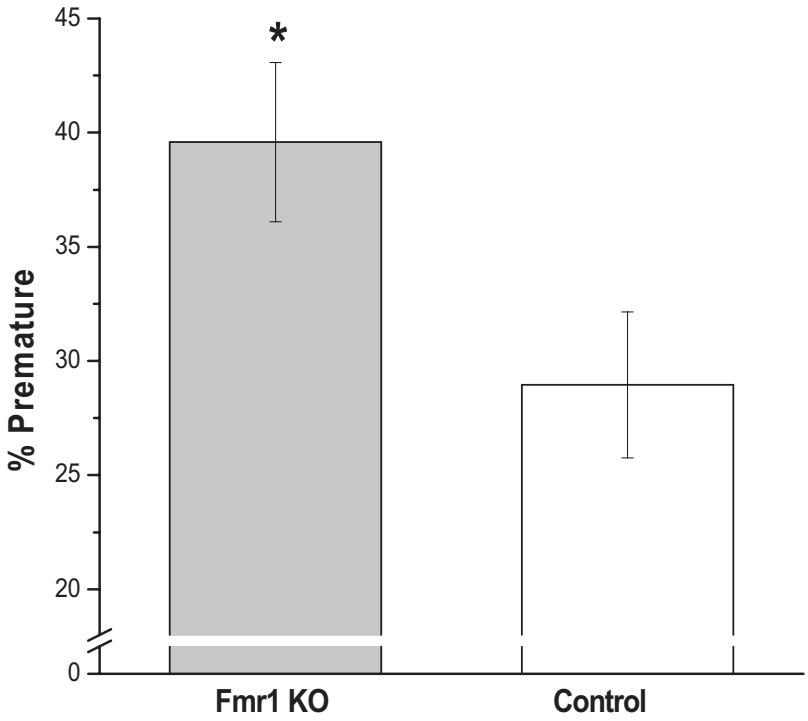

Figure 1. Mean (± *SE*) percentage of premature responses in attention task 1. During this task, the first in which a delay was imposed prior to cue onset, the knockout (KO) mice committed a higher rate of premature responses than controls, indicative of impaired inhibitory control. *Fmr1* = fragile X mental retardation 1. $^{*}p = .03$.

Sustained Attention Task

Premature responses. The analysis of percentage of premature responses for the 20 sessions of the sustained attention task revealed a significant interaction of genotype and delay, $F(2, 76) = 3.26$, $p = .04$ (see Figure 2); the increase in premature response rate from trials with a 0-s precue delay to those with a 2-s delay was significantly greater for the KO mice than for controls ($p = .015$), indicative of impaired inhibitory control. The increase from 2 to 4 s was comparable for the two groups. Group differences were significant only at the 2-s delay, because of the greater variance seen at the 4-s delay.

Inspection of average performance across the 20 test sessions revealed that the group difference in this dependent measure was most pronounced on the first session of the task. The KO group rapidly improved, with only subtle differences being apparent later in testing. One possible explanation for this pattern is that the KO mice, like humans with FXS (Kau, Reider, Payne, Meyer, & Freund, 2000) and autism (Rogers, Wehner, & Hagerman, 2001), had difficulty dealing with change. Note, however, that the only difference between the sustained attention task and the prior attention task (attention task 1) was that cue duration now varied randomly from trial to trial, rather than being constant; all other characteristics were identical. To directly test the effect of changing this one aspect of the task, we conducted an additional analysis, which included the final session of attention task 1 and the first session on the sustained attention test, so that these two consecutive test sessions could be statistically compared. This analysis revealed a significant interaction of genotype and task, $F(1, 37) = 5.82$, $p = .02$ (see Figure 3). The rate of premature responses increased significantly across these two sessions for the KO mice ($p = .0001$), but not for the controls ($p = .52$).

Inaccurate responses. The analysis of percentage of inaccurate responses revealed a main effect of genotype, $F(1, 38) = 4.16$, $p = .048$, and a borderline two-way interaction between genotype and trial block, $F(2, 1012) = 2.58$, $p = .06$. Overall, the *Fmr1* KO mice committed a higher percentage of inaccurate responses than the controls ($p = .048$). The borderline interaction between genotype and trial block ($p = .06$) reflected that the impairment of the KO mice, relative to controls, was most pronounced in the final block of trials in each testing session (Trials 51–70). In this final block of trials, the KO mice, on average, committed a higher rate of inaccurate responses than controls ($p = .008$). Although the average difference in inaccurate response rate was relatively small (3.4%), a comparison of the distributions of the two groups suggests that this difference could translate into a functionally important deficit for affected children. As seen in Figure 4, only 23.8% of the control mice had scores above the overall median, whereas 63.1% of the KO mice had scores above this value.

Baseline Task (Prior to the Distraction Task)

The WT and KO mice did not differ for percentage of premature responses or percentage of inaccurate responses. The only error type that revealed a genotype-related effect was percentage of omission errors; the analysis of this measure revealed a significant

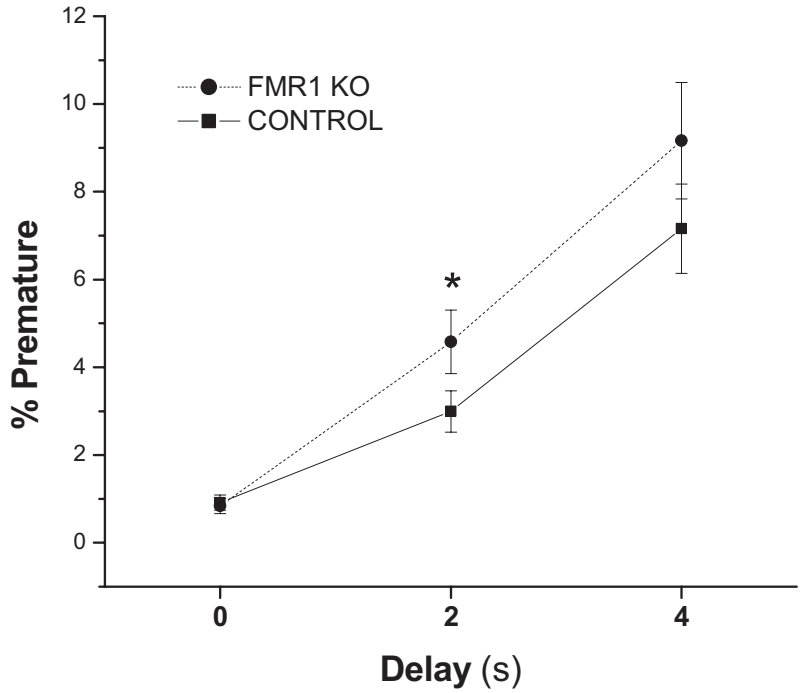

Figure 2. Mean (± *SE*) percentage of premature responses in the sustained attention task. As the precue delay increased from 0 to 2 s, the rate of premature responses increased to a greater extent for the *Fmr1* KO mice than for controls, indicating impaired inhibitory control. *Fmr1* KO = fragile X mental retardation 1 knockout. $^{*}p$ = .015.

interaction of genotype and previous trial outcome, $F(1, 430) = 7.49$, $p = .006$. Although both groups made more omission errors on trials following an error than on trials following a correct response, this increase in omission errors for trials following an error was greater for the KO mice than for the controls (see Figure 5). This finding indicates that the disruptive effect of committing an error was more pronounced for the KO mice than for the controls.

Distraction Task

A preliminary analysis used a seven-level session block variable to assess the change in performance across the 20 sessions of testing on this task (3 sessions in each of the first six blocks and 2 sessions in the final block). This analysis revealed a significant interaction between distraction condition and session block, $F(6, 1928) = 3.09$, $p = .005$, reflecting the fact that the difference in performance between the distraction and nondistraction trials was most pronounced during the first session block and was constant, at a slightly lower value, for the remaining six session blocks. Therefore, subsequent models used a two-level session block variable in which the first 3 sessions were designated *Session Block 1*, and the final 17 sessions were designated *Session Block 2*.

Inaccurate responses. The analysis of percentage of inaccurate responses did not reveal a main effect of genotype, $F(1, 38) < 1.0$, *ns*, but the two-way interaction of genotype and session block, $F(1, 39) = 4.25$, $p = .04$, and the three-way interaction of genotype, session block, and distraction condition, $F(1, 491) = 16.61$, $p = .01$, were significant. The three-way interaction reflected that the two genotypes differed in the rate of inaccurate responses only for the nondistraction trials in Session Block 1 ($p < .0001$). They did not differ on distraction trials in either session block, nor on nondistraction trials for Session Block 2.

The fact that the KO mice were impaired relative to controls during the nondistraction trials of Session Block 1, whereas their performance did not differ from controls during the baseline task (identical trial characteristics), suggests that for the KO mice the unpredictable presentation of the distractors produced a generalized disruption in performance that extended beyond the distraction trials into the nondistraction trials. An additional analysis was conducted in which the nondistraction trials of Session Block 1 in the distraction task were directly compared with the final session of the baseline task. This analysis revealed a significant interaction between genotype and trial type, $F(2, 95) = 3.99$, $p = .02$ (see Figure 6). The two groups differed only for the nondistraction trials of the distraction task, not for the final session of the baseline task or the distraction trials. For the KO mice, the rate of inaccurate responses increased significantly from the final session of the baseline task to the nondistraction trials during Session Block 1 of the distraction task ($p = .006$) but not for the controls ($p = .32$).

Premature responses. A borderline effect of genotype was detected, $F(1, 41) = 3.71$, $p = .06$, reflecting the fact that the *Fmr1* KO mice tended to commit more premature responses than controls in this task. Because the two groups of mice did not differ for this measure in the baseline task, this finding indicates a generalized disruption in response to the unpredictable presentation of the

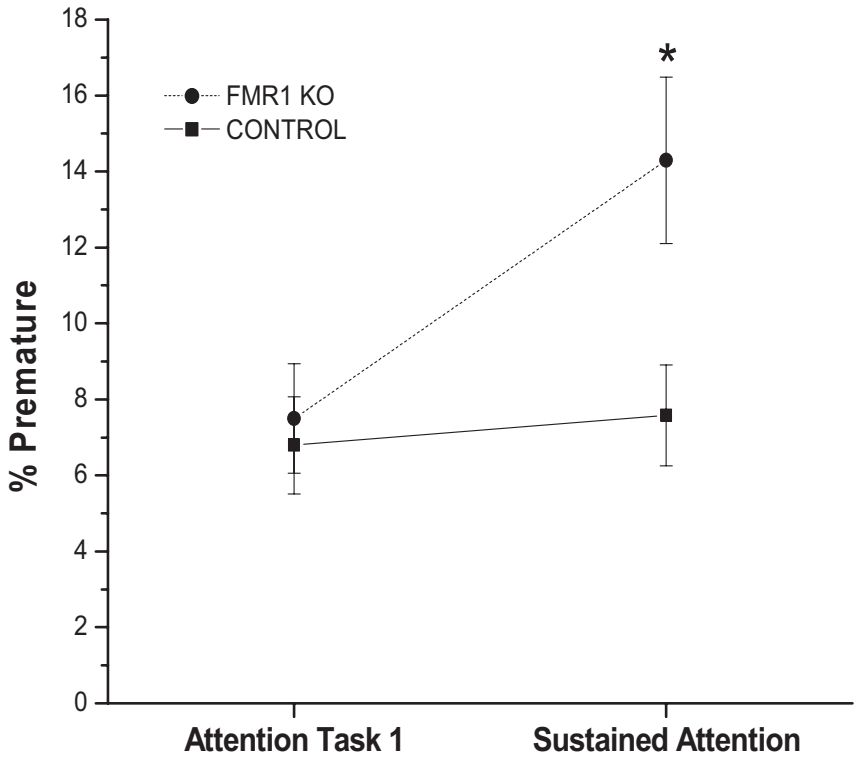

Figure 3. The increase in mean (± *SE*) percentage of premature responses from the final session of attention task 1 to the first session of the sustained attention task (consecutive sessions) was significantly more pronounced for the *Fmr1* KO mice than for controls. *Fmr1* KO = fragile X mental retardation 1 knockout. $^{*}p = .02$.

olfactory distractors, which then manifested as deficient inhibitory control. The fact that the interaction of genotype and distraction condition was not significant, $F(1, 115) = 1.25$, $p = .26$, indicates that the increase in premature responses was not limited to the distraction trials, supporting this interpretation.

Analysis of the Videotapes

As a result of apparatus malfunction, videotapes were not available for all mice. For these analyses, the sample size was 12 WT and 13 KO mice.

Wall climbing increased in both groups in response to the presentation of the distractor ($p < .05$) and on trials following an error ($p < .01$), indicating that this behavior was reflective of disruption experienced by the mouse. The increase in wall climbing on distraction trials was comparable for the two groups ($p = .24$), but the increase observed on nondistraction trials (relative to the baseline task) was significantly greater for the KO mice than for the WT controls ($p < 0.05$). As seen in Figure 7, 62% of the KO mice had difference scores greater than zero, whereas only 25% of the controls had a difference score greater than this value. This finding provides further evidence that the unpredictable presentation of the distractors produced a more generalized disruption in performance for the KO mice than for the controls.

Discussion

The present findings implicate impairments in inhibitory control and attention in the *Fmr1* mutant mice, which were most pronounced during the first few sessions of a new task immediately following a change in task characteristics. This pattern—whereby inhibition deficits and attentional dysfunction become manifest under times of arousal and when confronted with changing task demands—recapitulates findings from humans with FXS whereby overstimulation and difficulty with change led to loss of behavioral control and attentional dysfunction (Cornish, Munir, & Cross, 2001; Hagerman, 1996; Mazzocco, Pennington, & Hagerman, 1993; Merenstein et al., 1994; Munir, Cornish, & Wilding, 2000; Schapiro et al., 1995). The evidence for dysfunction in each of these domains is delineated below.

Impaired Inhibitory Control

A prominent aspect of the *Fmr1* mutant phenotype revealed in this task series is impaired inhibitory control or impulsivity. In attention task 1, the first task in which a delay was imposed between trial initiation and cue presentation, the KO mice committed 30% more premature responses than the WT controls, indicative of an impaired ability to withhold responding. This deficit was transient, however; the two groups did not differ in premature response rate by the final testing session on this task. In

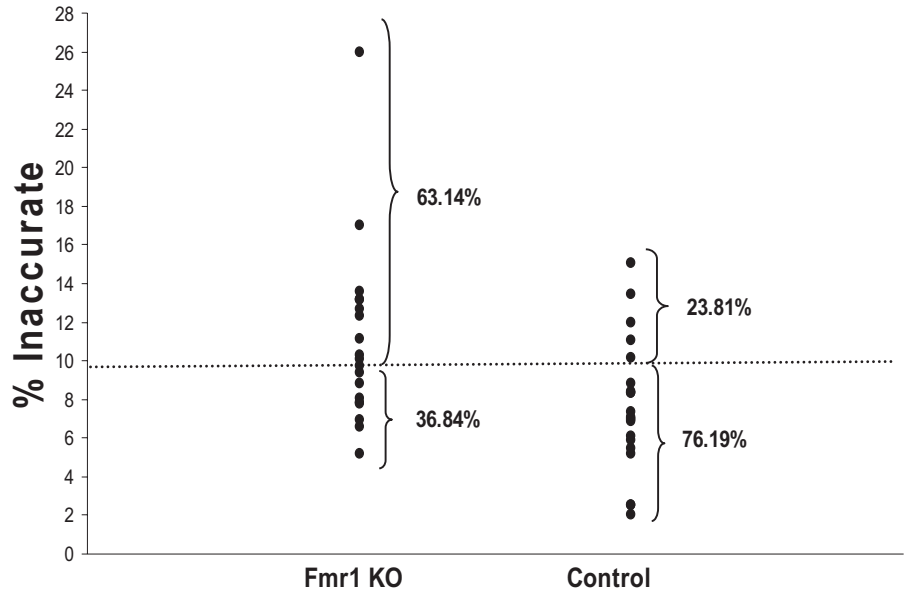

Figure 4. Average percentage of inaccurate responses during the third block of trials in each session (Trials 51–70) of the sustained attention task. Each dot represents the average for each mouse across the 20 sessions on the task. In this final block of trials, the *Fmr1* KO mice, on average, committed a higher percentage of inaccurate responses than controls ($p = .008$). Only 23.81% of the control mice had scores above the overall median (denoted by the dotted line), compared with 63.14% of the mutant mice. *Fmr1* KO = fragile X mental retardation 1 knockout.

light of this pattern, impaired learning of the task contingencies could be responsible for the higher rate of premature responses (i.e., learning that now, on some trials, the cue would be presented after a delay). However, the normal learning rate of these same mice in a series of olfactory discrimination and reversal learning tasks, as well as in an olfactory learning set task (Moon, Ota, Levitsky, Crnic, & Strupp, 2006), suggests that basic associative ability is not impaired in these mice, consistent with prior studies

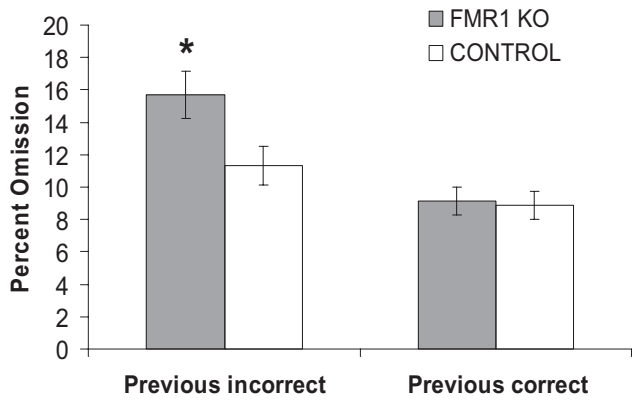

Figure 5. Mean (± *SE*) percentage of omission errors for trials following an error versus trials following a correct response, during the baseline task. Committing an error on the previous trial increased the rate of omission errors to a greater extent for the *Fmr1* KO mice than for controls (Genotype × Previous Trial Outcome, $p = .003$). *Fmr1* KO = fragile X mental retardation knockout. $^{*}p = .006$, KO versus control mice for trials following an error.

of F1 hybrid *Fmr1* mutant mice (e.g., Yan et al., 2004). On the basis of the pattern of results in the present series of tasks (discussed in more detail below), a more likely explanation is that the neural systems underlying inhibition are abnormal in the KO mice, but that deficient inhibitory control is evident only under conditions that arouse and/or disturb the mice, such as when task characteristics change. In the present case, attention task 1 was the first task in which the mice were required to wait on some trials for the cue to be presented, a profound change in task characteristics that was accompanied by a drop in reinforcement from 80% correct (on the prior training tasks) to 10% correct in the early sessions on attention task 1. The videotape data from the baseline and distraction tasks, described below, support the inference that changing task contingencies produced arousal in all mice but that the WT mice were better able to regulate this increased arousal than the KO mice.

This interpretation is supported by the fact that two other instances of impaired inhibitory control reemerged when task characteristics changed. The analysis directly comparing performance on the final session of attention task 1 with the first session of the sustained attention task (consecutive sessions) revealed a pronounced increase in premature response rate for the KO mice, whereas no change was seen for the controls across these two sessions. Because the two groups had not differed in premature response rate for the final session of attention task 1, the increased premature response rate of the mutant mice early in the sustained attention task appears to result from the slight change in task characteristics. The subtlety of the change in task parameters is notable: The only characteristic that differentiated the two tasks was that cue duration varied randomly across trials in the sustained

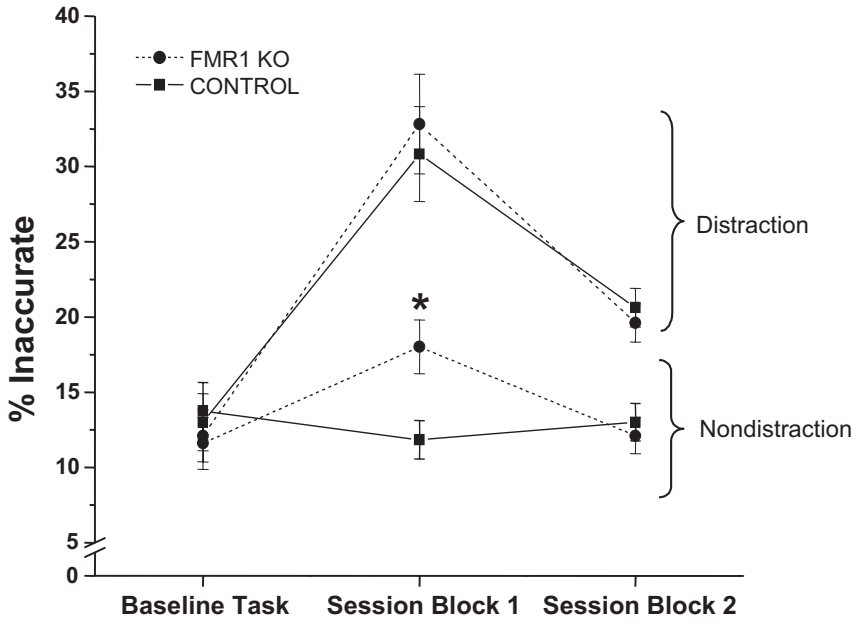

Figure 6. Performance on the final session of the baseline task compared with the two blocks of sessions in the distraction task. The mean (± *SE*) number of inaccurate responses (responses made to an incorrect port after cue onset) was significantly higher for the *Fmr1* KO mice than for wild-type controls during the first three sessions of the distraction task (Session Block 1), specifically on the trials without olfactory distractors (the nondistraction trials). *Fmr1* KO = fragile X mental retardation 1 knockout. $^{*}p < .0001$.

attention task, whereas it was constant in attention task 1; the four precue delays were identical in the two tasks, as were the basic contingencies. Note too that the poorer performance of the KO mice on this first session was not specific to any cue duration; premature responses are, by definition, independent of the duration of the visual cue on a given trial. Thus, the subtle change in task characteristics appears to have generally disrupted the KO mice, which manifested as impaired inhibitory control. A slight increase in premature response rate of the KO mice, relative to controls, was also evident throughout the distraction task. Because this was not seen in the prior baseline task, this reemergence of an increased premature response rate appears to reflect the arousal caused by the unpredictable presentation of the olfactory distractors.

Impaired Attention

The sustained attention task placed the greatest demand on sustained attention or vigilance, as the precue delays were often long, and the cue duration was variable and sometimes very brief. As noted above, the most robust differences between the KO and WT mice in this task were seen on the first session, indicative of difficulty in dealing with the change in task characteristics. This performance drop on Session 1 was primarily driven by the increase in premature response rate and is therefore indicative of impaired impulse control, rather than impaired attention per se. However, analysis of performance across the 20 sessions on this task revealed evidence for attentional dysfunction in the KO mice: They committed a higher rate of inaccurate responses than controls overall, with group differences being most pronounced during the final third of each testing session, a pattern that specifically indicates impaired sustained attention.

Two other instances of impaired attention in the KO mice appear to be the indirect result of impaired arousal regulation and inhibitory control. First, during the first three sessions of the distraction task, the KO mice committed a significantly higher rate of inaccurate responses than the WT mice, specifically on the trials without distractors. Impaired accuracy was not seen later in the task, following some degree of habituation to the olfactory stimuli, nor during the baseline task, a task that was identical to the nondistraction trials of the distraction task. This pattern, although unexpected, appears to indicate that whereas the distractors disrupted performance of the WT mice only on the distraction trials, they disrupted performance of the KO mice on both the distraction and nondistraction trials. This generalized disruption seen in the KO mice seems to reflect two factors: (a) the arousal caused by the change in task characteristics (as seen in the transition from attention task 1 to the sustained attention task), mirroring the impaired ability to deal with change seen in humans with FXS and autism (Kau et al., 2000; Rogers et al., 2001), and (b) hypersensitivity to potent sensory stimuli, a prominent feature of humans with FXS (Baranek & Berkson, 1994; Cohen et al., 1988; Hagerman, 1996; Miller et al., 1999). Studies of humans with FXS suggest that sensory processing alterations not only tax cognitive function but also lead to emotional arousal, which is incompatible with focused attention (Cornish, Sudhalter, & Turk, 2004; Hagerman, 1996; Merenstein et al., 1994).

The videotape data provide converging evidence that the KO mice were more aroused by the olfactory distractors and/or change in task characteristics than the controls. Wall climbing increased early in the distraction task relative to the baseline task for both groups of mice, indicating that the rate of this behavior provides an

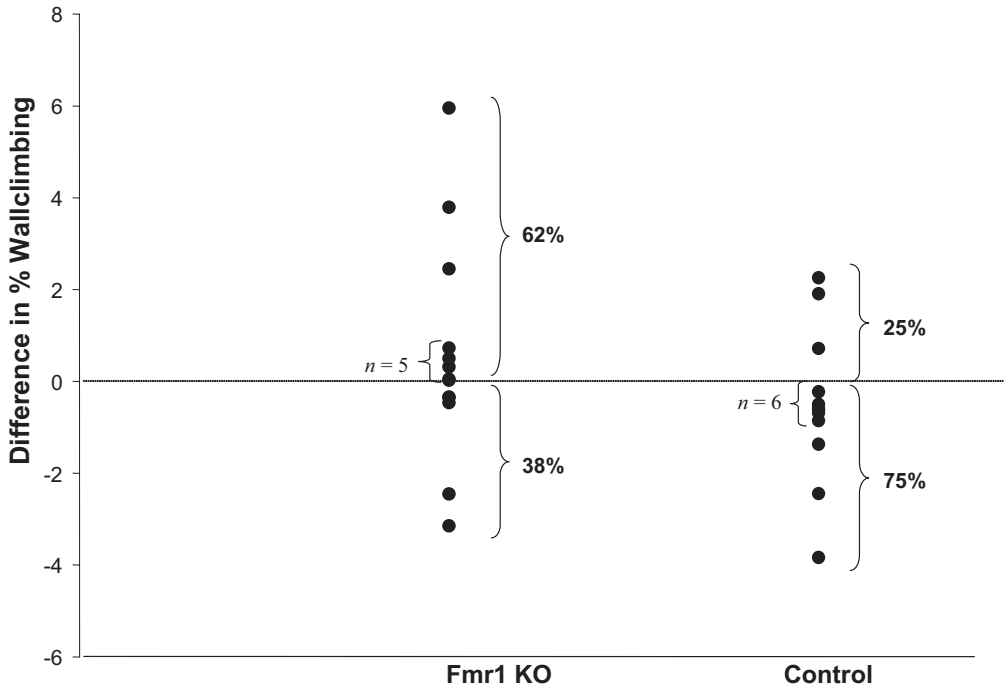

Figure 7. The difference in wall climbing between the baseline task and the nondistraction trials of the distraction task was significantly greater for the *Fmr1* KO mice than for controls ($p = .05$). Sixty-two percent of the KO mice had difference scores greater than zero (i.e., a higher percentage of wall climbing on the nondistraction trials), in contrast to 25% of the wild-type controls. This finding provides further evidence that the unpredictable presentation of the distractors produced a generalized disruption in performance for the *Fmr1* KO mice but not for the wild-type controls. Note that the overall rate of wall climbing was relatively low (*Mdn* = 4% per trial, during the baseline task); thus, a difference score of 2% represents, on average, a 50% increase in this behavior. *Fmr1* KO = fragile X mental retardation 1 knockout.

index of the arousal produced by the unpredictable presentation of the olfactory stimuli. For this measure, the increase (relative to the baseline task) was similar for the two groups on the distraction trials, but was significantly greater for the KO mice than for controls on the nondistraction trials, indicative of generalized arousal, as seen for accuracy of responding.

Analysis of performance as a function of the outcome of the prior trial (correct or incorrect) revealed another instance of attentional dysfunction that appears to be secondary to impaired regulation of arousal or negative affect. For both groups of mice, performance was significantly disrupted by committing an error (i.e., all types of errors increased on trials following an error, relative to trials that followed a correct response). Whereas this basic pattern was seen for both groups, the increase in omission error rate on post-error trials was more pronounced for the KO mice than for controls in the baseline task. Although some studies with human subjects have reported an exceptionally low error rate on trials following an error (e.g., Laming, 1979; Robertson, Manly, Andrade, Baddeley, & Yiend, 1997), indicating the operation of an executive error-correction system localized to the anterior cingulate cortex (see Bush, Luu, & Posner, 2000; Fernandez-Duque, Baird, & Posner, 2000), the finding uniformly seen in our rodent studies—increased error rate on post-error trials (Gendle et al., 2003, 2004; Morgan et al., 2001, 2002)—has also been reported in some human studies (e.g., Elliott et al., 1996; Rabbitt & Rogers, 1977). This pattern likely reflects a dominant influence of the emotional reaction engendered by committing an error. Consistent with this view, an electrophysiological measure of error detection, termed the *error-related negativity*, varies as a function of individual differences in negative affect and emotionality (Luu et al., 2000). Similarly, depressed subjects exhibit a more pronounced increase in error rate on post-error trials than nondepressed subjects (Elliott et al., 1996). Thus, the current finding that the attentional disruption seen on post-error trials was more pronounced for the KO mice than WT controls provides converging evidence for dysregulation of affect in the KO mice (for additional discussion, see Strupp & Beaudin, 2006).

Although these latter two instances of impaired performance in the KO mice seem most appropriately viewed as the indirect consequence of impaired arousal regulation and/or inhibitory control, it is notable that the resulting impairment was attentional in nature. This inference, based in part on the characteristics of these tasks, gains support from other findings from this same cohort of mice (Moon et al., 2006). In an olfactory reversal learning task, the KO mice exhibited more pronounced behavioral disruption than controls when the contingencies were reversed, and the reinforcement rate consequently dropped from 80% to 0% correct. Coded videotapes of the mice performing this task revealed that all mice exhibited higher rates of wall climbing early in the task (relative to later in the task, after the contingencies had been mastered) and

that this early behavior change was more pronounced for the KO mice. However, neither response accuracy nor learning rate differentiated the groups in this task. Thus, in a task without attentional demands, the impaired arousal regulation of the KO mice did not impair performance, in contrast to the attention tasks described here, in which the mice were required to wait for and then detect brief visual cues, which were unpredictable in onset time and location.

Although this is the first demonstration of impaired attention in *Fmr1* KO mice, several prior findings concerning this mouse model may also reflect impaired regulation of arousal. For example, this area of dysfunction may underlie the impaired performance of these KO mice in a radial arm maze task (Yan et al., 2004). This interpretation is suggested by the pattern of findings: The *Fmr1* KO mice committed a higher rate of errors than WT controls only during the first few sessions, a period of training generally associated with high arousal. Similarly, in some studies of Morris maze performance, *Fmr1* KO mice have been found to perform less well than controls specifically when the location of the platform has been moved (reversal trials), a pattern that may implicate impairments in arousal regulation and/or inhibitory control (D'Hooge et al., 1997; Paradee et al., 1999).

Conclusions and Implications

This study demonstrates that the hallmark deficits in human FXS—impaired attention, inhibitory control, arousal regulation, and adaptability to change—are also seen in the *Fmr1* KO mouse model of FXS. The present findings also suggest that impaired regulation of arousal or affect is a critical factor underlying the appearance of impaired attention and inhibitory control in the KO mice.

Although the nature of the dysfunction observed here provides strong encouragement for the validity of this mouse model, large deficits were seen only for a session or two, immediately following a change in task characteristics; the lasting deficits (i.e., seen across days or weeks of testing) were relatively small in magnitude. This aspect of the findings may indicate that a neural system playing a key role in FXS symptomatology is not used in a comparable way in the mouse. For example, as noted by Yan et al. (2004), if dysfunction of the neocortex is central to the deficits in human FXS, it is possible that the functional effects of this type of damage might be much less significant for the mouse, which has a much smaller and less complex neocortex. It is also possible that certain characteristics of the present tasks may have led to an underestimate of the degree of impairment of the mutant mice in terms of inhibitory control and attention. Each of these tasks assessed the ability to attend to a single stimulus in relatively calm, noncomplex testing conditions. Moreover, each task was administered for many sessions, allowing the characteristics of the task to become rote and predictable. These characteristics contrast with everyday life in which the cues to which one must attend occur amidst a complex background, with new stimuli constantly entering one's perceptual world. On the basis of the present findings that the attentional and inhibitory control deficits of the mutant mice were most pronounced at the beginning of each new task, particularly when confronted with novel distractors, it is likely that these mice would be more impaired, relative to controls, if their attentional abilities were tested in a more complex environment and the contingencies of the tasks were frequently changed (i.e., conditions that more closely approximate the complexity of the real world). Nonetheless, the fact that significant deficits in these key domains were detected in the present study, despite these task design limitations, provides strong support for the validity of this animal model.

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