Lithium treatment alleviates impaired cognition in a mouse model of Fragile X Syndrome

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Abstract

Fragile X Syndrome (FXS) is caused by suppressed expression of fragile X mental retardation protein (FMRP), which results in intellectual disability accompanied by many variably manifested characteristics, such as hyperactivity, seizures, and autistic-like behaviors. Treatment of mice that lack FMRP, *Fmr1* knockout (KO) mice, with lithium has been reported to ameliorate locomotor hyperactivity, prevent hypersensitivity to audiogenic seizures, improve passive avoidance behavior, and attenuate sociability deficits. To focus on the defining characteristic of FXS, which is cognitive impairment, we tested if lithium treatment ameliorated impairments in four cognitive tasks in *Fmr1* KO mice, tested if the response to lithium differed in adolescent and adult mice, and tested if therapeutic effects persisted after discontinuation of lithium administration. *Fmr1* KO mice displayed impaired cognition in the novel object detection task, temporal ordering for objects task, and coordinate and categorical spatial processing tasks. Chronic lithium treatment of adolescent (from 4–8 weeks of age) and adult (from 8–12 weeks of age) mice abolished cognitive impairments in all four cognitive tasks. Cognitive deficits returned after lithium treatment was discontinued for 4 weeks. These results demonstrate that *Fmr1* KO mice exhibit severe impairments in these cognitive tasks, that lithium is equally effective in normalizing cognition in these tasks whether it is administered to young or adult mice, and that lithium administration must be continued for the cognitive improvements to be sustained. These findings provide further evidence that lithium administration may be beneficial for individuals with FXS.

Keywords

Introduction

Fragile X syndrome (FXS) is the most common cause of inherited intellectual disability and is the most prevalent monogenetic cause of autism spectrum disorders. FXS is caused by a trinucleotide CGG repeat expansion on the X chromosome that suppresses expression of Fragile X Mental Retardation Protein (FMRP) (Verkerk *et al*. 1991; Pierreti *et al*. 1991), which is thought to cause the intellectual, behavioral, and physical abnormalities characteristic of FXS. In mouse hippocampus, FMRP expression is highest at postnatal day 7 (Lu *et al*. 2004), and FMRP is important for establishing functional neuronal networks (Gatto & Broadie 2009). Since individuals with FXS lack FMRP during postnatal

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development, crucial questions are whether cognitive deficits can be ameliorated pharmacologically, and if improvements depend on early intervention.

FXS is modeled in *Fmr1* knockout (KO) mice (Bakker *et al*. 1994) that display several characteristics of FXS, including impaired social interactions, locomotor hyperactivity, and decreased passive avoidance learning (Kooy *et al*. 1996; Mineur *et al*. 2002; Yan *et al*. 2004). Remarkably, all of these behavioral phenotypes are normalized in *Fmr1* KO mice by lithium treatment (Min *et al*. 2009; Mines *et al*. 2010; Yuskaitis *et al*. 2010a; Liu *et al*. 2011). Furthermore, lithium is the only agent that has improved performance on a cognitive task in FXS patients in a formal trial setting (Berry-Kravis *et al*. 2008). Since lithium is safely used in patients with bipolar disorder, including children and adolescents (Alessi *et al*. 1994; Ryan *et al*. 1999; Findling *et al*. 2011), these findings suggest that lithium is a promising therapeutic agent for FXS.

Here we tested if lithium treatment can reverse several cognitive deficits in *Fmr1* KO mice. We also tested if the beneficial effects of lithium treatment on cognitive tasks in *Fmr1* KO mice differ between young and adult mice, since the lack of FMRP during development may have established irreversible morphological and neuronal abnormalities that preclude effective intervention in adults. Administration of lithium to lactating mothers or to pups immediately upon weaning at 3 weeks of age results in retarded growth of the pups (Min et al., 2009), therefore lithium administration was initiated when mice reached 4 weeks of age to compare its effects on performance in cognitive tasks in young (from 4–8 weeks of age) mice with adult-treated (from 8–12 weeks of age) mice. Additionally, the effects of lithium withdrawal from mice treated during adolescence were examined to test if lithium-induced improvements in cognitive task performance by *Fmr1* KO mice required continual lithium treatment or if they remained stable once repaired, which may occur if lithium treatment resulted in long-lasting repairs of deficits in neural circuitry or neurogenesis in *Fmr1* KO mice. Significant deficits in *Fmr1* KO mice were found in object novelty detection, temporal order memory, and spatial learning tests, and each of these was improved by chronic lithium treatment of adolescent and adult mice, whereas the cognitive deficits were reinstated after four weeks of lithium withdrawal. These results further support the potential benefits of lithium treatment in FXS.

Materials and methods

Mice

This study used male C57Bl/6J littermates, with or without a disruption of the *Fmr1* gene (originally kindly provided by Dr. W. Greenough, University of Illinois). Mice were weaned 3 weeks after birth, group housed, tested between 1000 and 1400, and 7–20 mice were used in each experiment as described in the figure legends. The *Fmr1* KO mice were generated by breeding male C57BL/6J hemizygous *Fmr1* KO mice and female C57Bl/6J heterozygous *Fmr1* KO mice to generate male homozygous *Fmr1* KO mice and wild-type (WT) littermates. Genotypes were determined by PCR using the Jackson Laboratory protocol for genotyping *Fmr1* mice. To test chronic lithium treatment, *Fmr1* KO mice and WT mice were given water *ad libitum*, and were fed either normal 18% protein rodent diet or the same diet with 0.2% lithium carbonate (both from Teklad, Madison, WI) with provision of an additional bottle containing saline to prevent hyponatremia. This is a therapeutically relevant treatment regimen that produces serum lithium concentrations of 0.6–0.8 mM (Chen *et al*. 2000; O'Brien *et al*. 2004; Shaltiel *et al*. 2008; Jope 2011; Contestabile *et al*. 2013), within the 0.5–1.2 mM range that is therapeutic in human patients. Adult mice were treated with lithium for 4 weeks and throughout the behavioral tests. For lithium treatment during adolescence, mice were treated with lithium from 4 until 8 weeks of age and throughout the behavioral tests, then lithium was discontinued for 4 weeks, and the mice were retested.

Mice were housed in light and temperature controlled rooms and treated in accordance with NIH and University of Miami Institutional Animal Care and Use Committee regulations.

Object novelty detection task

Recognition memory for a novel object compared to a familiar object was assessed by the object novelty detection task (Hoge & Kesner 2007; Hunsaker & Kesner 2008; Hunsaker *et al.* 2012). For this task, a Plexiglas box (26 cm long × 20 cm wide × 16 cm tall) and four objects in duplicate (4–6 cm diameter × 2–6 cm height) were used. During the first session, two copies of Object 1 were placed at each end of the box, and the mouse was allowed to explore the objects for 5 min. The mouse was then removed to an opaque holding container for 5 min, and the objects were replaced with two copies of Object 2 for the next session. After 5 min exploring during session 2, the mouse was placed in the holding container and the copies of Object 2 were replaced with duplicates of Object 3. Following exploration during session 3, the mouse was removed and the objects were replaced by an unused copy of Object 1 and a novel Object 4 for the mouse to explore during the 5 min test session. More time exploring the novel Object 4 compared to the familiar Object 1 indicates that the mouse remembered previously exploring Object 1, but equal exploration time between the two objects indicates that the mouse has impaired recognition memory. Object exploration was defined as the mouse sniffing or touching the object with its nose, vibrissa, mouth, or forepaws, and time spent near or standing on top of the objects without interacting with the object was not counted as exploration. Exploration time of the novel and familiar object is presented, and changes in object exploration ratio were calculated as: (exploration time of Object 4 − exploration time of Object 1)/ (exploration time of Object 1 + exploration time of Object 4). This calculation constrains the ratios to be between −1 and 1, and a ratio approaching 1 indicates an intact memory of Object 1.

For this and all other behavioral assessments, the sessions were filmed, a white noise generator (55 dB) was used, and each apparatus and object was cleaned with 70% ethanol between each test session.

Temporal ordering for objects task

Temporal order memory was assessed using the temporal ordering for objects task (Mitchell & Laiacono, 1998; Hannesson *et al.*; 2004; Hoge & Kesner 2007; Hunsaker *et al.* 2012). Similar to the object novelty detection task, the same box was used and a mouse received three sessions to explore two copies of a new set of objects (Objects 5, 6, 7). For the 5 min test session, an unused copy of Object 5 and an unused copy of Object 7 were placed in the box and the mouse was allowed to explore. A mouse with normal temporal order memory spends more time exploring the first object (Object 5) presented compared to the most recent object (Object 7). Time exploring Object 5 and Object 7 are presented, and changes in object exploration ratio were calculated as: (exploration time of Object 5 − exploration time of Object 7)/ (exploration time of Object 5 + exploration time of Object 7).

Coordinate spatial processing task

Spatial memory was assessed in mice using the coordinate and categorical spatial processing tasks (Goodrich-Hunsaker *et al.* 2005; Goodrich-Hunsaker *et al.* 2008; Hunsaker *et al.* 2009; Hunsaker *et al.* 2012). The coordinate spatial processing task consisted of a 15 min habituation session, a 5 min holding time, and a 5 min test session. For the habituation session a mouse was placed at the edge of the table facing 2 different objects spaced 45 cm apart, and the mouse was allowed to explore the table and the objects for 15 min. Then the mouse was placed in an opaque holding container for 5 min. For the test session, the objects were moved closer together so that they were 30 cm apart, and the mouse was allowed to explore the objects for 5 min. Mice that have intact spatial memory display increased

exploration of the objects during the test session compared with the last 5 min of the habituation session. For the coordinate spatial processing task, the exploration ratio was calculated as: (exploration time during the 5 min test session)/ (exploration time during the 5 min test session + exploration time during the last 5 min of the habituation session). Increased exploration during the 5 min test session compared to the last 5 min of the habituation session is indicated by a ratio >0.5.

Categorical spatial processing task

Like the coordinate spatial processing task, the categorical spatial processing task (Goodrich-Hunsaker *et al*. 2005; Goodrich-Hunsaker *et al*. 2008; Hunsaker *et al*. 2009; Hunsaker *et al*. 2012), is used to assess spatial memory with 2 novel objects, which are different from the objects used in the coordinate spatial processing task. For the habituation session, a mouse was placed on the edge of the table facing 2 different objects that were spaced 45 cm apart and allowed to explore the table and objects for 15 min. Then the mouse was placed in an opaque container for 5 min, and the position of the objects was interchanged, while the distance was maintained. For the test session, the mouse was allowed to explore the objects for 5 min. Increased exploration of the objects during the test session compared with the last 5 min of the habituation phase indicates that the mice remember the object positions. The same exploration ratio was calculated for the categorical spatial processing task as in the coordinate spatial processing task.

Statistical analysis

Statistical significance was assessed by two factor ANOVA with genotype and treatment as factors followed by Bonferroni's multiple comparison tests (for lithium treatment in WT and *Fmr1* KO adult and adolescent mice), or by one factor ANOVA (for discontinued lithium treatment in WT and *Fmr1* KO mice), or Student's t-test (for time spent with each object in the object novelty detection task and the temporal ordering for objects task).

Results

Chronic lithium treatment significantly improves object novelty detection in Fmr1 KO mice

We tested if cognition was impaired in *Fmr1* KO mice in four hippocampus-dependent learning tasks, if chronic lithium treatment repaired cognitive deficits in *Fmr1* KO mice and if there were different outcomes after lithium was administered to adolescent mice (from 4–8 weeks of age) or adult mice (from 8–12 weeks of age). The object novelty detection task is a dentate gyrus-dependent task that assesses the ability to discriminate between familiar and novel objects, indicated by more time spent exploring a novel object than a familiar object (Otto & Eichenbaum 1992; Knight 1996; Dolan & Fletcher 1997; Lisman 1999; Hunsaker & Kesner 2008). Previous reports show that novel object recognition is impaired in *Fmr1* KO mice (Ventura *et al*. 2004; Pacey *et al*. 2011; Bhattacharya & Klann 2012). WT mice spent significantly more time exploring the novel object than the familiar object, whereas *Fmr1* KO mice spent equivalent amounts of time exploring each object (Figure 1A). Thus, there was a significant interaction between genotype and treatment and the object exploration ratio differed between *Fmr1* KO and WT mice (Figure 1B). The impairment in object novelty detection in *Fmr1* KO mice was corrected by lithium treatment, and lithium was equally effective after administration to adult or adolescent mice (Figure 1A). Lithium treatment of adolescent or adult WT mice did not alter performance in the object novelty detection task. In either adolescent or adult *Fmr1* KO mice that were treated with lithium, the exploration ratio was significantly increased to a level equivalent to that of WT mice (Figure 1B). These results demonstrate that object novelty detection is impaired in *Fmr1* KO mice, and that lithium treatment of adolescent or adult *Fmr1* KO mice corrects this impairment.

Lithium treatment normalizes temporal order memory in Fmr1 KO mice

The temporal ordering for objects is a hippocampal CA1-dependent task that is exhibited by mice spending less time with an object most recently presented in the previous habituation session (Honey *et al.* 1998; Wallenstein *et al.* 1998; Lisman 1999; Rolls & Kesner 2006; Hoge & Kesner 2007; Hunsaker *et al.* 2008; Hunsaker *et al.* 2012). WT mice, but not *Fmr1* KO mice, spent more time exploring the first object presented than the most recent object presented (Figure 2A). There was a significant interaction between genotype and treatment in the temporal ordering task, and the object exploration ratio differed significantly between *Fmr1* KO and WT mice (Figure 2B). The impairment in temporal order memory in *Fmr1* KO mice was corrected by lithium treatment, and lithium was similarly effective after administration to adult mice or adolescent mice (Figure 2A). Lithium treatment of adolescents or adults significantly increased the exploration ratio in *Fmr1* KO mice, whereas lithium treatment did not affect the performance of WT mice in this task (Figure 2B). Thus, temporal order memory is impaired in *Fmr1* KO mice, and is repaired by lithium treatment of adolescent or adult *Fmr1* KO mice.

Treatment with lithium repairs spatial memory impairment in Fmr1 KO mice

The coordinate and categorical spatial learning tasks assess metrical and topological spatial pattern separation, respectively, in similar spaces (Save *et al.* 1992; Tsien *et al.* 1996; Long & Kesner 1996; Lisman 1999; Goodrich-Hunsaker *et al.* 2005; Hunsaker *et al.* 2009; Goodrich-Hunsaker *et al.* 2008; Hunsaker *et al.* 2009; Hunsaker *et al.* 2012). The coordinate spatial learning task involves measuring the time spent exploring two objects after the objects have been moved closer together compared to the last 5 min of the habituation period. There was a significant interaction between genotype and treatment in the coordinate spatial learning task indicating that *Fmr1* KO mice exhibited an impaired object exploration ratio compared to WT mice, demonstrating a deficit in coordinate spatial memory in *Fmr1* KO mice (Figure 3A). Lithium treatment of adult or adolescent *Fmr1* KO mice normalized coordinate spatial memory to that of WT mice, but did not affect the performance of WT mice.

The categorical spatial learning task assesses the time spent exploring two objects after the position of the objects is transposed, with the distance unchanged, following the habituation phase. *Fmr1* KO mice spent significantly less time than WT mice exploring the objects after the objects had been transposed, and there was a significant interaction between genotype and treatment (Figure 3B), revealing impaired categorical spatial memory in *Fmr1* KO mice. Administration of lithium did not alter the amount of time that WT mice spent exploring the objects that were transposed, but lithium treatment of adult or adolescent *Fmr1* KO mice significantly increased the exploration ratio. Thus, the results of the coordinate and categorical spatial learning tests reveal impaired spatial pattern learning in *Fmr1* KO mice, and that this is significantly improved by lithium treatment of either adolescents or adults.

Learning deficits in Fmr1 KO mice are reinstated following lithium withdrawal

To determine if lithium's enhancing effects on cognition in *Fmr1* KO mice are sustained following lithium withdrawal, chronic lithium treatment was discontinued after the behavior tests in mice treated from 4 weeks until 8 weeks of age. Four weeks later the *Fmr1* KO and WT mice were retested in all cognitive tasks. Prior testing in the same paradigms had no effect on re-test performance in the WT mice or *Fmr1* KO mice that were not treated with lithium (Figures 4 and 5). Following lithium withdrawal, WT mice exhibited normal object novelty detection, indicating that there was no effect of lithium withdrawal in WT mice (Figure 4A). However, *Fmr1* KO mice that were withdrawn from lithium treatment spent significantly less time exploring the novel object, revealing that impaired object novelty detection returned following lithium withdrawal in *Fmr1* KO mice. *Fmr1* KO mice that had

been withdrawn from lithium demonstrated a significantly reduced object exploration ratio compared to WT mice that had been withdrawn from lithium (Figure 4B). The results show that the impairment in object novelty detection in *Fmr1* KO mice returned following lithium withdrawal.

Discontinuation of lithium treatment also reinstated the temporal order memory deficit in *Fmr1* KO mice without affecting WT mice. *Fmr1* KO mice that were discontinued from lithium treatment demonstrated deficient temporal order memory, whereas temporal order memory was unaltered by lithium withdrawal in WT mice (Figure 4C). The object exploration ratio was significantly reduced in *Fmr1* KO, compared with WT, mice withdrawn from lithium (Figure 4D). Thus the effect of lithium in the temporal order task was not sustained in *Fmr1* KO mice following four weeks of lithium withdrawal.

Coordinate and categorical spatial memory impairments also returned in *Fmr1* KO mice after lithium was withdrawn. Although WT mice maintained intact spatial memory, untreated and previously treated *Fmr1* KO mice displayed significantly reduced object exploration ratios compared to WT mice in the coordinate spatial task (Figure 5A) and in the categorical spatial task (Figure 5B). Thus, the improvements in coordinate and categorical spatial processing in *Fmr1* KO mice induced by lithium treatment were reversed when lithium treatment was discontinued.

Discussion

Here we report impaired cognition in adult *Fmr1* KO mice in four hippocampus-dependent learning and memory tasks, and that each of these was significantly improved by chronic lithium treatment. Furthermore, lithium treatment was equally effective in ameliorating cognitive deficits when administered to adult or adolescent *Fmr1* KO mice. Importantly, lithium administration did not affect the performance of adult or adolescent WT mice in these cognitive tasks, demonstrating an *Fmr1* KO-specific improvement in learning and memory. Discontinuation of lithium treatment caused cognitive impairments to return in *Fmr1* KO mice, but lithium withdrawal did not alter the performance of WT mice.

Although the predominant characteristic of FXS is intellectual disability, severe cognitive deficits were initially difficult to identify in the *Fmr1* KO mouse model. *Fmr1* KO mice display modest cognitive deficits in several hippocampus-dependent tasks, such as the Morris water maze, radial arm maze, and operant conditioning paradigms (Bakker 1994; Kooy *et al*. 1996; D'Hooge *et al*. 1997; Fisch *et al*. 1999; Paradee *et al*. 1999; Peier *et al*. 2000; Mineur *et al*. 2002). *Fmr1* KO mice also exhibit deficits in fear motivated learning tasks, including passive and active avoidance behaviors, and contextual, conditioned and trace fear memory (Yan *et al*. 2004; Qin *et al*. 2005; Zhao *et al*. 2005; Brennan *et al*. 2006; Hayashi *et al*. 2007; Baker *et al*. 2010; Guo *et al*. 2011). Recently, severe deficits in non-aversive learning and memory tasks, including novel object recognition and context discrimination, have been identified in *Fmr1* KO mice (Pacey *et al*. 2011; Eadie *et al*. 2012; Bhattacharya & Klann 2012). In the present study, *Fmr1* KO mice exhibited significant impairments in recognition memory, working memory, and short-term memory that are assessed in the object novelty detection task and the temporal ordering for objects task, and spatial memory measured in the coordinate and categorical spatial processing tasks (Goodrich-Hunsaker *et al*. 2005; Hoge & Kesner 2007; Hunsaker & Kesner 2008; Goodrich-Hunsaker *et al*. 2008; Hunsaker *et al*. 2009; Hunsaker *et al*. 2012). As previously discussed (Mineur *et al*. 2002; Ventura *et al*. 2004; Spencer *et al*. 2008; Hagerman *et al*. 2009; Baker *et al*. 2010; Bhogal & Jongens 2010; Guo *et al*. 2011; Bagni *et al*. 2012), similar deficits have been identified in patients with FXS, such as impaired recognition memory, working memory, short-term memory, and spatial memory (Kemper *et al*. 1988; Cornish *et al*. 1999;

Orstein *et al*. 2008; Gatto & Broadie 2009). Thus, the cognitive deficits displayed by *Fmr1* KO mice may model some of the impairments in nonverbal measures of cognitive functions in FXS patients.

Chronic lithium treatment proved to be remarkably effective in essentially normalizing severe deficits in *Fmr1* KO mice in novel object detection, temporal ordering for objects, and coordinate and categorical spatial processing tasks. The lithium treatments were designed to test the hypothesis that treatment of younger *Fmr1* KO mice would be more effective than treatment of adult *Fmr1* KO mice. This was based on the finding that FMRP is more highly expressed in young than adult mouse brain (Lu *et al*. 2004), raising the possibility that its absence may produce irreversible deficits in adult *Fmr1* KO mice. However, lithium treatment was equally effective in adolescent and adult *Fmr1* KO mice in reversing cognitive deficits. This is an encouraging finding that suggests some cognitive impairments may be pharmacologically reversible even with post-adolescent administration in FXS, although caution must be exercised in translating results from *Fmr1* KO mice. However, it is encouraging that lithium administration improved performance on a cognitive task in a small trial in FXS patients (Berry-Kravis *et al*. 2008).

The improvements in cognitive tasks reported here add to an extensive number of abnormal phenotypes that are improved by lithium treatment of *Fmr1* KO mice. Phenotypes in *Fmr1* KO mice that have been reported to be improved by lithium treatment include locomotor hyperactivity, audiogenic seizure hypersensitivity, increased spine density, macroorchidism, excess protein synthesis, social behavior deficits, deficient passive avoidance learning, and synaptic plasticity (Min *et al*. 2009; Yuskaitis *et al*. 2010a, Yuskaitis *et al*. 2010b; Mines *et al*. 2010; Liu *et al*. 2011; Choi *et al*. 2011; Liu *et al*. 2012). Improvement of cognition by lithium treatment correlates well with previous findings in *Fmr1* KO mice of altered synaptic plasticity, measured as long-term potentiation (LTP) and long-term depression (LTD). *Fmr1* KO mice display enhanced metabotropic glutamate receptor (mGluR)-dependent LTD at hippocampal CA1 synapses (Huber *et al*. 2002; Hou *et al*. 2006; Nosyreva & Huber 2006) and deficient LTP at medial perforant path synapses in the dentate gyrus (Eadie *et al*. 2012). Lithium treatment in adolescent *Fmr1* KO mice (from 5–6 weeks of age until 9–11 months of age) or adult *Fmr1* KO mice (from 8 weeks of age to 4–5 months of age) normalized mGluR-dependent LTD in the hippocampus, without affecting WT mice (Choi *et al*. 2011). Lithium inhibits glycogen synthase kinase-3 (GSK3) (Klein & Melton 1996), lithium treatment reduces abnormally hyperactive GSK3 in *Fmr1* KO mice (Min *et al*. 2009; Yuskaitis *et al*. 2010a), and hyperactive GSK3 impairs LTP and promotes LTD (Hooper *et al*. 2007; Zhu *et al*. 2007). Taken together, these findings suggest that inhibition of GSK3 by lithium contributes to the normalization of synaptic plasticity and cognition in *Fmr1* KO mice, although this conjecture will require further examination. The cognitive-enhancing actions of lithium in *Fmr1* KO mice are clearly dependent on the continued presence of lithium, since cognitive deficits were equivalent in *Fmr1* KO mice withdrawn from lithium and *Fmr1* KO mice that had never been given lithium. Thus, lithium treatment must be sustained in *Fmr1* KO mice for cognitive benefits to persist.

In summary, lithium treatment of adolescent or adult *Fmr1* KO mice is safe, and effectively remediates performance in several cognitive tasks, as well as providing many previously reported beneficial effects in *Fmr1* KO mice. These results extend previous findings that lithium ameliorates synaptic plasticity and/or cognitive deficits in *Fmr1* KO flies (McBride *et al*. 2005), mice (Yuskaitis *et al*. 2010a; Liu *et al*. 2011; Choi *et al*. 2011) and FXS patients (Berry-Kravis *et al*. 2008). Thus, there is increasing evidence that lithium may provide therapeutic benefits in FXS.

Acknowledgments

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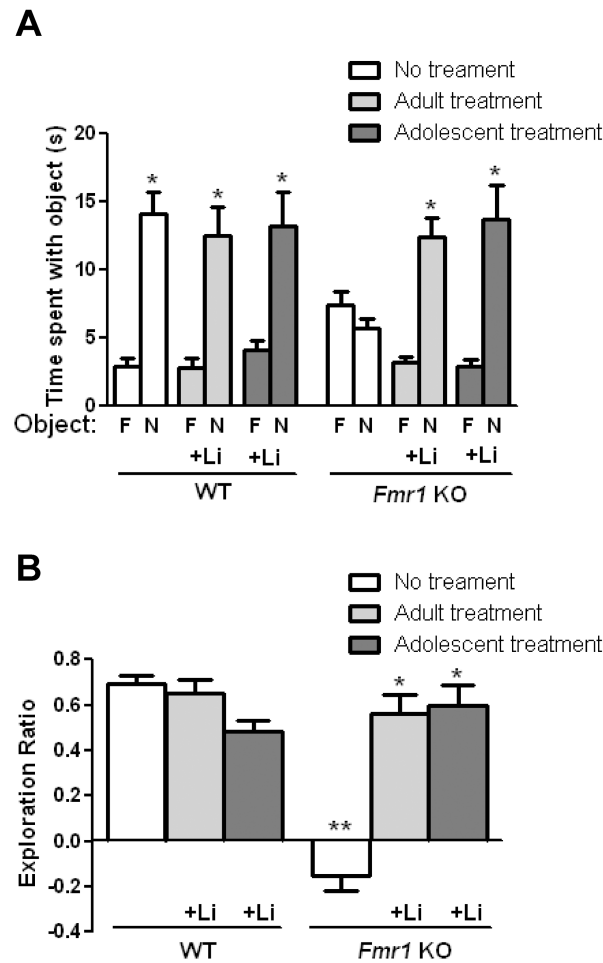
A

B

Figure 1. Chronic lithium treatment of adult or adolescent *Fmr1* KO mice reverses impaired discrimination in the object novelty detection task

Lithium was administered for four weeks to adult (from 8 to 12 weeks of age) and adolescent (from 4 to 8 weeks of age) male *Fmr1* knockout (KO) and wild-type (WT) mice prior to testing. (A) Times spent exploring the novel (N) and familiar (F) object. (Student's t-test; *p<0.05 compared to time spent with familiar object; WT no treatment: n=20, t(46)=6.51, p<0.05; WT adult lithium treatment: n=10, t(18)=4.29, p<0.05; WT adolescent lithium treatment: n=9, t(10)=3.47, p<0.05; *Fmr1* KO no treatment: n=20, t(48)=1.42, p>0.05; *Fmr1* KO adult lithium treatment: n=10, t(18)=6.20, p<0.05; *Fmr1* KO adolescent lithium treatment: n=9, t(16)=4.19, p<0.05). (B) Exploration ratio. (two-way ANOVA (genotype x treatment) followed by post hoc Bonferroni's multiple comparison test; F(2,72)=33.02, p<0.05; **p<0.05 compared to untreated WT mice; *p<0.05 compared to same genotype without treatment).
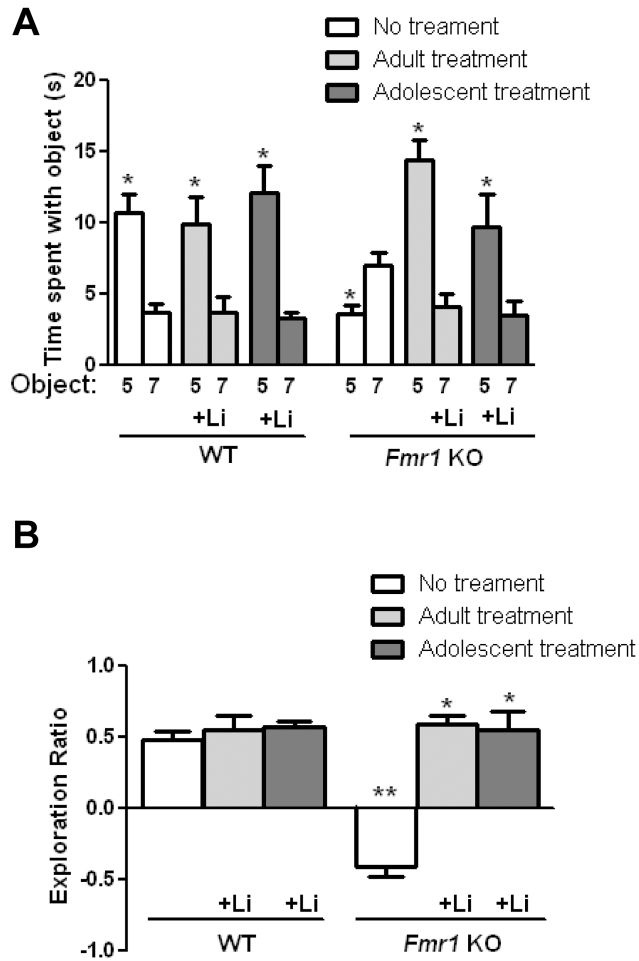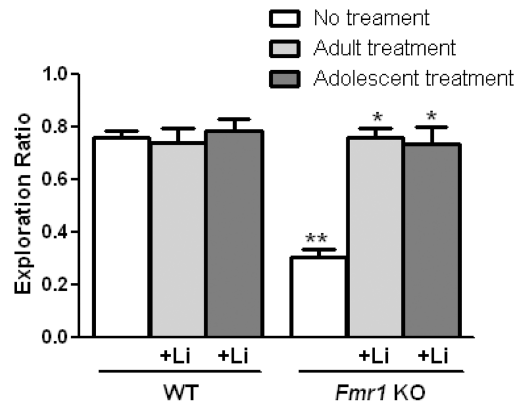
Figure 2. Chronic lithium treatment of adult or adolescent *Fmr1* KO mice ameliorates temporal order memory deficits

Adult and adolescent male *Fmr1* KO and WT mice were treated with lithium for 4 weeks prior to testing. (A) Times spent exploring the first object presented (Object 5) and the object most recently explored (Object 7). (Student's t-test; *p<0.05 compared to time spent with Object 7; WT no treatment: n=20, t(38)=4.82, p<0.05; WT adult lithium treatment: n=10, t(18)=2.74, p<0.05; WT adolescent lithium treatment: n=9, t(12)=4.56, p<0.05; *Fmr1* KO no treatment: n=20, t(38)=3.16, p<0.05; *Fmr1* KO adult lithium treatment: n=9, t(16)=6.21, p<0.05; *Fmr1* KO adolescent lithium treatment: n=9, t(16)=2.38, p<0.05). (B) Exploration ratio. (two-way ANOVA (genotype x treatment) followed by post hoc Bonferroni's multiple comparison test; F(2,75)=27.48, p<0.05; **p<0.05 compared to untreated WT mice; *p<0.05 compared to same genotype without treatment).

A Coordinate spatial processing
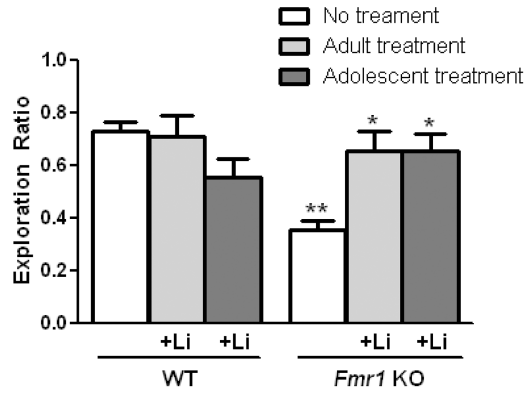
B Categorical spatial processing

Figure 3. Chronic lithium treatment of adult or adolescent *Fmr1* KO mice alleviates spatial processing impairments in *Fmr1* KO mice

Adult and adolescent male *Fmr1* KO and WT mice were treated with lithium for 4 weeks prior to testing. (A) Exploration ratio in the coordinate spatial processing task (two-way ANOVA (genotype x treatment) followed by post hoc Bonferroni's multiple comparison test; F(2,68)=10.68, p<0.05). (B) Exploration ratio in the categorical spatial processing task (two-way ANOVA (genotype x treatment) followed by post hoc Bonferroni's multiple comparison test; F(2,69)=24.93, p<0.05). **p<0.05 compared to untreated WT mice; *p<0.05 compared to same genotype without treatment; n=20 WT no treatment; n=10 WT adult treatment; n=9 WT adolescent treatment; n=20 *Fmr1* KO no treatment; n=10 *Fmr1* KO adult treatment; n=9 *Fmr1* KO adolescent treatment.
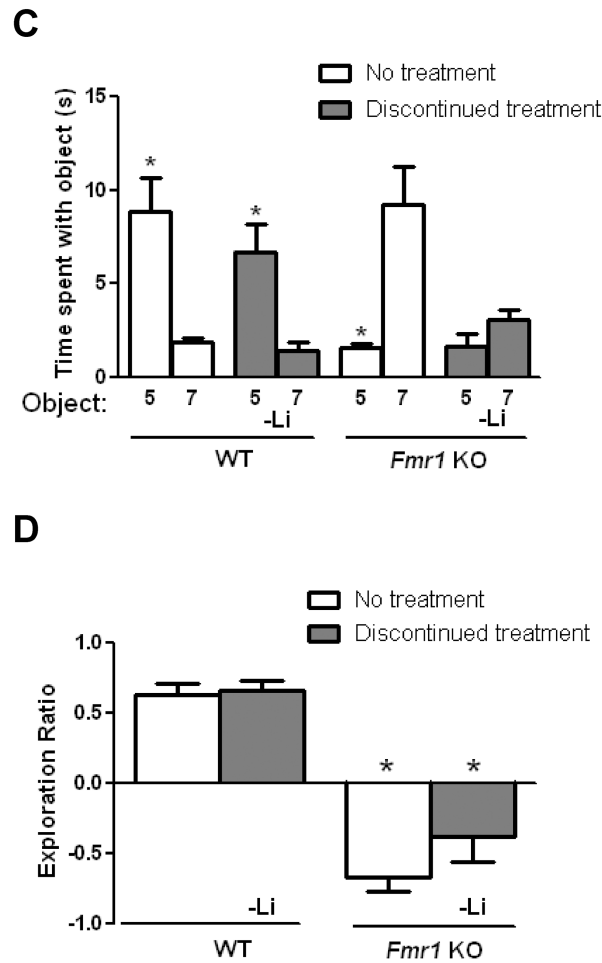
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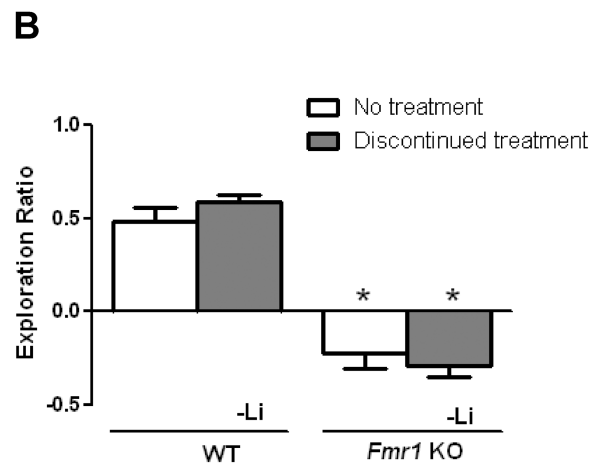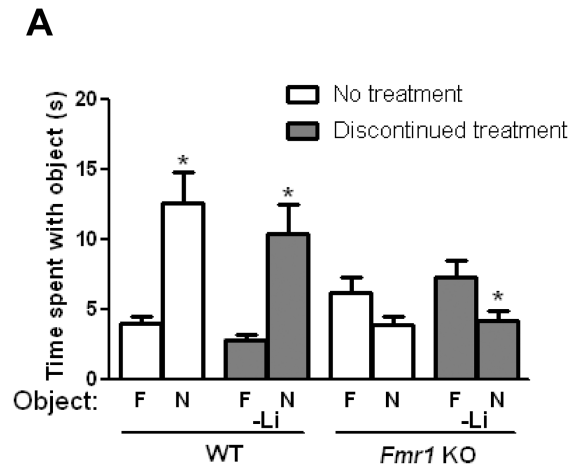
Figure 4. Impaired cognitive deficits are reinstated in *Fmr1* KO mice following discontinuation of lithium in the object novelty task and temporal ordering for objects task
Adolescent male *Fmr1* KO and WT mice were treated with lithium for 4 weeks. After testing, lithium treatment was discontinued for 4 weeks and the mice were retested. Prior testing in the same paradigms had no effect on re-test performance in untreated WT mice or *Fmr1* KO mice (Student's t-test, p>0.05 compared to retest; object novelty detection task: WT no treatment: n=7, t(12)=0.78, p>0.05; FX no treatment: n=9, t(16)=0.29, p>0.05; temporal ordering for objects task: WT no treatment: n=7, t(12)=0.68, p>0.05; FX no treatment: n=9, t(16)=1.42, p>0.05). (A,B) Performance in the object novelty detection task. (A) Times spent exploring the novel (N) and familiar (F) object. (Student's t-test; *p<0.05 compared to time spent with familiar object; WT no treatment: n=7, t(12)=3.78, p<0.05; WT discontinued lithium treatment: n=7, t(12)=3.57, p<0.05; *Fmr1* KO no treatment: n=9, t(16)=1.87, p>0.05; *Fmr1* KO discontinued lithium treatment: n=9, t(16)=2.28, p<0.05) (B) Exploration ratio. (one-way ANOVA followed by post hoc Bonferroni's multiple comparison test; F(3,28)=47.41). *p<0.05 compared to matched WT mice. (C,D) Performance in the temporal ordering for objects task. (C) Times spent exploring the first object presented (Object 5) and the object most recently explored (Object 7). (Student's t-test; *p<0.05 compared to time spent with Object 7; WT no treatment: n=7, t(12)=3.87, p<0.05; WT discontinued lithium treatment: n=9, t(12)=3.48, p<0.05; *Fmr1* KO no treatment: n=9, t(16)=3.75, p<0.05; *Fmr1* KO discontinued lithium treatment: n=9, t(16)=1.75, p>0.05). (D) Exploration ratio. (one-way ANOVA followed by post hoc

Bonferroni's multiple comparison test; $F_{(3,28)}=28.80$, $p<0.05$). *$p<0.05$ compared to matched WT mice.
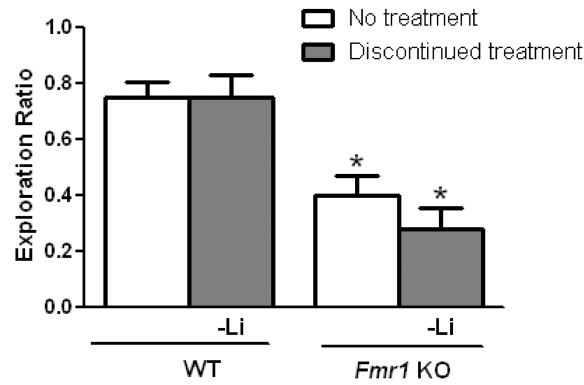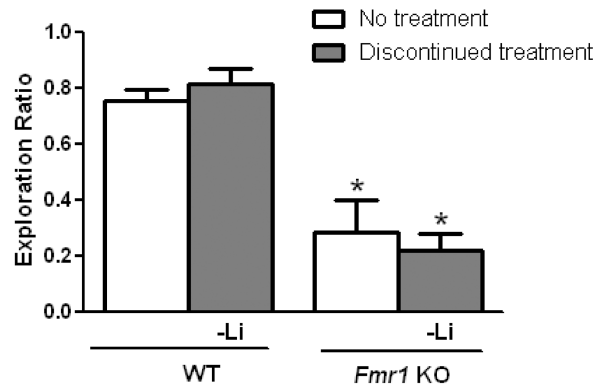
A Coordinate spatial processing

B Categorical spatial processing

Figure 5. Spatial processing impairments return in *Fmr1* KO mice following discontinuation of lithium

Adolescent male *Fmr1* KO and WT mice were treated with lithium for 4 weeks and then following testing, lithium treatment was discontinued for 4 weeks and the mice were retested. Prior cognitive testing in the same paradigms had no effect on re-test performance in untreated WT mice or *Fmr1* KO mice (Student's t-test, $p>0.05$ compared to retest; coordinate spatial processing task: WT no treatment: n=7, $t(12)=0.32$, $p>0.05$; FX no treatment: n=9, $t(16)=0.27$, $p>0.05$; categorical spatial processing task: WT no treatment: n=7, $t(12)=0.24$, $p>0.05$; FX no treatment: n=9, $t(16)=0.28$, $p>0.05$). (A) Exploration ratio in the coordinate spatial processing task. (one-way ANOVA followed by post hoc Bonferroni's multiple comparison test; $F(3,29)=11.34$, $p<0.05$). (B) Exploration ratio in the categorical spatial processing task. (one-way ANOVA followed by post hoc Bonferroni's multiple comparison test; $F(3,27)=17.29$, $p<0.05$). *$p<0.05$ compared to matched WT mice. n=7 WT no treatment; n=7 discontinued lithium treatment; n=9 *Fmr1* KO no treatment; n=9 *Fmr1* KO discontinued lithium treatment.