Vortioxetine alleviates PTSD-like behaviors by regulating ABPs through up-regulating FMRP in the hippocampus of rats exposed to SPS

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Research Article

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Abstract

Posttraumatic stress disorder (PTSD) is a mental disorder that may lead to a series of changes in the central nervous system, including damage to synaptic plasticity, loss of neuronal dendritic spine, increased apoptosis or inflammation. However, the pathogenesis of PTSD is still unclear and there are few efficacious pharmacological treatments for PTSD. In the present study, we found that intraperitoneal injection of vortioxetine could alleviate PTSD-like behaviors including depression-like behavior and fear memory extinction disorder of rats exposed to single-prolonged stress (SPS). This effect might be related to the upregulation of FMRP in the hippocampus of SPS rats. Furthermore, vortioxetine could also increase the dendritic spine density and the expression of synapse-related proteins, including PSD95 and synapsin I and Actin-binding proteins (ABPs), such as drebrin1, profilin2 and p-cofilin1 in hippocampal cell of rats exposed to SPS. The results of this study indicated that vortioxetine might alleviate PTSD-like behaviors by regulating neuronal morphology in the hippocampus of rats exposed to SPS through regulating FMRP, which might be related to the regulation of synapse-related proteins and ABPs.

Introduction

Post-traumatic stress disorder (PTSD) is a mental disorder that occurs after an individual encounters or witnesses a traumatic event, whose main symptoms are trauma re-experience, avoidance, excessive arousal and cognitive impairment, accompanied by persistent fear memory, headache, sleep and emotional disorders [1–3]. The incidence rate of PTSD continues to rise and it seriously affects the quality of life of patients. However, the pathogenesis of PTSD is still unclear and there are few efficacious pharmacological treatments for PTSD and many patients fail to benefit from existing treatments.

Selective serotonin reuptake inhibitors (SSRIs) are an evidence-based pharmacological treatment for PTSD. However, the treatment mechanism is not fully understood, and the treatment response of individual patients is also different[4]. Vortioxetine, a recently developed antidepressant, acts as a serotonin regulator by inhibiting the effects of serotonin transporters and actions at multiple types of serotonin receptors as 5-HT1A receptor agonist, 5-HT1B receptor partial agonist, and 5-HT1D, 5-HT3, and 5-HT7 receptor antagonist [5–7]. It has been shown that vortioxetine could improve cognitive function by regulating different neurotransmitters and regulate memory potentially and was be involved in the modulation of synaptic plasticity, neurogenesis and episodic memory and improve cell proliferation in the dentate gyrus in major depressive disorder (MDD) rats [8, 9]. Additionally, vortioxetine treatment could improve deteriorated recognition memory induced by stress and this effect might be related to the downregulation of apoptosis level of cell and upregulation of brain-derived neurotrophic factor (BDNF) in frontal cortex[10]. However, whether vortioxetine can alleviate the behavioral abnormalities of PTSD and the interactions between vortioxetine and its biological targets need further study.

Fragile X mental retardation protein (FMRP) is an RNA-binding protein encoded by the long arm FMR1 gene of X chromosome, which can bind and regulate the targeted transport and translation of multiple mRNA in the cytoplasm of neurons, and exercise the function of regulating the morphology and plasticity
of neurons by regulating the expression of synapse-related proteins[11, 12]. It has been indicated that FMRP could regulate the expression of multiple functional proteins at synaptic sites, and its deletion can lead to cognitive dysfunction such as learning and memory[13, 14]. In addition, FMRP is expressed in dendritic spines and regulates the translation of mRNAs in neuronal dendrites and the deletion of FMRP not only affects the phenotype of hippocampal dendritic spines, but also hinders the maturation of dendritic spines[15–17]. In the previous study, we found that the expression of FMRP was downregulated in the hippocampus of PTSD model rats, exposed to single prolonged stress (SPS) procedure, and this indicated that FMRP might participate in the pathological process of PTSD[18]. However, the upstream and downstream regulation pathways of FMRP in PTSD are still not clearly studied. Interestingly, it has been shown that vortioxetine could increase hippocampal expression of several neuroplasticity-related genes including *Fmr1* in middle-aged mice[19].

In the present study, the purpose was to investigate the possible protective effects of vortioxetine on rats exposed to SPS program in terms of behavioral functions including depression-like behavior and fear memory extinction disorder, and the possible interaction between vortioxetine and FMRP, which might be one of the regulators of the hippocampal dendritic spines and act as a target of vortioxetine. Furthermore, we found vortioxetine could increase the expression of synapse-related proteins, including PSD95 and synapsin I and Actin-binding proteins (ABPs), such as drebrin1, profilin2 and p-cofilin1 in hippocampal cell of rats exposed to SPS and this effect could be reversed by the downregulation of FMRP.

**Materials and methods**

**Animal and Experimental group**

The rats used in this experiment were 12-week-old healthy male SD rats of 250-300g, which were purchased from Liaoning Changsheng Biotechnology Co (Liaoning, China), and kept in the Laboratory Animal Department of China Medical University. The animals were provided with adequate food and water. All animal experimental procedures are approved by the Animal Use and Feeding Committee of National Medical University, and the experimental procedures strictly follow animal ethics. The rats meeting the criteria were randomly divided into 5 experimental groups (Fig. 1a-b): (1) Control group: control rats without any treatment, n = 24; (2) PTSD group: SPS treated PTSD model rats, n = 24; (3) SPS + Vehicle group: SPS rats were intraperitoneally injected with hydroxypropyl-beta-cyclodextrin, n = 9; (4) SPS + VOR group: SPS rats intraperitoneally injected with vortioxetine, n = 12. (5) SPS + VOR + shFmr1 group: sh-Fmr1 plasmid was transfected into the lateral ventricle of SPS rats after intraperitoneal injection of vortioxetine in order to test the relationship between vortioxetine and FMRP, and the group was named SPS + VOR + shFmr1 group (n = 6).

**Preparation of PTSD model rats**

The rats were restrained in plastic vessel (keeping limbs and bodies immobile but leaving holes through the nose for breathing) for 2 hours; Then the rats were forced to swim for 20 minutes immediately after
being unbound. After the rats were taken out and put back into the cage for a rest of 15 minutes, they were anesthetized with ether until they lost consciousness (Fig. 1b). The stimulated rats were put back into the cage and fed normally for 7 days for follow-up experiment, during which they were not given any stimulation.

**Mode of administration and dosage**

Vortioxetine was dissolved in 10% hydroxypropyl-beta-cyclodextrin solution with a concentration of 10mg/kg at a dose of 1ml per day. Intraperitoneal injection was administered at a fixed time daily for 7 days after the end of the SPS program, and behavioral testing was performed 24 hours after the last dose.

**Behavioral test**

Behavioral testing was performed 7 days after SPS stimulation or 24 hours after the last dose of vortioxetine, and animals were placed in the behavioral room for 30 minutes to familiarize themselves the day before and on the day of the test (Fig. 1a). After each rat is tested, the excreta in the tank should be carefully cleaned and the residual odor should be removed with 70% alcohol. All behavioral tests were performed by XinRun software (Xin Run, Shanghai).

**Open field test**

The Open field test (OPT) was used to detect depression-like behavior in rats[20–22]. The black experiment box of 72×72×40cm was divided into 16 squares, and the central (four squares in the middle), four corners (four squares in total in the four corners of the experiment box) and four surrounding areas (the remaining eight squares) were set. The rats were placed in a fixed area of the chamber and allowed to explore freely for 10 minutes. SuperMaze software was used to record latency time, average speed, the number of rearing.

**Forced swimming test**

The Forced swimming test (FST) was used to detect depression-like behavior in rats. The rats were placed in a glass bucket (height 40cm, diameter 20cm) filled with clean water (23 ± 1°C, water level 30cm) and forced to swim for 6 minutes. The floating time of the rats was recorded in the following 4 minutes using SuperFst software.

**Fear Conditions test**

The rats were placed in a cube box and the freezing time of the rats was recorded during the experiment. On the first day of the experiment, the rats were placed in a palisade floor and a white background environment. The rats were given three auditory stimuli (75dB, 30 seconds), and a foot electric shock (0.8mA) was inserted in the last two seconds of each auditory stimulus. The interval between two acoustic stimuli was 30 seconds, and the freezing time of the rats was recorded during this process. Twenty-four hours later, the rats were placed in the same background (but without acoustic stimulation or electric shock) for six minutes as an association test, and the freezing time was recorded. One hour after
the rats were put back in the cage, the environment of the box was changed into a black floor and a black and white striped background. The rats were allowed to move freely for 3 minutes, and then three auditory stimuli (75dB, 30 seconds) were given, with an interval of 30 seconds between the two stimuli as an auditory cue test. The freezing time of the rats during this process was recorded.

**Western Blot**

Total protein was extracted from hippocampal tissue of rats in each group by RIPA (Radioimmunoprecipitation assay buffer, Beyotime Biotech, Shanghai, China) and PMSF (Phenyl methane sulfonyl fluoride, Beyotime Biotech, Shanghai, China) and the protein concentration in the supernatant was determined by BCA method. 50ug total protein was added into SDS-PAGE gel hole for electrophoresis and then the protein was transferred to PVDF membrane after SDS-PAGE electrophoresis. The membrane was incubated overnight at 4°C with primary antibody FMRP (1:5000, Abcam), PSD95 (1:5000, Abcam), Synapsin (1:5000, Abcam), Drebrin1 (1:2000, Boster Biological Technology, Wuhan, China), Profilin2 (1:2000, Boster), p-cofilin1 (1:1000, Zenbioscience, Chengdu, China), Coflin1 (1:2000, Boster) and GAPDH (1:50,000, Proteintech Group, Inc), β-Actin (1:10000, Proteintech) after blocking by 5% milk powder. The membrane was incubated with the secondary antibody in 37°C for 1.5 hours. The Enhanced chemiluminescence solution (ECL, Proteintech) was used for protein development on the ChemiDoc XRS+ imaging system, and Image J was used for band gray value detection.

**Immunofluorescence staining**

After the intact brains were removed, the brains were soaked in 4% paraformaldehyde solution and gradient sucrose solution for dehydration (15%→25%→30%). After that, the brains were cut into slices 15um thick along the coronal plane in a frozen microtome and stored at -20°C. The slices were washed in phosphoric acid buffer (PBS) for 3 times and then put in 0.2% Triton-100 for 10 minutes. After blocking by 5% bovine serum protein for 1 hour, the slices were incubated with mouse β-Tubulin (1:300, Abcam), rabbit FMRP (1:300, Abcam), rabbit PSD95 (1:300, Abcam), rabbit Synapsin primary antibody (1:300, Abcam) and rabbit Drebrin1 primary antibody (1:200, Boster) at 4°C overnight. The secondary antibodies consisted of goat anti-mouse 488-conjugated secondary antibody (1:500, Abcam) and donkey anti-rabbit 594-conjugated secondary antibody (1:400, Abcam) were used to incubate slices at 37°C for 2 hours. The nuclei were stained with DAPI and the image was captured by Olympus microscope.

**Golgi-cox staining**

After deep anesthesia with pentobarbital sodium, the rats were sacrificed for neck dislocation, the intact brain was removed quickly, the excess blood was rinsed with normal saline, and the rats were treated according to the Hito Golgi-Cox OptimStain Kit (Hitobiotech Corp, USA) treated the brain tissue. Then the brain tissue was divided into 100um slices at -20°C and placed on gelatin coated slides. The slices were dyed according to kit instructions and seal with slides and high concentration of neutral gum. And then the slices were taken pictures with Olympus microscope and image J was used to analyze the density and morphology of dendritic spines.

**Plasmid construction and extraction**
The shFmr1 gene silencing sequence was composed of target sequence which was designed in the Thermo Fisher Sciencec website (https://rnaidesigner.thermosher.com) complementary sequence, stem ring and double restriction site (Xho (Takara, Beijing) and BamH (Takara)). The recombinant plasmid shRNA-Fmr1 and shRNA-NC was synthesized by pscioR-GFP vector. The shRNA sequence we designed is as follows:

Fmr1: sense: 5′-GCACCAAGTTGTCTCTTATAC-3′
NC: sense: 5′-GAGAACAACGTAACTTAGTCT-3′

The sequencing was performed by GENEWIZ (Azenta Life Science, Tianjin), and the plasmid extraction was extracted according to the instructions of EndoFree Maxi Plasmid Kit (TIANGEN, Beijing).

**Lateral ventricle stereotactic injection**

After the rats were deeply anesthetized with sodium pentobarbital, their head hair was shaved and fixed to a stereoscope, The 5000ng shRNA-Fmr1 plasmid was mixed with 7µl Entranster in vivo Transfection Reagent DNA transfection reagent (Engreen Biosystem Co.Ltd), and supplemented with normal saline to 10µl. The rats were stereotactically positioned in the bilateral lateral ventricles of the rats, with parameters of 1.2mm behind the fontanelle, 2.0mm on the left and right sides, and 3.8mm in depth. The injection dose was 5µl on each side. The rats were put back into the cage after the operation, and no stimulation was given during the period.

**Statistical analysis**

All statistical analysis in this study was performed using GraphPad Prism 7.0. The major statistical methods consisted of unpaired student's t-test, one-way analysis of variance (ANOVA) followed by post-hoc test (Dunnett's test) and two-way ANOVA. All data were expressed as mean ± standard error (mean ± SEM), and $P < 0.05$ was considered statistically significant.

**Results**

**Vortioxetine alleviated depression-like behavior and fear memory extinction disorder in rats subjected to SPS**

In order to test whether vortioxetine can improve PTSD-like behaviors in SPS rats, we conducted behavioral tests on the animals after continuous intraperitoneal injection of vortioxetine for one week. The results of OFT showed that the latency time of rats in PTSD group was higher than Control and the average speed and rearing numbers of rats in PTSD group were significantly lower than the animals of Control group, while the results above could be reversed by vortioxetine (latency time: ($F$ (3, 20) = 8.033, $P = 0.001$, Fig. 1d; average speed: $F$ (3, 20) = 8.279, $P = 0.0009$, Fig. 1e; rearing numbers: $F$ (3, 20) = 10.700, $P = 0.0002$, Fig. 1f). Additionally, The results of FST showed that the immobility time of rats in PTSD group was higher than Control group, this trend could also be reversed by vortioxetine ($F$ (3, 20) = 6.616, $P$...
Furthermore, we tested fear memory regression of rats in each group through FCT and the results showed that the freezing times in rats exposed to SPS were significantly longer than the control rats in both the contextual fear and cued conditioning test, while the freezing time was significantly shortened by vortioxetine (contextual fear: $F(3, 20) = 11.93, P = 0.0001$, Fig. 1h; cued conditioning test: $F(3, 20) = 6.24, P = 0.0036$, Fig. 1i).

These results indicated that vortioxetine could alleviate depression-like behavior and promote fear extinction in rats exposed to SPS.

**Vortioxetine improved the mature spine in the hippocampus of SPS rats**

The previous study indicated that the density of dendritic spines and the proportion of mature dendritic spines in the hippocampal cells of PTSD model rats were decreased, which may be one of the mechanisms that the abnormal behaviors of rats SPS. In order to explore the possible mechanism of vortioxetine alleviating the PTSD-like behaviors of SPS rats, we further detected the morphology of dendritic spines of neurons in the hippocampus of rats in Control group, PTSD group and VOR group by Golgi staining. Compared with the Control group, the density of dendritic spines of hippocampal neurons in PTSD model rats was significantly decreased, while it could be increased by vortioxetine ($F(2, 6) = 23.21, P = 0.0025$, Fig. 2d). Dendritic spines can be roughly divided into four types including filopodia, thin, stubby and mushroom according to the proportion of their head and neck width (Fig. 2a). The results showed that the proportion of mature dendritic spines (mushroom) in hippocampal neurons of rats in PTSD group was significantly lower than that in Control group. While the proportion of immature dendritic spines (filopodia) was significantly higher than that of in Control group. The administration of vortioxetine could significantly reverse this trend (mushroom: $F(2, 6) = 12.18, P = 0.0077$, Fig. 2e; filopodia: $F(2, 6) = 6.621, P = 0.0303$, Fig. 2f).

The above results showed that the intraperitoneal injection of vortioxetine could alleviate the PTSD-like behaviors of rats exposed to SPS procedure and it might be achieved by regulating the density of dendritic spines and the proportion of mature dendritic spines of hippocampal neurons in rats.

**Vortioxetine upregulated the expression of FMRP in the hippocampus of SPS rats**

Next, we explored the effect of vortioxetine on the expression of FMRP in the hippocampus of PTSD model rats through Western blot and immunofluorescence staining. The results of Western blot showed that the protein expression level of FMRP in the hippocampus of rats in the PTSD group was significantly decreased compared with the control group, while this trend could be significantly reversed by vortioxetine ($F(3, 20) = 15.99, P < 0.0001$, Fig. 3a and b). According to the result that there is no significant difference between PTSD group and Vehicle group through Western blot, we selected Control, PTSD and VOR group for performing immunofluorescence staining. The results of immunofluorescence staining
showed that FMRP was expressed in hippocampal neurons of each group and the fluorescence intensity in PTSD group was lower than other two groups (Fig. 3c).

These results indicated that the downregulation of FMRP expression in the hippocampus of rats exposed to SPS could be increased by vortioxetine and FMRP might be one of biological targets for vortioxetine.

**Vortioxetine up-regulated the expression of PSD95 and synapsin I in the hippocampus of PTSD model rats**

The postsynaptic density (PSD), which is composed of receptors, kinases, structural proteins and signal molecules related to synaptic plasticity, is a dense localized area in the dendritic spines of excitatory synapses[23, 24]. Postsynaptic density protein 95 (PSD95), as a post-synaptic dense substance, is enriched in the head of mature dendritic spines and plays an important role in determining the size and shape of dendritic spines [25]. The functional property of synapsin I is related to the organization of small synaptic vesicles (SVs) and the actin cytoskeleton in synapses in the central nervous system (CNS) [26]. It has been reported that synapsin I was related to formation and/or stabilization of synaptic contacts [27]. To explore whether vortioxetine can affect the expression of synapse-related protein in the hippocampus of PTSD model rats, we detected the expression level of PSD95 and synapsin I by Western blot and immunofluorescence staining. The results of Western blot showed that the expression level of PSD95 and synapsin I in the hippocampus of rats in PTSD group was significantly lower than that in the Control group, while the protein expression level of PSD95 and synapsin I in PTSD group was significantly increased by vortioxetine (PSD95: F (3, 20) = 7.272, \( P = 0.0017 \), Fig. 4a, b; synapsin I: F (3, 20) = 6.201, \( P = 0.0037 \), Fig. 4a, c). Furthermore, we selected Control, PTSD and VOR group for performing immunofluorescence staining according to the results of Western blot. The results of immunofluorescence staining showed that PSD95 (Fig. 4d) or synapsin I (Fig. 4e) expressed in the hippocampal neurons of each group and the fluorescence intensity in PTSD group was lower than that in the Control group, which could be reversed by vortioxetine.

The above results suggested that vortioxetine might regulate synaptic plasticity by regulating the expression of synapse-related proteins, such as PSD95 and synapsin I, and thus participate in the regulation of synaptic plasticity of hippocampal neurons in PTSD rats.

**Vortioxetine increased the expression of ABPs in the hippocampus of SPS rats**

The structure of dendritic spine is tightly linked to its synaptic function and filamentous (F)-actin is highly enriched in dendritic spines, where it functions as the major cytoskeletal component that governs spine morphogenesis and spine plasticity[28, 29]. The dynamic remodeling of actin cytoskeleton is considered to be the driving force for the structural change of dendritic spines, which depends on the regulatory role of ABPs, which contain coflin1, profilin2, drebrin1 and so on[30]. In order to explore whether vortioxetine has effect on the regulation of the morphology of dendritic spines of neurons in the hippocampus of PTSD model rats, we detected the expression level of ABPs including p-cofilin1, profilin2, drebrin1 in each
group by Western blot or immunofluorescence staining. The results of Western blot showed that the expression levels of drebrin1 and profilin2 and the proportion of p-cofilin1/cofilin1 in the hippocampus of rats in the PTSD group were significantly decreased compared with that in the Control group, while vortioxetine could significantly reverse this trends (Drebrin1: $F (3, 20) = 6.044, P = 0.0042$, Fig. 5a, c; Profilin2: $F (3, 20) = 11.09, P = 0.0002$, Fig. 5a, d; p-cofilin1/Cofilin1: $F (3, 20) = 6.063, P = 0.0042$, Fig. 5b, e). Drebrin1 can stabilize the morphology of dendritic spines by promoting actin assembly and aggregation of PSD95 in dendritic spines[31] and we further observed the location and expression of drebrin1 in the hippocampus by immunofluorescence staining. The results of immunofluorescence showed that drebrin1 positive cells were found in the hippocampus of each group and the fluorescence intensity in the PTSD group was lower than Control group and it could be recovered by vortioxetine (Fig. 5f).

These results indicated that vortioxetine might affect the morphology of dendritic spines of neurons by increasing the expression level of ABPs in the hippocampus of PTSD model rats, and thus alleviate the behavioral abnormality of PTSD.

### Vortioxetine modulated the expression of ABPs in the hippocampus of SPS rats by upregulating FMRP

To determine whether FMRP is involved in the regulatory mechanism of vortioxetine on ABPs, the shRNA-Fmr1 plasmid was transfected into the hippocampus of rats with PTSD who received vortioxetine by intraperitoneal injection and the transfection effect is shown in Fig. 6a. Then the behavioral tests were performed and the results of OFT showed that the latency time of rats transfected with shRNA-Fmr1 plasmid was significantly increased ($F (4, 25) = 17.77, P<0.0001$, Fig. 6c), while the average speed ($F (4, 25)= 10.15, P<0.0001$, Fig. 6d) and rearing numbers ($F (4, 25) = 13.53, P<0.0001$, Fig. 6e) were significantly decreased compared with the rats treated with vortioxetine. In addition, vortioxetine significantly alleviated freezing time in rats with PTSD in conditioned fear experiments, but freezing time in rats was significantly increased after lateral ventricle transfection with shRNA-Fmr1 in both contextual condition test ($F (4, 25) = 5.556, P = 0.0024$, Fig. 6f) and cue test ($F (4, 25) = 22.23, P<0.0001$, Fig. 6g). Furthermore, the expression levels of related proteins were detected by Western Blot and the results showed that the expression of FMRP ($t = 4.625, P = 0.0003$, Fig. 6h i), Drebrin1 ($t = 3.751, P = 0.0017$, Fig. 6h j), PSD95 ($t = 4.546, P = 0.0011$, Fig. 6k l) and Synapsin ($t = 3.706, P = 0.0041$, Fig. 6k m) of rats in the SPS + VOR + shFmr1 group were significantly lower than those in SPS + VOR group. These results showed that shRNA-Fmr1 decreased the therapeutic effect of vortioxetine on PTSD rats.

These results suggest that the therapeutic effect of vortioxetine on abnormal behaviors in PTSD rats and the regulation of related proteins in hippocampus region might be related to FMRP.

### Discussion

Many treatment approaches have been proposed to cure PTSD patients. Selective serotonin reuptake (SSRI), which could promote nerve growth in the hippocampus and cortex, has been approved to treat
PTSD although the remission of PTSD is about 30% with them[32–35].

However, these drugs currently cannot completely treat the symptoms of PTSD and they have side effects[33, 36]. Vortioxetine is a novel multimodal antidepressant that has been developed for the treatment of major depressive and anxiety disorders [37] and its antidepressant action is related to a direct regulation of serotonergic receptor activity and inhibition of the serotonin transporter[38, 39]. The mechanism of vortioxetine action is not fully understood actually. In the present study, we found that intraperitoneal injection of vortioxetine could alleviate the PTSD-like behaviors including depression, anxiety and memory impairment of rats exposed to SPS procedure.

It has been reported that the generalization of fear memory, one of the basic clinical characteristics of PTSD, was related to the regions of brain including hippocampus, amygdala and prefrontal cortex, which work together in a circuit-dependent manner[40, 41]. The clinical data proved that the volume and neuronal integrity of the hippocampus were decreased and the function of the hippocampus was damaged in patients with PTSD[42–44]. In addition, it has been indicated that the core problem of disability related to traumatic stress is caused by the failure of normal operation of various reasonably determined neural networks, which is caused by the improper degeneration or growth of dendritic spines that support these networks[45]. Alterations in spine density, morphology, and maturation strongly correlate with neuronal disorders, such as Alzheimer's disease, Fragile-X syndrome, mental retardation, Down's syndrome, epilepsy, pointing to the central role of these structures in neuronal function[46–50]. In order to explore the possible mechanism of vortioxetine on the improvement of behavioral abnormity of PTSD model rats, we observed the effect of vortioxetine on the density and morphology of dendritic spines of hippocampal neurons by Golgi staining. The results indicated that vortioxetine could increase the density of dendritic spines and the proportion of mature dendritic spines in hippocampal neurons through some pathway.

It has been shown that chronic vortioxetine treatment of middle-aged mice could increase hippocampal expression of \textit{Fmr1}, belonged to transcription and translation factors[19]. FMRP is localized to dendrites and synapses and the loss of FMRP function in the fragile X syndrome (FXS) leded to a pathological hyperabundance of long thin immature dendritic protrusions[51–53]. Furthermore, we tested whether the hippocampal expression level of FMRP could be affected by vortioxetine through Western blot and immunofluorescence staining and the results in the present study showed that intraperitoneal injection of vortioxetine could upregulated the expression level of FMRP, whose level was decreased in the hippocampus of rats exposed to SPS reported by our previous study[54]. We speculated that vortioxetine might participate in the regulation of dendritic spine morphology by up-regulating the expression of FMRP in hippocampal neurons of PTSD model rats.

It has been reported that FMRP could selectively stabilize the PSD-95 mRNA in the hippocampus and the hippocampal levels of PSD-95 mRNA and protein was reduced in FMRP-deficient mice[55]. Dendritic spines mature with an enrichment of postsynaptic scaffolding proteins, among which PSD95 localized mostly in the dendritic shaft or present in mature spine-heads rather than the immature dendritic
protrusions[56, 57]. Synapsin I is a regulator of neuronal development such that higher levels of synapsin I can accelerate the development of synapses[58]. In the present study, we tested the expression of pre- and post-synaptic markers, synapsin I and PSD95, in the hippocampus of PTSD model rats, and the results showed a decreased level that could be reversed by vortioxetine. These results suggested that vortioxetine might participate in the regulation of synaptic morphology and further affect neural function, whose impairment might be related to the pathological process of abnormal behaviors of SPS rats. We speculated that this function of vortioxetine might be correlated with the upregulation of FMRP and confirmed this hypothesis by intraperitoneal injection of vortioxetine into SPS rats treated with sh-FMR1.

Actin and ABPs are particularly abundant in PSD and accumulate in the heads of dendritic spines, and F-actin dynamics are the driving force for spinal morphological remodeling[59–63]. In fact, the actin cytoskeleton maintains the formation of dendritic spines during neuronal development, and maintains the enlargement and shrinkage of dendritic spines as synaptic activity increases and decreases, respectively[29, 64–67]. Cofilin1 mainly promotes the rapid depolymerization of actin by decomposing or cutting off microfilaments, while phosphorylation reduces or even deactivates cofilin1 activity[68, 69]. Cofilin localizes within the postsynaptic density of dendritic spines in neurons and inactive cofilin mutant can results in shorter protrusions and more mature spines[70, 71]. Profilin2 is brain-specific and its induced enrichment at spine heads appears to be accompanied by the stabilization of spine morphology[72]. It has been reported that profilin2 was translocated into spines of the lateral amygdala under fear-inducing conditions[73]. Drebrin1 has been found in the function of maintaining long-term memory in vitro and is distributed in the neurons of the brain, especially in dendritic spines[74, 75]. It stabilizes the morphology of dendritic spines by promoting actin assembly and the aggregation of PSD95 in dendritic spines[31]. In our present study, we found decreased expression levels of p-cofilin1, profilin2 and drebrin1 in the hippocampus of rats exposed to SPS procedure, which could be recovered by vortioxetine in vivo. However, this effect of vortioxetine could be blocked by sh-FMR1. These results indicated that the structure of synapses in the hippocampal neurons of PTSD model rats might be improved by vortioxetine and the mechanism might be related to the expressions of ABPs, which were also be regulated by FMRP.

The present study found that vortioxetine alleviated the behavioral abnormality in rats with PTSD, which might be due to its increased expression of FMRP in hippocampal neurons of rats and thereby affecting the morphology of neuronal dendritic spines. However, the molecular mechanism of vortioxetine action is not limited to this and the morphological regulation of neuronal dendritic spines are affected by multiple factors. In addition, FMRP might be a new target for the prevention and treatment of PTSD, but a more comprehensive and specific mechanism still needs further research.

**Conclusions**

In summary, the present study showed that vortioxetine alleviated the PTSD-like behaviors by upregulating the expression level of FMRP in hippocampal neurons. Furthermore, we provided a possible mechanism by which vortioxetine regulated the morphology of dendritic spines through upregulating the
expression level of synaptic associated proteins and ABPs, which lead to morphological changes in hippocampal neurons in rats and this function of vortioxetine might related to FMRP. However, other detailed signaling cascades involved in the regulation of alleviating PTSD-like behaviors of SPS rats by vortioxetine need to be explored in further experimental research. These works will provide evidence that FMRP might be a potential target for PTSD prevention or treatment in the future.

**Declarations**

**Ethics Approval**

The experimental procedures were approved by the Ethics Committee of China Medical University (Shenyang, China: Approval No.: KT2022451)

**Consent to Participate**

Not applicable.

**Consent for Publication**

All authors consent to the publication of this

**Availability of Data and materials**

The datasets used during the present study are available from the corresponding author upon reasonable request.

**Competing Interests**

The authors declare no competing interests.

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**Authors’ Contributions**

LL J and ST Y designed the research; ST Y, Q A, CF C, JQ K and LL J performed the research and analyzed the data; M L provided suggestions for the experimental design and manuscript; ST Y and LL J wrote the manuscript.

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Not applicable.
References


**Figures**
**Figure 1**

**Vortioxetine alleviated depression-like behavior and fear memory extinction disorder in rats subjected to SPS**

a The procedure and timelines of experiments. b Schematic diagram of SPS procedure. c Representative tracts in OFT. d Latency time e average speed f rearing time in OFT. g The immobility time in FST. h The freezing time on the contextual test. i The freezing time on the cue test. n = 6 rats per genotype. Each bar indicates the mean ± SEM. One-way ANOVA followed by Dunnett’s test. *P < 0.05, **P < 0.01, ***P < 0.001
Figure 2

**Vortioxetine improves morphological abnormalities of dendritic spine in hippocampal neurons in PTSD rats.**

- **a** Images of Golgi-Cox-stained neuron in hippocampus and the identification of dendritic spine type.
- **b** Representative images of Golgi-Cox-stained neuron in Control, PTSD and VOR group.
- **c** Representative images of Golgi-Cox staining basilar dendrites.
- **d** Dendritic spine number of hippocampal neurons per 10μm in each group. The proportion of **e** mushroom and **f** filopodia dendritic spine in each group. n = 3 rats per genotype. Bar graphs represent the mean ± SEM. One-way ANOVA followed by Bonferroni's post hoc test; *P < 0.05, **P < 0.01
Figure 3

Vortioxetine increased the expression level of FMRP in the hippocampus of PTSD model rats. a Representative band and b the relative level of FMRP tested by Western blot analysis in the hippocampus of rats in each group. GAPDH was served as a loading control. n = 6 rats per genotype. Bar graphs represent the mean ± SEM. One-way ANOVA followed by Bonferroni’s post hoc test; **P < 0.01. c Representative images of immunofluorescence staining showing the expression of β-tubulin III (green), FMRP (red) or DAPI (blue) in the hippocampus of rats in Control, PTSD and VOR group.
Vortioxetine upregulated the expression level of PSD95 and synapsin I in the hippocampus of PTSD model rats. a Representative band of PSD95 and synapsin I in the hippocampus of rats in each group tested by Western blot. The relative level of b PSD95 and c synapsin I in the hippocampus of rats in each group. GAPDH was served as a loading control. n = 6 rats per genotype. Bar graphs represent the mean ± SEM. One-way ANOVA followed by Bonferroni’s post hoc test; *P < 0.05, **P < 0.01. Representative images
of immunofluorescence staining showing the expression of β-tubulin III (green), d PSD95 (red), e synapsin I (red) or DAPI (blue) in the hippocampus of rats in Control, PTSD and VOR group.

![A] (Control, PTSD, Vehicle, VOR)

![B] (Control, PTSD, Vehicle, VOR)

![C] (Control, PTSD, Vehicle, VOR)

![D] (Control, PTSD, Vehicle, VOR)

![E] (Control, PTSD, Vehicle, VOR)

![F] (Control, PTSD, VOR)

Figure 5

Vortioxetine upregulated the expression level of ABPs including drebrin1, profilin2 and p-cofilin1 in the hippocampus of PTSD model rats. a Representative band of drebrin1, and profilin2 in the hippocampus of rats in each group tested by Western blot. β-actin was served as a loading control. b Representative band of p-cofilin1 and coflin1 in the hippocampus of rats in each group tested by Western blot. GAPDH
was served as a loading control. The relative level of c drebrin1 d profilin2 or e p-cofilin1 in the hippocampus of rats in each group. n = 6 rats per genotype. Bar graphs represent the mean ± SEM. One-way ANOVA followed by Bonferroni’s post hoc test; *P < 0.05, **P < 0.01. Representative images of immunofluorescence staining showing the expression of β-tubulin III (green), f drebrin1 (red) or DAPI (blue) in the hippocampus of rats in Control, PTSD and VOR group.

**Figure 6**
Vortioxetine modulated the expression of ABPs in the hippocampus of SPS rats by upregulating FMRP. The transfection effect of the shRNA-Fmr1 plasmid in the hippocampus of rats with PTSD. Representative tracts in OFT. Latency time in OFT. Average speed in OFT. Rearing time in OFT. The freezing time on the contextual test. The freezing time on the cue test. n = 6 rats per genotype. Representative band of FMRP and drebrin1 in the hippocampus of rats in each group tested by Western blot. GAPDH was served as a loading control. The relative level of FMRP or drebrin1 in the hippocampus of rats in each group. Representative band of PSD95 and Synapsin I in the hippocampus of rats in each group tested by Western blot. GAPDH was served as a loading control. The relative level of PSD95 or Synapsin I in the hippocampus of rats in each group. n = 6 rats per genotype. Bar graphs represent the mean ± SEM. One-way ANOVA followed by Bonferroni's post hoc test; *P < 0.05, **P < 0.01, ***P < 0.001.