Available online at www.sciencedirect.com

Journal of Acupuncture and Meridian Studies

journal homepage: www.jams-kpi.com

RESEARCH ARTICLE

Soluble N-ethylmaleimide-sensitive Factor Attachment Receptor (SNARE) Protein Involved in the Remission of Depression by Acupuncture in Rats

Ling Fan [1,2], Zhao Chen [3], Wenbin Fu [1,*], Nenggui Xu [4], Jianhua Liu [1], Aiping Lu [5], Ziping Li [1], Shengyong Su [6], Taixiang Wu [7], Aihua Ou [8]

[1] *Department of Acupuncture and Moxibustion, Guangdong Provincial Hospital of TCM, Guangzhou, China*
[2] *Postdoctoral Research Station of Guangzhou University of Chinese Medicine, Guangzhou, China*
[3] *Division of Epidemiology and Biostatistics, Mel and Enid Zuckerman College of Public Health, University of Arizona, Tucson, AZ, USA*
[4] *College of Acupuncture and Moxibustion, Guangzhou University of Chinese Medicine, Guangzhou, China*
[5] *Department of Acupuncture and Moxibustion, School of Chinese Medicine, Hong Kong Baptist University, Kowloon Tong, Hong Kong*
[6] *Department of Acupuncture and Moxibustion, The First Affiliated Hospital of Guangxi University of Chinese Medicine, Nanning, China*
[7] *Primary Registry of WHO ICTRP, Chinese Clinical Trial Registry, Chinese Evidence-based Medicine Center, Chinese Cochrane Center, West China Hospital, Sichuan University, Chengdu, China*
[8] *Department of Epidemiology Center, Guangdong Provincial Academy of Chinese Medical Sciences, Guangzhou, China*

Available online 26 April 2016

* Corresponding author. Department of Acupuncture and Moxibustion, Guangdong Provincial Hospital of Traditional Chinese Medicine, 111 Dade Road, Yuexiu District,Guangzhou 510120, China.
E-mail: 546624680@qq.com (W. Fu).

pISSN 2005-2901 eISSN 2093-8152
http://dx.doi.org/10.1016/j.jams.2016.04.002

Received: Oct 20, 2015
Revised: Apr 9, 2016
Accepted: Apr 15, 2016

KEYWORDS
acupuncture;
antidepressant;
botulinum toxin A;
riluzole

Abstract
This study aims to investigate the molecular mechanisms of acupuncture in the remission of depression. A depressive disorder model was induced by exposing Sprague—Dawley rats to chronic unpredictable stress. The rats were divided into five groups: healthy (blank group) and stressed rats (model group), and stressed rats treated with acupuncture (acupuncture group), riluzole (riluzole group), acupuncture combined with botulinum toxin A (BTX-A) injection (acupuncture + BTX-A group) or riluzole combined with BTX-A injection (riluzole + BTX-A group). Behavioral analysis showed significant differences in sucrose consumption, weight, and horizontal or vertical movements between the model and both the riluzole and acupuncture groups. No obvious differences between the riluzole + BTX-A and acupuncture + BTX-A groups were found. Moreover, no significance differences in glutamate content in the hippocampus were found among the riluzole + BTX-A, acupuncture + BTX-A and model groups ($p > 0.05$). Western blots and reverse transcription polymerase chain reactions were employed to detect protein and mRNA expressions of VGLUT2, SNAP25, VAMP1, VAMP2, VAMP7, and syntaxin1; no obvious differences among the riluzole + BTX-A, acupuncture + BTX-A and model groups were found. These data suggest that soluble N-ethylmaleimide-sensitive factor attachment receptor proteins are involved in the remission of depression in rats treated with acupuncture.

1. Introduction

Depressive disorder is a mental disorder characterized by persistent low mood and is very difficult to cure. So far, the etiology of depression remains unclear. According to a World Health Organization report, the rate of global depression is about 11%, which makes it the world's fourth major disease [1]. Antidepressants, such as triple-reuptake inhibitors and riluzole, are the mainstay in the treatment of depressive disorders. However, the outcomes of treatment are unsatisfactory [2—4]. Therefore, alternative treatments for depressive disorders must be sought. Previous studies have found acupuncture to be an effective treatment modality for remitting stress disorders [5]. However, the mechanism underlying this effect remains ambiguous.

Extremely high levels of glutamate have been found in depressive patients, and clinical studies have indicated that depressive disorders are associated with increased glutamatergic activity [6]. Glutamatergic neurotransmission occurs predominantly within the confines of a tripartite synapse. It is then recycled through the glutamate—glutamine cycle by glutamate receptors in postsynaptic neurons. The interactions among synaptic soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) proteins, synaptobrevin (vesicle-associated membrane protein, VAMP), synaptosomal associated protein 25 (SNAP-25), and syntaxin in the plasma membrane play important roles in glutamate neurotransmission. Dysregulation of the glutamatergic system has been found to be an important factor in depressive disorders [7].

Acupuncture is an ancient Chinese medical technic that can be traced back at least 2500 years. It is now widely used as a form of complementary and alternative medicine in many countries [8,9]. Clinical studies have found acupuncture to be an effective treatment modality for remitting stress disorder, anxiety, and depression [10—13]. Electroconvulsive therapy might regulate the expression of distinct neurotrophic signaling pathways, including the metabotropic glutamate receptor, neuron glucose transporter, and Gamma aminobutyric acid A (GABA-A) receptor. Despite the effectiveness of acupuncture in the treatment of depressive disorders, very few studies have investigated the underlying mechanisms.

To probe into the mechanism of acupuncture in the treatment of depressive disorders, we investigated synaptic SNARE proteins and the glutamate—glutamine cycle. Botulinum toxin A (BTX-A), secreted by the deadly botulinum, is a highly toxic bacterial endotoxin. Evidence has shown that BTX-A is able to specifically hydrolyze SNARE proteins [14,15]. In this study, BTX-A was used to regulate the SNARE protein content and to investigate the molecular mechanisms of acupuncture.

2. Materials and methods

2.1. Animals

Adult, male Sprague—Dawley rats (180—220 g) were used in this experiment. Animals were obtained from the Experimental Animal Center of Guangzhou University of Chinese Medicine, Guangzhou, China. Both the animal's care and the study protocol were conducted according to the Helsinki Declaration Accords and followed the guidelines of the Committee on the Care and Use of Laboratory Animals at the Experimental Animal Center of Guangzhou University of Chinese Medicine. Animals were housed under a 12 hour light/dark cycle at a constant room temperature of 23°C.

2.2. Depressive disorder model and treatment

Depression disorder was induced in the rats by exposing them to chronic unpredictable stress (CUS) for 21 days [16]. The CUS included cold swims (5 minutes), water deprivation

(24 hours), food deprivation (24 hours), tail pinching (1 minute), tail shaking (1 time/s, 15 minutes), reverse day and night, and electric shock (5 minutes). Only one form of CUS was conducted each day at a frequency of 21 times a day.

After 21 days of CUS, the model was assessed by using the sucrose preference test and the open-field test [17]. The stressed rats were then randomized into five groups (8 rats per group): untreated stressed rats (model group) and stressed rats treated with only acupuncture (acupuncture group), with only riluzole (riluzole group), with riluzole + BTX-A injection (riluzole + BTX-A group), and with acupuncture + BTX-A injection (acupuncture + BTX-A group). Acupuncture intervention in the acupuncture group was conducted each day for 3 consecutive weeks. The body acupoints for acupuncture were Hegu (LI4) and Taichong (LR3) on both sides of the body (Fig. 1). The intensity was adjusted to induce a slight muscle contraction of the hind limb. At the same time, intracerebroventricular injection of BTX-A was implemented for 3 consecutive weeks in the acupuncture + BTX-A group. In the riluzole group, rats were orally administrated riluzole at 1.8 mg/kg for 3 consecutive weeks. In the riluzole + BTX-A group, rats were intragastrically administered riluzole (1.8 mg/kg dissolved in saline) combined with an intracerebroventricular injection of BTX-A for 3 consecutive weeks. Eight healthy rats that received no treatment were used as a blank control.

2.3. Behavioral assays

Sucrose preference test: On the 19th day, rats were habituated to 1% (wt/vol) sucrose solution (for 3 days) to prevent neophobia during testing. On the 22nd day, following 2 hours of water deprivation, rats were presented with two bottles containing 1% sucrose or water and allowed to drink for 1 hour. Sucrose and water intake were recorded, and this test was performed for 3 consecutive days (Days 22−24). Sucrose intake was averaged across experiments.

Open-field test: Rats were subjected to an open-field test before, and 7 days, 14 days, and 22 days after exposure to CUS. The open-field test was performed in a square iron box (80 × 80 × 40 cm^3) with a black base. The base was divided into 25 mini squares (4 cm × 4 cm each). Each animal was tested in the open-field four times (3 minutes each time). The crossing score was determined as the number of squares crossed during the test, and the rearing score was determined as the number of times the rat stood up. The box was cleaned with 70% ethanol after each time.

2.4. Analysis of glutamate in the hippocampus

Rats were anesthetized with CO_2 and decapitated. The hippocampus was dissected on ice and rapidly frozen by using liquid nitrogen. The samples were weighed and homogenized in 90% cold ethyl alcohol. The samples were then centrifuged at 1,500 g for 10 minutes, and the resulting supernatant was analyzed. The precipitation was extracted for glutamate three times, and all the supernatant was collected into one tube. The supernatant was filtered with a 0.45-μm pore filter before loading (Millipore, Boston, MA, USA). Quantification of glutamate was performed by using a high performance liquid chromatography system (GILSON, Madison, Wisconsin, USA) with fluorescence detection on a Hypersil ODS column (Thermo Fisher Scientific, Waltham, MA, USA) (4.0 mm × 125 mm, 5 μm). The mobile phase consisted of Mobile Phase A [10-mmol/L Na_2HPO_4 (PB), pH 7] and Mobile Phase B (PB:methanol:acetonitrile = 50:35:15, V/V). Mobile Phase B was elevated from 0% to 100% within 25 minutes at a flow rate of 1.0 mL/min. The excited and the emitted wavelengths were selected as 340 nm and 450 nm, respectively.

2.5. Western blot analysis

The hippocampus was lysed in Radio Immunoprecipitation Assay Buffer. Protein concentrations were determined by using a Bicinchoninic acid (BCA) protein assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The protein extraction (30 μg/lane) was separated by using 12% (w/v) gradient sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% (w/v) skimmed milk in 0.05% tris-buffered saline with Tween-20

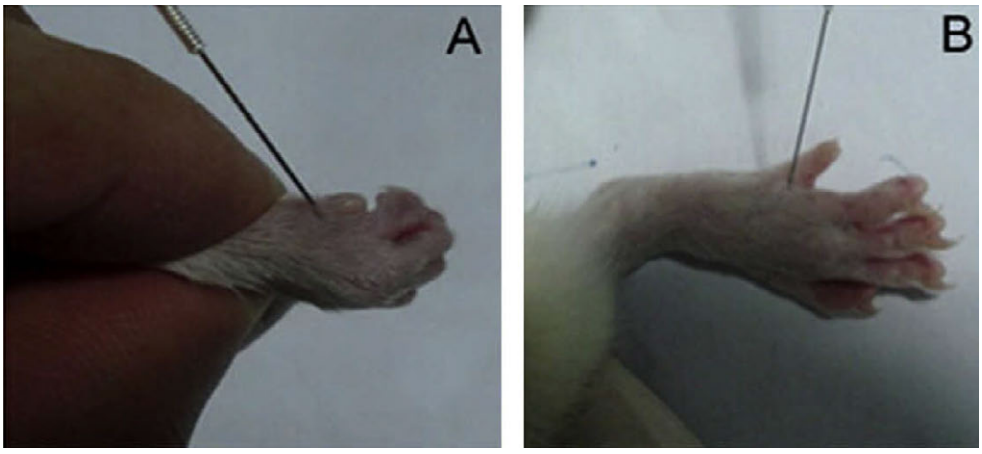

Figure 1 Body acupoints for acupuncture. (A) Hegu acupoint. (B) Taichong acupoint.

Table 1 Primers used in this study.

Primer	Forward (5′—3′)	Reverse (5′—3′)
VGLUT1	CCACGACCAATGTGCGAAAGC	ACAGCCAGGACCAGGAAGGA
VGLUT2	CCTCTCCAACTCACAGCCTTG	CTCGTGTCCAGCCTTACCAGA
VAMP1	GCGTCCCACATGCTTCTGACT	GCGTGTTCCTGAGAGCCTGTT
VAMP2	GGTGGATGAGGTGGTGGACAT	CGCTTGAGCTTGGCTGCACTT
VAMP7	GCCCAAGTGGACGAACTGAAAGG	GGTGACGGACGAATCTACGAGGT
Syntaxin 1	GTGGAACACGCTGTGGACTAC	CGATGGTGGAGGCGATGATGAT
SNAP-25	GCAGGTGAGCGGCATCAT	GCACGTTGGTTGGCTTCATC
18S	GAATTCCCAGTAAGTGCGGGTCAT	CGAGGGCCTCACTAAACCATC

Primers were designed using Primer Express version 2.0 software (Foster, CA, USA). Primer specificity was confirmed using Primer-BLAST web software (National Centre for Biotechnology Information, Bethesda, Maryland, USA).

at room temperature for 2 hours and were incubated with rabbit polyclonal anti-V-GLUT1 (Abcam, 1:3000), V-GLUT2, syntaxin-1A, VMAP1, VMAP2, VMAP7, or SNAP-25 (Abcam, 1:2000) in phosphate buffer solution (0.25% Triton X, 1% bovine serum albumin) overnight at 4°C. Membranes were then incubated with antirabbit horseradish peroxidase-conjugated secondary antibody (1:5000, Boster, Wuhan, China) for another 2 hours at room temperature. Blots were visualized by using enhanced chemiluminescence and film exposures. Blots were scanned with a ChemiDoc image analysis system (Bio-Rad Laboratories, Hercules, CA, USA) and analyzed with ImageJ software (National Institutes of Health, Bethesda, MD, USA).

2.6. Quantitative real-time polymerase chain reaction analysis

Total RNA was extracted from the hippocampus by using an RNA Extraction Kit (Invitrogen, Thermo Fisher Scientific, Waltham, MA, USA) according to manufacturer's instructions. The concentration of total RNA was quantified by measuring the absorbance at 260 nm. For cDNA synthesis, 1.5 μg of total RNA were used with oligo dT primers and SuperScript II reverse transcriptase (Invitrogen) and was subsequently diluted with nuclease-free water to obtain 10-ng/uL cDNA. The primers used in this study are shown in Table 1. Real-time Quantitative Polymerase Chain Reaction

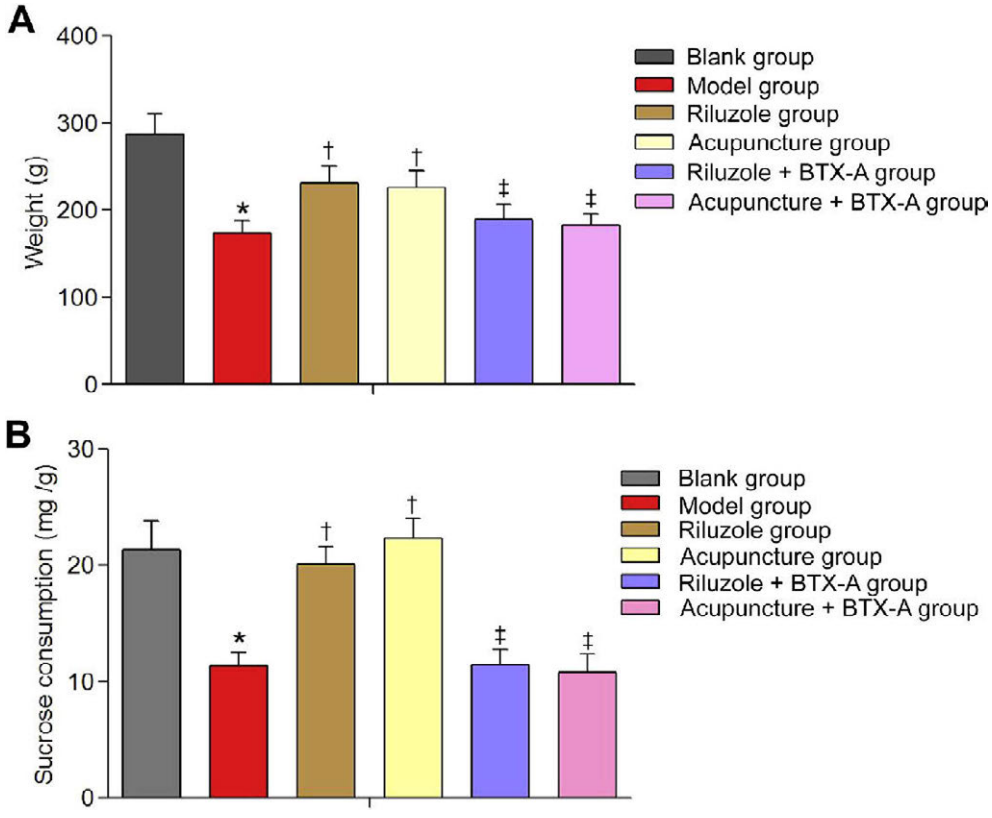

Figure 2 Behavioral assays: (A) weight changes for each group and (B) changes in sucrose consumption for each group. Data are presented as mean ± standard error of the mean. * $p < 0.05$ versus control group. † $p < 0.05$ versus model group. ‡ $p > 0.05$ versus model group.

Table 2 Horizontal or vertical movements of rats in each group (squares/3 min; $n = 8$).

	Groups	Before therapy	7 d	14 d	22 d
Vertical movements	Blank group	14.2 ± 2.2	14.2 ± 1.7	13.7 ± 1.4	13.9 ± 1.7
	Model group	7.5 ± 1.5	5.5 ± 1.3	4.3 ± 0.7	3.5 ± 1.1&
	Riluzole group	7.6 ± 1.2	5.4 ± 1.7	6.2 ± 1.6	7.8 ± 1.5
	Acupuncture group	7.7 ± 1.3	5.3 ± 1.5	6.9 ± 1.4	7.2 ± 1.4
	Riluzole + BTX-A group	7.7 ± 1.2	5.3 ± 1.2	4.9 ± 0.5	3.2 ± 0.7
	Acupuncture + BTX-A group	7.5 ± 1.4	5.4 ± 1.2	4.1 ± 1.4	3.4 ± 0.9
Horizontal movements	Blank group	71.1 ± 7.2	69.8 ± 6.8	70.3 ± 7.5	70.6 ± 7.9
	Model group	55.8 ± 5.3	46.0 ± 5.1	41.7 ± 5.9	33.8 ± 5.7
	Riluzole group	56.3 ± 6.4	45.2 ± 5.6	52.4 ± 5.3	60.8 ± 5.3
	Acupuncture group	54.8 ± 5.9	46.2 ± 5.4	45.4 ± 6.2	54.7 ± 6.2
	Riluzole + BTX-A group	56.3 ± 6.5	45.2 ± 5.3	40.6 ± 5.8	34.6 ± 4.8
	Acupuncture + BTX-A group	55.7 ± 6.3	45.4 ± 4.3	40.6 ± 5.6	34.7 ± 5.2

Data are presented as mean ± standard error of the mean.

(qPCR) was performed by utilizing a hot-start SYBR-green-based method (Invitrogen). Gene fold changes were determined by utilizing the ΔΔCt method with 18srRNA for normalization. DNA was amplified with an initial denaturation at 94°C for 3 minutes, followed by 35 cycles at 94°C (15 seconds) and 60°C (15 seconds). All experiments were performed in triplicate and repeated twice.

2.7. Statistical analysis

The data are presented as means ± standard errors of the mean and were analyzed by using SPSS version 13.0 statistical software (SPSS Inc., Chicago, IL, USA). The statistical significance of the differences between groups was analyzed by using either the Student *t* test or the one-way analysis of variance. A value of $p < 0.05$ was considered as statistical significance.

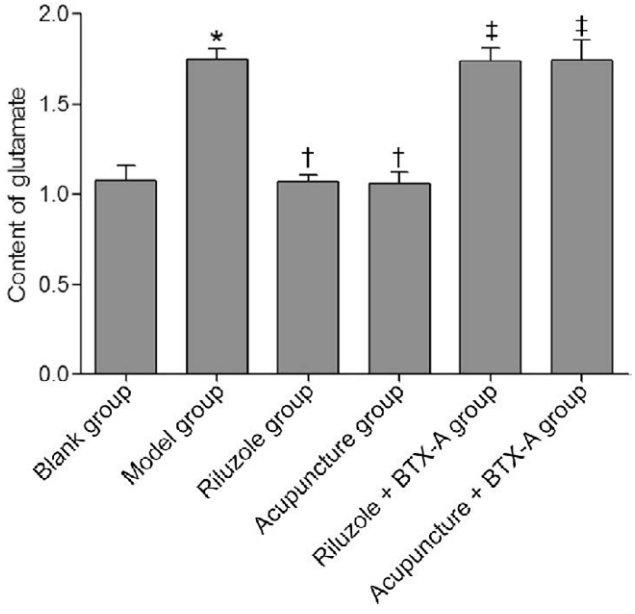

Figure 3 Glutamate content in the hippocampus in each group. Data are presented as mean ± standard error of the mean. * $p < 0.05$ versus control group, † $p < 0.05$ versus model group, ‡ $p > 0.05$ versus model group.

3. Results

All rats developed depression-like behaviors after 21 days of exposure to CUS. The changes in the weights and sucrose consumptions in each group are shown in Fig. 2. The weights of the rats were significantly lower in the model group (165.5 ± 14.3 g) compared with the blank group (275.3 ± 15.5 g; $p < 0.05$). Moreover, compared with the blank group (21.3 ± 2.5 mg/g), sucrose consumption dropped markedly in the model group (11.3 ± 1.2 mg/g; $p < 0.05$). Acupuncture or riluzole treatment ameliorated the changes in weight and sucrose consumption ($p < 0.05$). By contrast, no obvious differences, including weight and sucrose consumption differences, were observed among the model, the acupuncture + BTX-A, and the riluzole + BTX-A group ($p > 0.05$).

Open-field tests were performed the day before treatment and 7 days, 14 days, and 22 days after treatment. After acupuncture or riluzole treatment, the horizontal and vertical movements were significantly increased compared with the model group ($p < 0.05$). By contrast, no distinct differences were observed among the model, the acupuncture + BTX-A, and the riluzole + BTX-A groups after acupuncture combined with BTX-A or riluzole combined with BTX-A treatment ($p > 0.05$; Table 2).

The content of glutamate in the hippocampus was very much higher after 21 days of exposure to CUS ($p < 0.05$). After 22 days of therapy, glutamate contents were 1.74 ± 0.078 mg/g in the riluzole + BTX-A group and 1.75 ± 0.11 mg/g in the acupuncture + BTX-A group; these values were similar to the value for the model group and much higher than the value for the blank group ($p < 0.05$). In addition, no obvious differences were noted among the blank, riluzole, and acupuncture groups ($p < 0.05$; Fig. 3).

Glutamicacid—glutamicacid (Glu—glu) cycle-related proteins were investigated before and after therapy. The protein and mRNA expression levels of synapsin, VGLUT1, and VGLUT2 were decreased after 21 days of exposure to CUS ($p < 0.05$; Figs. 4 and 5). However, VGLUT1 and VGLUT2

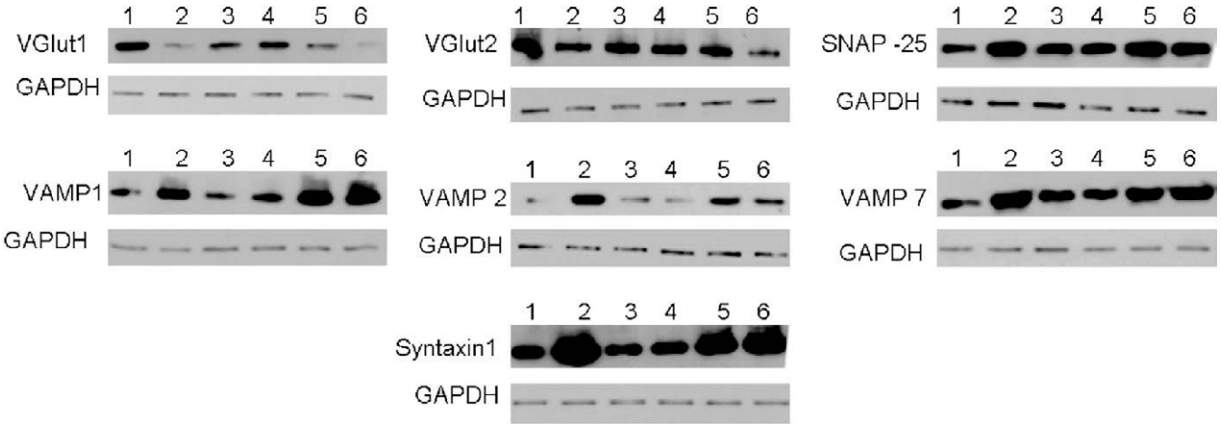

Figure 4 Protein expression levels of each group. 1 = blank group; 2 = model group; 3 = riluzole group; 4 = acupuncture group; 5 = riluzole + BTX-A group; 6 = acupuncture + BTX-A group.

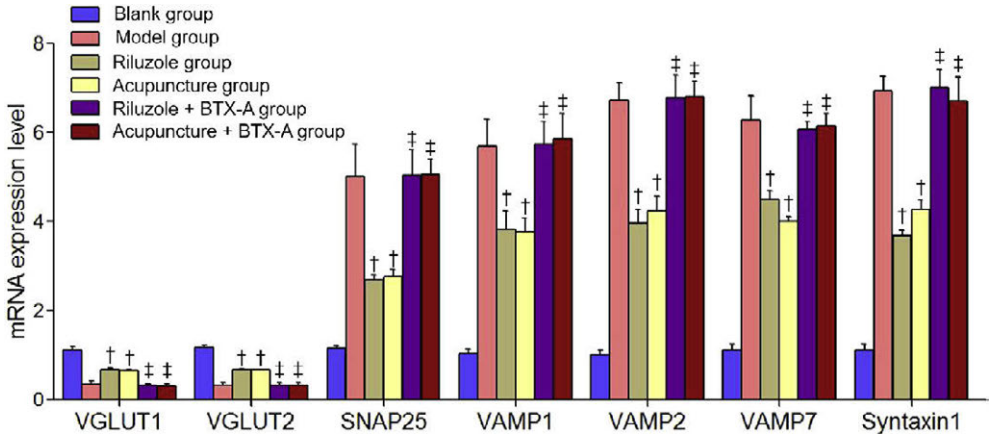

Figure 5 mRNA expression levels of each group. Data are presented as mean ± standard error of the mean. * $p < 0.05$ versus control group, † $p < 0.05$ versus model group, ‡ $p > 0.05$ versus model group.

were downregulated after riluzole + BTX-A or acupuncture + BTX-A treatment, similar to the model group. Furthermore, the model group exhibited upregulated SNARE complex-related genes and protein expressions including SNAP25, VAMP1, VAMP2, VAMP7, and syntaxin1 ($p < 0.05$). However, both riluzole and acupuncture treatments reduced these genes and protein expressions compared with the model group ($p < 0.05$). In addition, both the riluzole + BTX-A and the acupuncture + BTX-A treatments restored these deduction ($p > 0.05$).

4. Discussion

SNARE proteins are membrane-associated proteins that have been implicated as being central in most intracellular membrane trafficking events studied so far [18]. The synaptic proteins syntaxin, SNAP-25, and VAMP10 were the first SNAREs to be discovered. SNARE proteins play key roles in all eukaryotic cell endocytosis, secretion, and membrane fusion [19].

Neurotransmitter release is mediated by synaptic vesicle exocytosis at the active zone of a synapse. Glutamate is the major excitatory neurotransmitter in the brain, and SNARE proteins are involved in all glu–glu cycles. Furthermore, glutamate release is found to be sensitive to stress and glucocorticoids. Reagan et al [20] found that exposure to acute stress rapidly increased glutamate release in the hippocampus and the prefrontal cortex. Rats receiving CUS treatments showed significant increases in the amount of glutamate and the number of structural changes in the hippocampus [20]. In "the excitatory amino acid mechanism theory" Ongur et al [21] indicated that the etiology of depressive disorders among nervous-system disorders was associated with glutamate release. Danglot et al [22] constructed mutant mice lacking neuronal SNARE vesicles (TI-VAMP/VAMP7) to characterize forebrain neuronal synaptic release, and they found that the number of neurons was greatly reduced. Extremely high contents of glutamate have also been found in depressive patients, and clinical studies have indicated that depressive disorder is associated with increased glutamatergic activity [6].

Botulinum neurotoxins (BoNTs) represent a family of seven antigenically distinct molecules and are produced by clostridium botulinum bacteria strains. Due to their capacity to block neuromuscular signal transmission, BoNTs always lead to botulism [23]. BoNTs bind to cellular

receptors, causing cellular internalization or endosomal escape, finally resulting in SNARE-protein cleavage [24,25]. In this study, we injected BTX-A into the rat's encephalocele to hydrolyze SNARE proteins and found that acupuncture therapy and riluzole therapy for rats that had been injected with BTX-A were ineffectual in treating depression. Decreased rat weights, sucrose consumption, horizontal and vertical movements, together with increased amounts of glutamate in the hippocampus and glu—glu cycle-related gene expression levels in the BTX-A treatment groups indicated that SNARE proteins might be one of the targets of antidepression. However, single acupuncture or riluzole therapy affected SNARE proteins expression and showed efficacy in remitting depression syndrome. Furthermore, these results are also in accord with the results of our clinical, randomized, controlled trials of acupuncture for the treatment of depression [5].

In conclusion, this study suggests that SNARE proteins are involved in the remission of depression in rats treated by using acupuncture. Therefore, SNARE proteins might be targets of antidepression. However, we are still at the beginning of our understanding of how these proteins operate and how they are regulated through acupuncture. Further studies using gene-knockout mice are still needed for a deeper understanding of this antidepression mechanism of acupuncture. In addition, cautious and deeper research will be indispensable if the effect of acupuncture in treating depression in human is to be assessed.

Disclosure statement

The authors declare that they have no conflicts of interest and no financial interests related to the material of this manuscript.

Acknowledgments

The authors express their deepest appreciation to the staff of the Scientific Research Department of Guangdong Hospital of Traditional Chinese Medicine for its technical support and to the staff of the Division of Epidemiology and Biostatistics, Mel and Enid Zuckerman College of Public Health, University of Arizona, for its analysis of the data. This research was supported by (1) the Youth Fund Project of the Natural Science Foundation of China: Antidepressant mechanism of acupuncture based on SNARE proteins modulating the release of glutamic acid in presynaptic neurons (81303041); (2) A class general financial grant from the China Postdoctoral Science Foundation: Mechanism research of acupuncture on hippocampal tripartite glutamatergic synapses of depressive disorder rats (2012M511784); (3) Science Foundation of Outstanding Young Innovative Personnel, Department of Education, Guangdong Province: Clinical research of acupuncture in the treatment of mild to moderate depression (2012LYM_0043); (4) Special research foundation of the new teacher category for the doctoral program of higher school sponsored by the National Ministry of Education: The moderating effects of acupuncture for hippocampal tripartite glutamatergic synapses of depressive disorder rats (20124425120005); (5) Special financial grant from the China Postdoctoral Science Foundation: The modulating effects of acupuncture for glutamic acid in hippocampal presynaptic neurons of depressive disorder rats (2013T60793); and (6) Science Foundation of the Postdoctoral Researchers, Guangzhou University of Chinese Medicine, from Guangdong Provincial Department of Human Resources and Social Security Fund: Clinical study on acupuncture and moxibustion for treatment of depression based on patient reported outcome indicators (BBK429122K19).

References

[1] Lee CN. Reviewing evidences on the management of patients with motor neuron disease. *Hong Kong Med J*. 2012;18:48—55.
[2] Lane RM. Antidepressant drug development: Focus on triple monoamine reuptake inhibition. *J Psychopharmacol*. 2015;29:526—554.
[3] Blier P, Montigny C. Current advances and trends in the treatment of depression. *Trends Pharmacol Sci*. 1994;15:220—226.
[4] Arroll B, Macgillivray S, Ogston S, Reid I, Sullivan F, Williams B, et al. Efficacy and tolerability of tricyclic antidepressants and SSRIs compared with placebo for treatment of depression in primary care: a meta-analysis. *Ann Fam Med*. 2005;3:449—456.
[5] Fan L, Fu W, Xu N, Liu J, Ou A, Wang Y. Meta-analysis of 20 clinical randomized controlled trials of acupuncture for depression. *Neural Regener Res*. 2010;5:1862—1869.
[6] Croarkin PE, Nakonezny PA, Husain MM, Melton T, Buyukdura JS, Kennard BD, et al. Evidence for increased glutamatergic cortical facilitation in children and adolescents with major depressive disorder. *Jama Psychiatry*. 2013;70:291—299.
[7] Sanacora G, Zarate CA, Krystal JH, Manji HK. Targeting the glutamatergic system to develop novel improved therapeutics for mood disorders. *Nat Rev Drug Discov*. 2008;7:426—437.
[8] Hunt KJ, Coelho HF, Wider B, Perry R, Hung SK, Terry R, et al. Complementary and alternative medicine use in England: results from a national survey. *Int J Clin Pract*. 2010;64:1496—1502.
[9] Garcia MK, McQuade J, Lee R, Haddad R, Spano M, Cohen L. Acupuncture for symptom management in cancer care: an update. *Curr Oncol Rep*. 2014;16:418—427.
[10] Zhang J, Chen J, Li X, Lai X, Zhang S, Wang S. Early filiform needle acupuncture for poststroke depression: a meta-analysis of 17 randomized controlled clinical trials. *Neural Regen Res*. 2014;9:773—784.
[11] Mukaino Y, Park J, White A, Ernst E. The effectiveness of acupuncture for depression - a systematic review of randomised controlled trials. *Acupunct Med*. 2005;23:70—76.
[12] Altar CA, Laeng P, Jurata LW, Brockman JA, Lemire A, Bullard J, et al. Electroconvulsive seizures regulate gene expression of distinct neurotrophic signaling pathways. *J Neurosci*. 2004;24:2667—2677.
[13] Schloss P, Henn FA. New insights into the mechanisms of antidepressant therapy. *Pharmacol Ther*. 2004;102:47—60.
[14] Bonnet E, Martinez N, Martinez A, Marks PMH, Cadd VA, Elliott M, et al. A botulinum toxin—derived targeted secretion inhibitor downregulates the GH/IGF1 axis. *J Clin Invest*. 2012;122:3295—3306.
[15] Samuel M, Young J. Proteolysis of SNARE proteins alters facilitation and depression in a specific way. *Proc Natl Acad Sci*. 2005;105:2614—2619.
[16] Banasr M, Valentine GW, Li XY, Gourley SL, Taylor JR, Duman RS. Chronic unpredictable stress decreases cell proliferation in the cerebral cortex of the adult rat. *Biol Psychiat*. 2007;62:496—504.

[17] Risterucci C, Coccurello R, Banasr M, Stutzmann JM, Amalric M, Nieoullon A, et al. The metabotropic glutamate receptor subtype 5 antagonist MPEP and the Na(t) channel blocker riluzole show different neuroprotective profiles in reversing behavioral deficits induced by excitotoxic prefrontal cortex lesions. *Neuroscience*. 2006;137:211—220.

[18] Sollner T, Whiteheat SW, Brummer M, Erdjument-Bromage H, Geromanos S, Tempst P, et al. SNAP receptors implicated in vesicle targeting and fusion. *Nature*. 1993;362:318—324.

[19] Lonart G, Sudhof TC. Assembly of SNARE core complexes prior to neurotransmitter release sets the readily releasable pool of synaptic vesicles. *J Biol Chem*. 2000;275:27703—27707.

[20] Reagan LP, Reznikov LR, Evans AN, Gabriel C, Mocaer E, Fadel JR. The antidepressant agomelatine inhibits stress-mediated changes in amino acid efflux in the rat hippocampus and amygdala. *Brain Res*. 2012;1466:91—98.

[21] Ongur D, Drevets WC, Price JL. Glial reduction in the subgenual prefrontal cortex in mood disorders. *Proc Natl Acad Sci*. 1998;95:13290—13295.

[22] Danglot L, Zylbersztejn K, Petkovic M, Gauberti M, Meziane H, Combe R, et al. Absence of TI-VAMP/Vamp7 leads to increased anxiety in mice. *J Neurosci*. 2012;32:1962—1968.

[23] Young Jr MS. Proteolysis of SNARE proteins alters facilitation and depression in a specific way. *Proc Natl Acad Sci*. 2005;102:2614—2619.

[24] Sheng C, Barbieri JT. Engineering botulinum neurotoxin to extend therapeutic intervention. *Proc Natl Acad Sci*. 2009;106:9180—9184.

[25] Marinelli S, Vacca V, Ricordy R, Uggenti C, Tata AM, Luvisetto Siro, et al. The Analgesic Effect on Neuropathic Pain of Retrogradely Transported Botulinum Neurotoxin A Involves Schwann Cells and Astrocytes. *PloS One*. 2012;7:1—12.