Lithium ameliorates open-field and elevated plus maze behaviors, and brain phospho-glycogen synthase kinase 3β expression in fragile X syndrome model mice

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ABSTRACT

الأهداف: دراسة فيما إن كان الليثيوم يحسن سلوك المتاهة ذو الارتفاع الزائد وتعبير P–GSK3β الدماغي.

الطريقة: اشتملت الدراسة على 180 فئران برية ومنتفضة جينياً بلغت أعمارهم 30 يوم. في اليوم الخامس، تم استخدام المتاهة ذو الارتفاع الزائد لقياس السلوك واللطخة لقياس تعبير P–GSK3β. اشتملت الدراسة على مجموعة التحكم ومجموعة الليثيوم استخدمت الجرعات التالية 30، 60، 90، 120، 200 ملغرام/كلغ. أجريت التجربة في معهد العلوم العصبية، المستشفى التابع لجامعة قوانقتشو، الصين خلال الفترة من يناير حتى يونيو 2012م.

النتائج: يقلل الليثيوم من المسافة الكلية، ووقت المنطقة الوسطى، ودخول المركزي في اختبار الحقل المفتوح (p<0.05) ويقلل من ملاحقة الذراع المفتوحة ودخول الذراع ووقت الذراع المفتوح في المتاهة ذو الارتفاع الزائد (p<0.05) وذلك في الفئران البرية. وظهرت اختلافات إحصائية مهمة في الفئران البرية والمنتفضة جينياً في اختبارات السلوك كلها في مجموعات العلاج. كما أن الليثيوم يحسن تعبير P–GSK3β في الحصين لجميع مجموعات العلاج في الفئران البرية (p<0.05). ولكن الليثيوم لا يحسن من تعبير GSK3β في أنسجة الفئران البرية أو تعبير GSK3β و P–GSK3β في أنسجة الفئران البرية.

خاتمة: يحسن الليثيوم من سلوك المتاهة ذو الارتفاع الزائد في الفئران البرية. الأمر الذي يحسن من تعبير P–GSK3β. تشير نتائجنا أن الليثيوم له أثر علاجي لمتلازمة كروموسوم X المنكسر.

Objective To investigate whether lithium modifies open-field and elevated plus maze behavior, and brain phospho-glycogen synthase kinase 3 (P-GSK3β) expression in Fmr1 knockout mice.

Methods: One hundred and eighty FVB mice, including knockout and wild type, with an age of 30 days were used. An open-field and elevated plus maze was utilized to test behavior, while western blot was used to measure the P-GSK3β expression. Six groups were formed: control (saline), lithium chloride 30, 60, 90, 120, and 200 mg/kg. The experiments were carried out in the Institute of Neuroscience, Second Affiliated Hospital of Guangzhou Medical University, Guangzhou, China between January and June 2012.

Results: Lithium significantly decreased total distance, crossing, central area time, and center entry in the open-field test (p<0.05), and significantly reduced open-arm tracking, open-arm entry, and open-arm time in the elevated plus maze (p<0.05) in knockout mice. In wild type mice, significant changes were observed in both behavior tests in some treatment groups. Lithium ameliorated P-GSK3β expression in the hippocampus of all the treatment groups in knockout mice (p<0.05). However, lithium did not modify either GSK3β expression in tissues of knockout mice, or P-GSK3β or GSK3β expression in tissues of wild type mice.

Conclusion: Lithium ameliorated open-field and elevated plus maze behaviors of Fmr1 knockout mice. This effect may be related to its enhancement of P-GSK3β expression. Our findings suggest that lithium might have a therapeutic effect in fragile X syndrome.

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Fragile X syndrome (FXS) is one of the most common forms of inherited mental retardation. It is caused by an abnormal expansion of a trinucleotide repeat (CGG) in the 5' terminal untranslated region of fragile X mental retardation gene 1 (FMR1),[1] leading to a decrease or absence of expression of fragile X mental retardation protein (FMRP),[2] then causing the typical symptoms of FXS, including macro-orchidism, hypophrenia, learning and memory disorder, autism, anxiety with bipolar disorder, and hyperactivity.[3,4] Other investigators have also identified FMR1 to be an autism related gene.[5] The Fmr1 knockout mouse (KO mouse), in which Fmr1 has been silenced, has been generated and developed to be an effective model for investigating FXS.[6] It presents hyperactivity, and anxiety as in FXS patients.[6] Lithium, a mood stabilizer that has been using in humans for many years,[7] has been identified as having therapeutic effects in FXS mouse models.[8] However, the mechanism of lithium's therapeutic effect remains to be determined. It possibly attributes to lithium's inhibition of glycogen synthase kinase 3 (GSK3).[9] Glycogen synthase kinase 3, a serine/threonine protein kinase, which is involved in a number of central intracellular processes such as cellular proliferation, migration, glucose regulation, and apoptosis, consists of 2 subtypes, GSK3α and GSK3β.[10] It is regulated by phosphorylation on an N-terminal serine, serine-21-GSK3α and serine-9-GSK3β, which inhibits GSK3 activity.[11] The GSK3β is considered to play an important role in brain and behavior, furthermore, it is considered a target that responds to several antipsychotic drugs, including lithium.[12] The open-field test investigates the locomotor activity and general anxiety in rodents.[13] Total distance, central area time, and center entry are measured to evaluate the activity and general anxiety condition of the rodents.[13] The elevated plus maze is an apparatus, which can also measure the anxiety-like behavior in rodents.[14] It evaluates the anxiety status and exploratory behavior of the animal by calculating their activities in the open-arm and the closed-arm.[14] Previous research on the Fmr1 KO mouse has indicated that lithium can recover the characteristic features in Drosophila melanogaster[15] and mouse[8] models of FXS. For instance, it improved the memory and courtship behavior of Drosophila melanogaster,[15] as well as reduced audiogenic seizures and hyperactivity in open-field tests of FVB/NJ FXS mice models.[8] One report[16] showed that the inhibitory serine-phosphorylation of GSK3 is impaired in FVB/NJ Fmr1 KO mice.[16] Another previous study found that lithium chloride ameliorated learning and memory in FVB Fmr1 KO mouse.[17] To date, although many studies have been carried out to investigate the mechanism and treatment of FXS, it is still not clarified. In the current study, we applied open-field and elevated plus maze tests to investigate whether lithium ameliorated the hyperactivity, and anxiety behaviors of FVB KO mouse and modified its P-GSK3β expression. Also, with different concentrations of lithium chloride, we attempted to identify an optimal dosage for FXS mouse model treatment, and find more evidence for the research of therapy of FXS.

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Methods. The FVB Fmr1 KO and its wild type (WT) FVB inbred strain mice were kindly provided by Professor Oostra (Cellular Biology and Genetics Research Center, Erasmus University Rotterdam, Netherlands). Both KO and WT mice were caged separately and maintained on a 12-hour light-dark cycle in the Laboratory Animal Research Center of Guangzhou Medical University, Guangzhou, China. All mice had access to food and water ad libitum. A total of 90 KO and 90 WT mice with an age of 30 days of both genders were used in this study. The experiments were carried out in the Institute of Neuroscience, Second Affiliated Hospital of Guangzhou Medical University between January and June 2012. All the experimental mice were confirmed to be pure breed following a detection method used in previous studies.[18] Both KO and WT mice were randomly divided into 6 groups, with 15 mice in each group. Five treatment groups were intraperitoneally injected with 30, 60, 90, 120, and 200 mg/kg lithium chloride (Sigma, St. Louis, MO, USA),[16] and a control group was intraperitoneally injected with the same volume of normal saline for 6 consecutive days. Drugs were administrated at 9:00 every morning during the experimental procedure. Behavioral tests were carried out 30 minutes after lithium injection. The open-field test was carried out first, followed by elevated plus maze with a one hour break for each mouse between the 2 behavioral tests. Our study was reviewed and approved by the Institutional Animal Care and Use Committee of Guangzhou Medical University. All our experimental procedures were performed according to the NIH Guiding Principles in the Care and Use of Animals, and in accordance with the Guidance Suggestions of Institutional Animal Care and Use Committee of Guangzhou Medical University.

The open-field test was conducted with a black square plastic board measuring 72 cm x 72 cm and a 60 cm tall surrounding wall. The square board was marked with an 18 cm x 18 cm grid. An 18 cm x 18 cm square was set in the center of the field. Another 68 cm x 68 cm square was marked 2 cm away from the edge inside the field. Then from outside to inside the field was divided into 4 regions named region one to 4 in sequence. The fourth region was also called the center region. In this task, mice were placed in region one and moved freely in the field, each animal's movements were recorded by a camera, which was set on the top of the field for 5 minutes. After each trial, the field was cleaned with 75% ethanol. After the tests, the mice tracks were measured using the Smart video tracking system (Harvard Apparatus, Holliston, MA, USA).

The elevated plus maze is an apparatus modified into an elevated maze with 4 arms (2 open and 2 closed).[14] The 2 open-arms are set opposite to each other, and the 2 closed arms are also opposite each other, in a cross shape. The maze was placed in a luminous room, and a camera was set above to trace the rodents. The task began when the mice were placed into the central area of the maze. Each mouse's behavior was also traced by the Smart video tracking system for 5 minutes. To avoid interference of the results by the smell left by a previous mouse, 75% ethanol was used to clean the maze after each experiment. After the tests, the mice tracks were measured by Smart video tracking system manually.

On day 6, the mice were decapitated under deep anesthesia (chloral hydrate, intraperitoneally, Kemiou Chemical Reagent Co, Tianjin, China) and the brains were removed after 30 minutes. The cortex and hippocampus were dissected on ice. Every 100 mg of tissue was mixed with 1000 µl whole-cell lysis buffer, 10µl phenylmethanesulfonyl fluoride, and 10µl phosphatase inhibitor (Guangzhou Maygene Bio-Technology Company, Guangzhou, China), and then homogenated and centrifuged at 14,000 rpm at 4°C for 5 minutes. The supernatant was transferred into different centrifuge tubes and kept at -80°C. The concentration of protein of the supernatant was determined by bicinchoninic acid, protein assay kit (Maygene Bio-Technology Company, Guangzhou, China). Protein was separated in 10% SDS-polyacrylamide gels, and transferred to the polyvinylidene fluoride membranes. Blots were incubated with rabbit anti-GSK3β (1:6000 dilution, Cell Signaling Technology, Beverly, MA, USA) and anti-P-GSK3β (1:2000 dilution, Cell Signaling Technology, Beverly, MA, USA) and mouse GAPDH (1:4000 dilution, Proteintech Group Inc., Chicago, IL, USA) overnight in 4°C. Then, blots were developed by horseradish peroxidase-probed goat anti-rabbit secondary antibodies (1:2000 dilution, Biotime, China) for one hour, at 25°C, followed by detection with enhanced chemiluminescence. The results of the Western blot were quantified by Quantity One (Bio-Rad, Hercules, CA, USA). The results of the treatment and control groups were compared on the same gel and statistical analysis was performed using t-test.

All the results were presented as mean ± SD and analyzed by the Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL USA). In open-field and elevated plus maze test, results between homologous groups of KO and WT were analyzed by independent sample t-test. The results of the 5 tested groups and the control group of KO and WT mice were analyzed by one-way ANOVA. Independent sample t-test was used to analyze the KO and WT mice in Western blot. One-way ANOVA was performed to compare each dose group with the control group of the same strain.

Results. After the open-field tests, between the 2 control groups, the KO mice were more active than the WT mice (**Table 1**). In this task, total distance, crossing, and center entry of KO mice were significantly greater than in WT mice ($p<0.05$) (**Table 1**). However, there was no significant difference between KO and WT mice for the central area time of the field ($p>0.05$) (**Table 1**). After administration of lithium chloride, the KO treatment group had a significant decrease in total distance and crossing compared with the control group

Table 1 - Results of KO and WT control groups in the open-field test.

Groups	Total distance (cm)	Crossing (no. of times)	Central area time (seconds)	Center entry (no. of times)
WT	2083.31±304.71	125.73±9.24	2.48±0.82	4.47±1.13
KO	2688.49±197.04[a]	159.80±10.23[a]	2.91±0.50	5.60±1.05[a]

KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse. [a]$p<0.05$ compared with WT mouse, n=15 in each group

(p<0.05) (**Figure 1A & 1B**). In addition, they spent less time in the central area (p<0.05) (**Figure 1C**). Our results also showed a significant decrease among the 90, 120, and 200 mg/kg groups in total distance (p<0.05), as well as in the 200 mg/kg group in crossing (p<0.05) (**Figure 1D**) in WT mouse. In addition, WT groups with 30, 90 mg/kg lithium chloride injection recorded less central area time in the test (p<0.05) (**Figure 1C**). In both KO and WT mice, the 5 treatment groups showed significantly decreased center entry compared with the control groups (p<0.05) (**Figure 1D**).

For the elevated plus maze, we observed that KO mice had significantly more open-arm time (OT) and open-arm entry (OE), even open-arm tracking (OTR), than WT mice (**Table 2**). After treatment with different dosages of lithium chloride, as the dosage increased the KO mice demonstrated less and less exploration compared with their control groups. They showed significantly less OE and OTR in the open-arms, as well as OT, compared with their control groups (**Tables 3, 4, & 5**). However, we observed modified behaviors in WT mice. Lithium, ranging from 90 to 200 mg/kg, significantly reduced OT, OE, and OTR of WT mice (p<0.05) (**Tables 3, 4, & 5**). Although lithium improved the behavior of KO mice on the elevated plus maze, the KO mice still performed more OT, OE, and OTR than

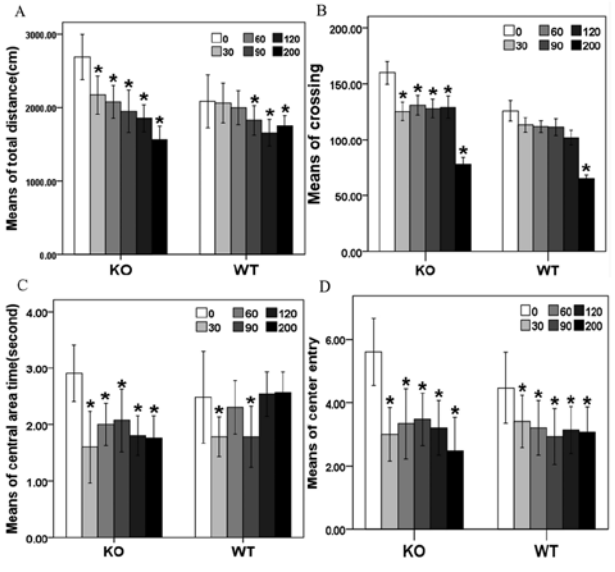

Figure 1 - Results of treatment groups of KO and WT mice in the open-field test. **A**) Total distance among treatment groups between KO and WT mice. **B**) Crossing among treatment groups between KO and WT mice. **C**) Central area time among treatment groups between KO and WT mice. **D**) Center entry among treatment groups between KO and WT mice. KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse. *p<0.05, compared with the control group, n=15 in each group.

Table 2 - Results of KO and WT control groups in the elevated plus maze test.

Variable	KO mice	WT mice
OTR	57.9±6.6[a]	48.7±9.2
OE	49.1±9.5[a]	30.5±2.3
OE/(CE+OE) (%)	62.68	55.83
OT (s)	198.8±16.9[a]	170.9±29.6
OT/(OT+CT) (%)	66.27	56.97

KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse, OTR - open-arm tracking, OE - open-arm entry, CE - close-arm entry, OT - open-arm time, CT - close-arm time. [a]p<0.05 compared with WT control group, n=15 in each group

Table 3 - Open-arm tracking (cm) among treatment groups between KO and WT mice.

Groups	KO mice	WT mice
Control	57.9±6.6[a]	48.7±9.2
30 mg/kg	56.3±4.9[a]	48.5±5.2
60 mg/kg	54.4±6.5[a,b]	47.8±5.3
90 mg/kg	51.6±4.8[a,b]	45.3±5.7[b]
120 mg/kg	49.3±6.2[a,b]	44.3±6.5[b]
200 mg/kg	47.6±4.3[a,b]	43.3±6.2[b]

KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse, [a]p<0.05, compared with homologous WT group, [b]p<0.05 compared with the control group of KO or WT mouse, n=15 in each group

Table 4 - Open-arm entry (OE) (no. of times) among treatment groups of KO and WT mice.

Groups	KO mice	WT mice
Control	49.1±9.5[a]	30.5±12.3
30 mg/kg	45.3±7.9[a,b]	28.5±5.2
60 mg/kg	43.4±8.5[a,b]	27.8±5.3
90 mg/kg	35.6±9.8[a,b]	26.3±5.7[b]
120 mg/kg	31.3±11.2[a,b]	24.3±6.5[b]
200 mg/kg	28.6±14.3[a,b]	22.3±6.2[b]

KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse, [a]p<0.05, compared with the homologous WT group, p<0.05 compared with the control group of KO or WT mouse, n=15 in each group

Table 5 - Open-arm time (OT) (seconds) among treatment groups of KO and WT mice.

Groups	KO mice	WT mice
Control	198.8±16.9[a]	170.9±29.6
30 mg/kg	195.3±13.9[a,b]	168.5±15.2
60 mg/kg	193.4±13.5[a,b]	167.1±15.3
90 mg/kg	185.6±10.8[a,b]	164.2±15.7[b]
120 mg/kg	171.3±11.2[a,b]	162.5±16.5[b]
200 mg/kg	168.6±14.3[a,b]	157.3±16.2[b]

KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse, [a]p<0.05 compared to the homologous WT group, [b]p<0.05 compared with the control group of KO or WT mice, n=15 in each group

their WT counterparts in all of the treatment groups (p<0.05) (**Tables 3, 4, & 5**).

After the behavioral tests, we investigated P-GSK3β and GSK3β expression in the cortex and hippocampus of FVB KO and WT mice. Compared with the WT control group, in both cortex and hippocampus, P-GSK3β expression of the KO control group were significantly decreased (p<0.05) (**Figure 2A**). Nevertheless, there were no significant differences in GSK3β expression in both brain regions between KO and WT mice (p>0.05) (**Figure 2B**). Then, after 6 days' treatment with 5 different dosages of lithium chloride, we found that the P-GSK3β expression increased significantly (p<0.05) in the cortex of the 120 mg/kg and 200 mg/kg treatment groups compared with the control group (**Figure 2D**). In the hippocampus of KO mice, the P-GSK3β expression increased significantly in all of the 5 treatment groups (p<0.05) (**Figure 2C**), compared with the control group. However, in comparison of WT mice and the control group, we observed no significant changes of P-GSK3β expression in either the cortex or hippocampus of all treatment groups (**Figures 2E & 2F**). We also did not find any significant changes in GSK3β expression in both the cortex and hippocampus among all of the treatment groups in both the KO and WT mice (**Figures 2E & 2F**).

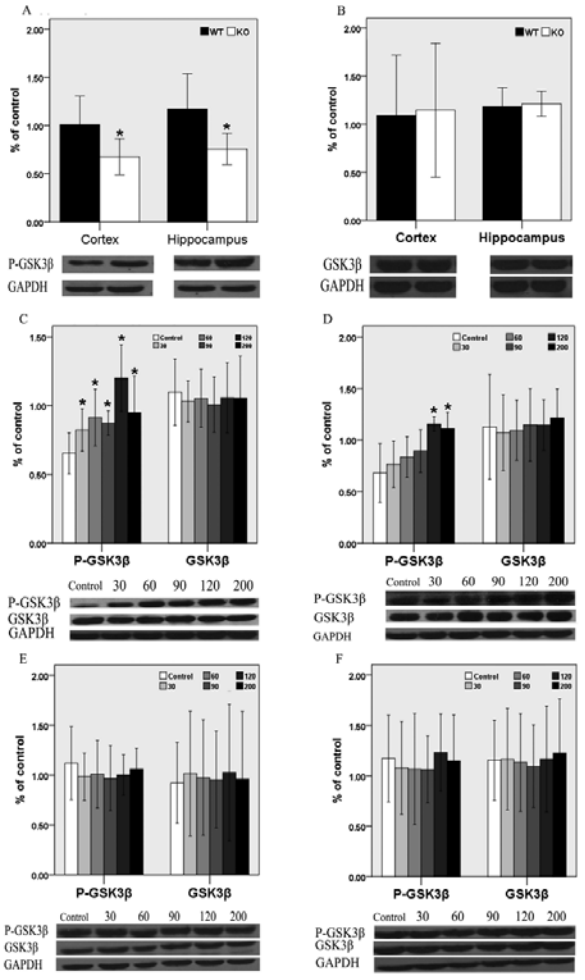

Figure 2 - Western blot results of KO and WT mice. **A)** Phospho-glycogen synthase kinase 3 (P-GSK3β) expression in cortex and hippocampus of WT and KO mice. **B)** Glycogen synthase kinase 3 (GSK3β) expression in cortex and hippocampus of WT and KO mice. **C)** P-GSK3β and GSK3β expression among all of the treatment groups in hippocampus of KO mice. **D)** P-GSK3β and GSK3β expressions among all of the treatment groups in cortex of KO mice. **E)** P-GSK3β and GSK3β expressions among all of the treatment groups in hippocampus of WT mice. **F)** P-GSK3β and GSK3β expressions among all of the treatment groups in cortex of WT mice. KO - FVB Fmr1 knockout mouse, WT - FVB wild type mouse. *p<0.05, compared with control group

Discussion. Fragile X syndrome manifests certain phenotypes such as mental retardation and hyperactivity, which are caused by decreasing or absence of FMRP expression.[19] However, to date there is no effective therapy. Recently, research has demonstrated that lithium might have a therapeutic effect in FXS.[11] Lithium salt has been considered an effective pharmacotherapy for treating bipolar disorder since 1949.[20] Lately, research has inferred that lithium's therapeutic effect in FXS might be partially due to the direct action of GSK3β inhibition.[9,21] Lithium has an indirect inhibitory effect on GSK3β via activation of the PI3K/Akt pathway increasing the production of inhibitory P-GSK3.[22] A previous study[23] showed that acute administration of lithium increased P-GSK3 in the mouse brain. Other studies[8] reported increased P-GSK3β in whole brain regions of FVB Fmr1 KO mice 30 minutes after lithium administration.[8] These effects were shown to involve both P-GSK3β and P-GSK3β expression, with increases occurring to variable extents in different brain regions, and similar reactions in Fmr1 KO and WT mice.[16] The GSK3β is considered an essential target for lithium treatment in research of cellular signaling, neuronal plasticity, and even bipolar disorder.[12] Therefore, we focused on the relationship between changes of the typical phenotype of KO mice and P-GSK3β and GSK3β expressions in the hippocampus and cortex in KO mice, after lithium chloride administration.

In the present study, we treated both KO and WT FVB mice intraperitoneally with 5 different dosages of lithium chloride continuously for 6 days. The cortex of KO mice showed significantly increased expression of P-GSK3β expression in the 120 mg/kg and 200 mg/kg treatment groups compared with the control group. In

the hippocampus of KO mice, P-GSK3β expression increased significantly in all of the 5 treatment groups compared with the control group. However, in WT mice in comparison with the control group, we did not observe significant changes of P-GSK3β expression in either the cortex or the hippocampus of all treatment groups. We found no significant changes in GSK3β expression in both the cortex and the hippocampus among all of the treatment groups of both KO and WT mice. Lithium chloride did not modify P-GSK3β expression in the WT mice, and our results are consistent with other studies, which indicate that lithium chloride merely has therapeutic effect on FVB Fmr1 KO mice, and has no effect on FVB WT mice.[16]

To investigate whether 5 days' lithium treatment would improve the behavioral phenotype of FVB KO mice, we used an open-field and elevated plus maze test. A previous study[24] showed that FVB Fmr1 KO mice appeared hyperactive in the open-field test, and spent more time in the open-arm of the elevated plus maze, when compared with WT mice,[25] which is consistent with our results. In the open-field test, compared with the KO control group, the 5 KO treatment groups showed a significant decrease in total distance and crossing. This indicates an effective reverse in specific hyperactivity of KO mice, inferring that the improvement of abnormal behavior of KO mice may be related to increased P-GSK3β expression in the brains of KO mice.

In the elevated plus maze, prolonged OT and OTR, as well as OE represents a reduction of general anxiety.[14] In our study, we found that in comparison with WT mice, the KO mice recorded significantly longer OT, OTR, as well as OE, in the open-arm, which is consistent with the previous studies.[16] This indicates that KO mice might have a reduced anxiety reaction and increasing exploratory behavior. After lithium chloride administration, all of the treatment groups had significantly decreasing OT, OTR, and OE in the open-arm compared with the control group indicating that in the range of 30-200 mg/kg, in addition to enhancing inhibitory P-GSK3β expression, lithium improved anxiety, and increased exploration of KO mice. However, despite effective therapy with lithium, the KO mice still recorded significant hyperactivity in the elevated plus maze test (**Tables 3, 4, & 5**). The results indicate that lithium could partly normalize the abnormal phenotype of KO mice in the elevated plus maze.

Interestingly, in both the open-field test and the elevated plus maze, we observed significant behavioral modification in WT mice after lithium administration. In the open-field test, we observed a significant decrease of total distance in WT mice treated with 90, 120, and 200 mg/kg of lithium chloride. Also, there was a significant decrease of crossing in 200 mg/kg lithium treated FVB WT mice. On the other hand, in the elevated plus maze, we found reduced OT, OE, and OTR of WT mice treated with 90-200 mg/kg of lithium chloride. However, those changes in behavior of WT mice were not accompanied with changes in brain P-GSK3β expressions. According to our previous research, 90 mg/kg lithium chloride is equivalent to a 2.34 mmol/L blood lithium concentration,[17] which is higher than the toxic concentration of lithium (1.5mmol/L).[26] Therefore, we inferred that the reduced activity of the WT mice may not be caused by increased P-GSK3β expression, but by the toxic sedation effect of lithium chloride. Previous research demonstrated that lithium had no effect on FVB WT mice.[16] However, according to our results, lithium ranging below 60 mg/kg, had no effect on the activity of WT mice, which is partially consistent with previous results.[16]

We speculated that GSK3β may contribute to anxiety and exploration behavior, whereas expression of P-GSK3β might maintain the natural anxiety reaction and exploration behavior. However, there are many other indicators for measuring anxiety in mice, for instance, number of head drips, rears, stretch-attend posture, and excretions. Hence, one of the limitations of our study was only recording the entry and time rates in open-field and elevated plus maze text. Future studies should investigate further parameters to provide supporting evidence.

In conclusion, our research indicates that there is an impairment of GSK3β serine-phosphorylation in FVB KO mice, and lithium therapy could reverse some of the behavioral phenotypes of KO mice, such as hyperactivity, anxiety, and increasing exploration. Lithium can also increase P-GSK3β expression and suppress GSK3β activity in the cortex and hippocampus in KO mice. This indicates that P-GSK3β expression is involved in the formation of hyperactivity and mood disorders. Our results indicate that lithium could ameliorate the irregular behavior of KO mice, demonstrating that lithium may be beneficial in treating FXS patients. We also concluded that 60 mg/kg of lithium would be the optimal dosage for FXS model mouse treatment.

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References

1. Peprah E. Fragile X syndrome: the FMR1 CGG repeat distribution among world populations. ***Ann Hum Genet*** 2012; 76: 178-191.
2. Garber K, Smith KT, Reines D, Warren ST. Transcription, translation and fragile X syndrome. ***Curr Opin Genet Dev*** 2006; 16: 270-275.
3. Berry-Kravis E, Sumis A, Hervey C, Mathur S. Clinic-based retrospective analysis of psychopharmacology for behavior in fragile x syndrome. ***Int J Pediatr*** 2012; 2012: 843016.
4. Boyle L, Kaufmann WE. The behavioral phenotype of FMR1 mutations. ***Am J Med Genet C Semin Med Genet*** 2010; 154C: 469-476.
5. Hagerman RJ, Ono MY, Hagerman PJ. Recent advances in fragile X: a model for autism and neurodegeneration. ***Curr Opin Psychiatry*** 2005; 18: 490-496.
6. Bhogal B, Jongens TA. Fragile X syndrome and model organisms: identifying potential routes of therapeutic intervention. ***Dis Model Mech*** 2010; 3: 693-700.
7. Chiu CT, Chuang DM. Neuroprotective action of lithium in disorders of the central nervous system. ***Zhong Nan Da Xue Xue Bao Yi Xue Ban*** 2011; 36: 461-476.
8. Min WW, Yuskaitis CJ, Yan Q, Sikorski C, Chen S, Jope RS, et al. Elevated glycogen synthase kinase-3 activity in Fragile X mice: key metabolic regulator with evidence for treatment potential. ***Neuropharmacology*** 2009; 56: 463-472.
9. Watase K, Gatchel JR, Sun Y, Emamian E, Atkinson R, Richman R, et al. Lithium therapy improves neurological function and hippocampal dendritic arborization in a spinocerebellar ataxia type 1 mouse model. ***PLoS Med*** 2007; 4: e182.
10. Paul JR, Johnson RL, Jope RS, Gamble KL. Disruption of circadian rhythmicity and suprachiasmatic action potential frequency in a mouse model with constitutive activation of glycogen synthase kinase 3. ***Neuroscience*** 2012; 226: 1-9.
11. Doble BW, Woodgett JR. GSK-3: tricks of the trade for a multi-tasking kinase. ***J Cell Sci*** 2003; 116: 1175-1186.
12. Benedetti F, Bollettini I, Barberi I, Radaelli D, Poletti S, Locatelli C, et al. Lithium and GSK3-beta promoter gene variants influence white matter microstructure in bipolar disorder. ***Neuropsychopharmacology*** 2013; 38: 313-327.
13. Liu ZH, Chuang DM, Smith CB. Lithium ameliorates phenotypic deficits in a mouse model of fragile X syndrome. ***Int J Neuropsychopharmacol*** 2011; 14: 618-630.
14. Rodrigues DH, Vilela Mde C, Lacerda-Queiroz N, Miranda AS, Sousa LF, Reis HJ, et al. Behavioral investigation of mice with experimental autoimmune encephalomyelitis. ***Arq Neuropsiquiatr*** 2011; 69: 938-942.
15. McBride SM, Choi CH, Schoenfeld BP, Bell AJ, Liebelt DA, Ferreiro D, et al. Pharmacological and genetic reversal of age-dependent cognitive deficits attributable to decreased presenilin function. ***J Neurosci*** 2010; 30: 9510-9522.
16. Yuskaitis CJ, Mines MA, King MK, Sweatt JD, Miller CA, Jope RS. Lithium ameliorates altered glycogen synthase kinase-3 and behavior in a mouse model of fragile X syndrome. ***Biochem Pharmacol*** 2010; 79: 632-646.
17. Chen S, Luo X, Yang Q, Sun W, Cao K, Chen X, et al. Lithium chloride ameliorates learning and memory ability and inhibits glycogen synthase kinase-3 beta activity in a mouse model of fragile X syndrome. ***Neural Regeneration Research*** 2011; 6: 2452-2459.
18. Xing Z, Sun W, Huang Y, Yi Y, Dai L, Chen S, et al. Genotype Analysis of Fmr1 Gene Knockout Mice with Polymerase Chain Reaction. ***Modern Hospital*** 2009; 9: 12-14.
19. Bagni C, Tassone F, Neri G, Hagerman R. Fragile X syndrome: causes, diagnosis, mechanisms, and therapeutics. ***J Clin Invest*** 2012; 122: 4314-4322.
20. Dunlop BW, Rakofsky JJ, Rapaport MH. A simple question answered: adding moderate-dosage lithium does not help patients with bipolar disorder. ***Am J Psychiatry*** 2013; 170: 9-11.
21. Freland L, Beaulieu JM. Inhibition of GSK3 by lithium, from single molecules to signaling networks. ***Front Mol Neurosci*** 2012; 5: 14.
22. De Sarno P, Li X, Jope RS. Regulation of Akt and glycogen synthase kinase-3 beta phosphorylation by sodium valproate and lithium. ***Neuropharmacology*** 2002; 43: 1158-1164.
23. Jope RS. Lithium and GSK-3: one inhibitor, two inhibitory actions, multiple outcomes. ***Trends Pharmacol Sci*** 2003; 24: 441-443.
24. Veeraragavan S, Graham D, Bui N, Yuva-Paylor LA, Wess J, Paylor R. Genetic reduction of muscarinic M4 receptor modulates analgesic response and acoustic startle response in a mouse model of fragile X syndrome (FXS). ***Behav Brain Res*** 2012; 228: 1-8.
25. Qin M, Smith CB. Unaltered hormonal response to stress in a mouse model of fragile X syndrome. ***Psychoneuroendocrinology*** 2008; 33: 883-889.
26. Pouladi MA, Brillaud E, Xie Y, Conforti P, Graham RK, Ehrnhoefer DE, et al. NP03, a novel low-dose lithium formulation, is neuroprotective in the YAC128 mouse model of Huntington disease. ***Neurobiol Dis*** 2012; 48: 282-289.

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