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ORIGINAL RESEARCH

Study on the Therapeutic Effect of Yu-Mu-Tiao-Shen Acupuncture on Rats with Autism Spectrum Disorder

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Purpose: Acupuncture has been shown to improve symptoms of Autism Spectrum Disorder (ASD), but its underlying mechanisms remain unclear. This study aims to explore the behavioral effects of Yu-Mu-Tiao-Shen Acupuncture on ASD model rats and investigate the potential molecular mechanisms based on SFRP5, β-catenin, and GSK-3β.

Patients and Methods: On gestation day 12.5, SD (Sprague-Dawley) rats were intraperitoneally injected with valproic acid (VPA), and their offspring were considered a reliable ASD rat model. The offspring were randomly assigned to the VPA group or the VPA_acupuncture group (n=7), with a normal group serving as the control. The VPA_acupuncture group underwent Yu-Mu-Tiao-Shen Acupuncture from postnatal days 8 to 28. All rats underwent behavioral testing, including social interaction, open field, and Morris water maze tests. Subsequently, hippocampal and prefrontal cortical tissues were extracted for histological analysis and RNA sequencing to assess gene expression differences across groups, as well as protein expression in hippocampal tissues.

Results: Behavioral tests demonstrated that the Yu-Mu-Tiao-Shen acupuncture technique improved spontaneous activity, abnormal social interaction, and alleviated learning and memory impairments in autistic rat models. In the VPA_acupuncture group, total travel distance, average speed, social interaction time, time spent exploring novel rats, and the number of platform crossings were significantly increased ($P < 0.05$). Transmission electron microscopy revealed abnormal synaptic and mitochondrial structures in the hippocampal tissue of the VPA group. In contrast, the SD and VPA_acupuncture groups showed relatively intact pre- and post-synaptic membrane structures and normal mitochondrial morphology. Differentially expressed gene (DEG) analysis revealed 111 significantly altered genes in the hippocampus and 282 in the prefrontal cortex between the VPA_acupuncture and VPA groups. Furthermore, in the VPA_acupuncture group, the hippocampal expression levels of SFRP5, β-catenin, and GSK-3β proteins were significantly reduced compared to the model group ($P < 0.05$).

Conclusion: Yu-Mu-Tiao-Shen Acupuncture can regulate the expression of relevant proteins, modulating synaptic plasticity in hippocampal and prefrontal neurons, which effectively improves motor, social cognitive, and spatial memory abilities in ASD rats. This study provides valuable insights for further exploration of ASD treatments.

Keywords: Yu-Mu points, sishencong points, acupuncture, ASD, autism spectrum disorder, behavioral experiments

Introduction

Autism Spectrum Disorder (ASD) is a severe neurodevelopmental disorder, with a steadily increasing global prevalence in recent years, resulting in a significant burden on affected families and society.[1,2] The core symptoms of ASD primarily include impairments in social communication, a narrow range of interests or activities, and repetitive, stereotyped

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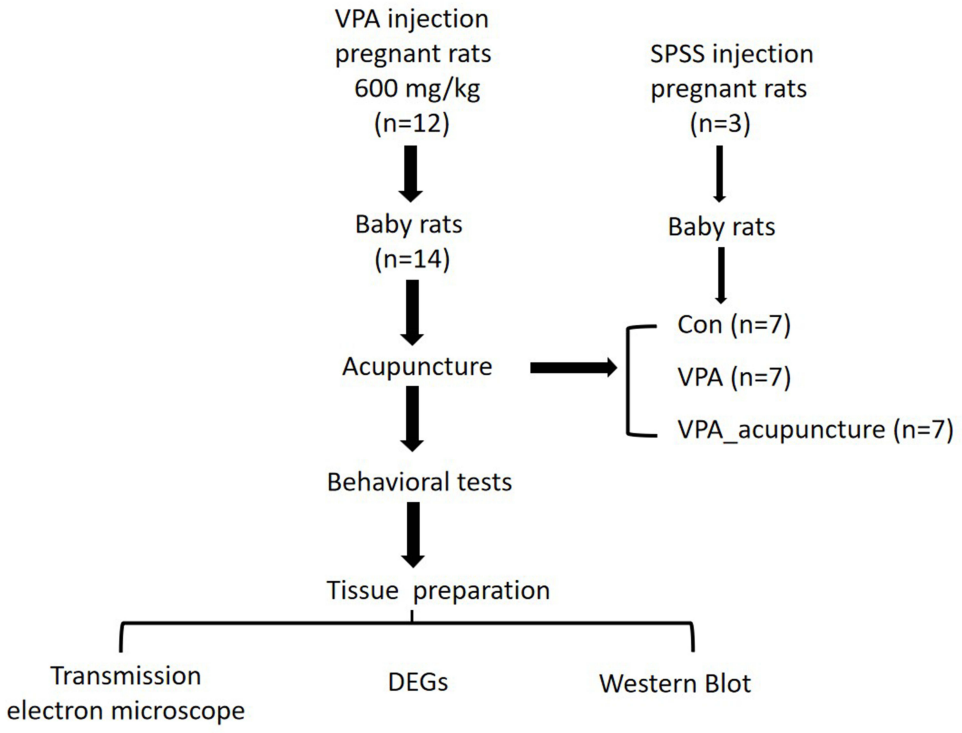
Graphical Abstract



behaviors, which severely impact the patient's daily life quality and social adaptability. Current treatment strategies primarily focus on behavioral therapy, pharmacological interventions, and educational support, which can partially alleviate symptoms but do not provide a complete cure.[3] Although medications such as bumetanide and memantine have demonstrated therapeutic potential in animal models, their efficacy in human clinical trials has been disappointing.[4–6] These drugs generally exhibit limited effectiveness and are associated with significant side effects.

As an important component of traditional Chinese medicine, acupuncture has gradually garnered increasing attention in the treatment of ASD.[7] By applying needles to specific acupoints, regulating qi and blood, and balancing the functions of internal organs, acupuncture is believed to yield significant therapeutic effects for various neurological disorders.[8,9] It primarily works by modulating neurotransmitter levels and neural pathway functions within the central nervous system, thereby improving patients' social behaviors, cognitive abilities, and overall functioning.[10,11] However, existing research findings are inconsistent. While some studies indicate that acupuncture has a positive impact on alleviating core symptoms of ASD, challenges remain due to significant individual variations, particularly in standardizing treatment protocols and exploring underlying mechanisms. There is also a lack of extensive exploration regarding combinations of acupoints.[12–14]

Traditional Chinese Medicine (TCM) theories such as the "Heart-Brain Connection Theory", "Xuanfu Theory", and the "Transformation and Steaming Theory" provide a theoretical basis for the Yu-mu-Tiao-shen acupuncture technique, elucidating its significance in regulating organ functions, balancing the flow of Qi, blood, and Yin-Yang, and improving neurological and psychiatric disorders. The "Shenyu" point helps to calm the Qi and regulate the mind. The "Pishu" point strengthens the spleen, generates blood, and nourishes the heart to calm the spirit. The "Shenshu" point has a tonifying effect on the kidneys and strengthens the marrow, promoting mental calmness. The "Mu points" are all located in the abdominal region around the gastric area, with the "Zhongfu" point regulating Qi and unblocking meridians, relieving liver Qi stagnation. The "Zhongwan" point, a Mu point of the stomach, adjusts the Qi mechanism of the five organs, strengthens the spleen and stomach, and generates Qi and blood. The "Guanyuan" point, a Mu point of the small intestine, supplements the original Qi, and tonifies the

kidneys, consolidating the foundation. The "Shuyu" and "Mu" points are two specific types of acupuncture points—one located anteriorly, the other posteriorly, one Yin and one Yang. When used together, they complement each other to harmonize Yin and Yang, facilitate the flow of Qi through the organs and meridians, regulate the balance of Qi and blood, and coordinate the mental state of the five organs. The "Four Spirit Needle" technique has various therapeutic effects, including sedating the spirit, opening the orifices and awakening the brain, calming the liver and extinguishing wind, dispelling wind and relieving pain, and promoting blood circulation and unblocking meridians.[15,16]

Clinically, the combination of "Shuyu" and "Mu" points is used to apply the corresponding acupoints of the affected organs in order to exert a synergistic effect for the treatment of various diseases. Studies have shown that rapid needling of the "Shuyu" and "Mu" points can improve sleep disorders in children with ASD and alleviate core symptoms.[17,18] Acupuncture with the "Four Spirit Needle" significantly improves behavioral symptoms in children, especially in areas such as sensory function, motor skills, self-care abilities, and the Childhood Autism Rating Scale (CARS) score.[19] Furthermore, clinical experiments involving the combined use of the "Four Spirit Needle" treatment and sandplay therapy for ASD children indicate that both methods can assist in improving language and social adaptability.[20,21] As a result, acupuncture's potential in treating ASD has been recognized, though further exploration of more acupoint combinations is necessary to validate its effectiveness and safety.

Functional imaging studies have confirmed that acupuncture specifically activates the left hippocampus and bilateral pons, enhancing the functional connectivity between the parietal and frontal lobes to improve language cognition.[22] Animal experiments show that acupuncture improves autism-like behaviors by repairing the synaptic function of prefrontal neurons and regulating immune inflammation.[23] Additionally, acupuncture activates the hypothalamic oxytocin system, significantly upregulating the levels of endogenous cannabinoids and serotonin (5-HT).[24] In clinical practice, the combination of "Shuyu" and "Mu" points regulates the HPA axis, with a 67.5% improvement in the abnormal circadian rhythm of cortisol, highlighting the potential for neuroendocrine regulation.[17] Although existing research has preliminarily elucidated the mechanisms of some acupuncture therapies, further studies on the evaluation of efficacy and molecular mechanisms are still needed.

Based on this, the present study proposes a multi-acupoint combination acupuncture treatment plan, which integrates the theory of "Shuyu and Mu point combination" in Traditional Chinese Medicine with the concept of "spiritual regulation". The "Yu-Mu-Tiao-Shen acupuncture" is used to improve the behavior, social cognition, and spatial memory function of rats in a VPA-induced ASD model. Additionally, RNA sequencing and Western blot (WB) techniques are employed to further explore the potential mechanisms, providing a more systematic and scientific reference for acupuncture treatment of ASD.

Materials and Methods

Animals and Grouping

The experiment involved 15 female SD rats and 15 male SD rats, all aged 6 weeks, with a weight of 200 ± 20g. The rats were kept in an environment with a temperature of 21–24°C, humidity of 50–60%, and a 12-hour light/dark cycle. They had free access to food and water. After 1 week of acclimatization, the rats were divided into groups and subjected to interventions. Vaginal smears were taken from female rats, and the day when sperm was observed under the microscope was marked as day 1 of pregnancy. Using the Matlab R2022a random number generation function, the pregnant rats were randomly assigned to two groups: the VPA group (n = 12) and the control group (n = 3). The VPA group received an intraperitoneal injection of valproic acid (600 mg/kg) on day 12.5 of pregnancy, which led to offspring showing neurodevelopmental abnormalities and displaying ASD-like behaviors at birth. The control group received an equal volume of saline injection at the same time. The expected duration of the study for the newborn rats was from PND 0 to PND 21. We followed the method described by Roulle et al.[24] After successful model establishment confirmed by developmental and social behavioral assessments, a total of 14 newborn rats delivered by dams in the VPA group were randomly assigned into two groups: the VPA group (n=7) and the VPA_acupuncture group (n=7). Additionally, 7 newborn rats from pregnant rats injected with saline were designated as the control group (n=7). A timeline and flowchart of the animal experiment are shown in Figure 1.

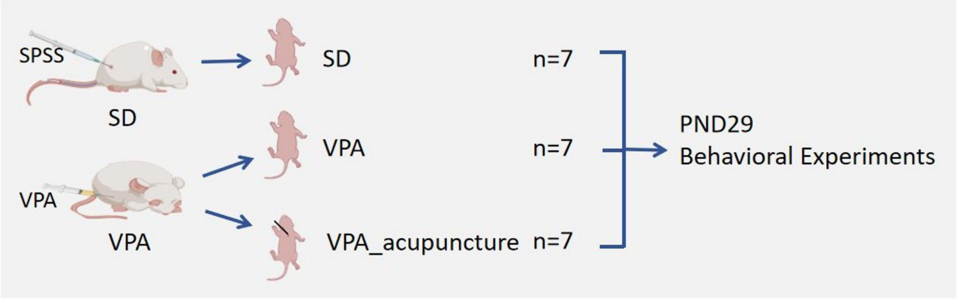


Figure 1 Model Preparation and Experimental Procedure.

All experiments followed the National Standards for Laboratory Animal Management. This study was approved by the Animal Ethics Committee of Henan University of Chinese Medicine (IACUC-202404016).

Intervention

During the period from PND 8 to 28, rats in the VPA_acupuncture group received daily acupuncture treatment. The acupuncture points included Sishen (EX-NH1), Feishu (BL 13), Shenshu (BL 23), Shanzhong (CV 17), Zhongwan (CV 12), Guanyuan (CV 4), Shenmen (HT 7), Pishu (BL 20), and Qimen (LR 14), with a needle depth of 3mm. During the Sishen acupuncture, the needle tip was directed toward Baihui (GV 20), and the needle was retained for 20 minutes. The procedure employed the horizontal insertion technique, with the needle positioned at a 15–20° angle to the skin surface, ensuring that the sensation of the needle was conducted toward the Baihui area. After needling in the other acupuncture points, rapid needling was performed, as shown in Figure 2.

Both the SD and VPA groups also underwent restraint to minimize the physiological responses caused by handling, which could introduce errors into the experimental results, thus improving the reliability of the findings.

Behavioral Experiments

Open Field Test

On the night of PND 29, the three groups of rats were placed in an open field (40cm × 40cm × 40cm) for an open field test. The walls and bottom of the box were black and opaque. The rats were allowed to explore freely for 10 minutes, and their movements were continuously monitored and recorded using tracking software (TopScanLite). We recorded the rats' movement trajectories, velocity, time spent in the center area of the field, and other behavioral parameters to assess their exploratory behavior and locomotor activity. The experiment was conducted quietly, and between two tests, the box was cleaned with 75% alcohol and cotton cloths to eliminate any odors.

Three-Chamber Social Test

On PND 30, the three-chamber social interaction test was conducted to evaluate the rats' social interest and social memory. The rectangular three-chamber apparatus, visible within the camera's field of view, was divided into three sections, each separated by transparent resin glass, with channels connecting them. The test subject was placed in the center chamber, and a same-strain, same-gender unfamiliar rat was placed in the upper right chamber. After the subject rat became familiar with the environment for 3–5 minutes, it was reintroduced into the center chamber, and its movement trajectories were recorded for social behavior evaluation. Following this, a social novelty preference test was performed. The experimental animal was placed in the central starting area, and the side compartment was closed off. An unfamiliar stimulus rat was introduced into the previously empty side chamber, making it the novel rat. We continuously recorded the movement trajectories and dwell times of the experimental subject in both side chambers to quantify its preference for the novel social stimulus. The experiment was conducted quietly, and between two tests, the box was cleaned with 75% alcohol and cotton cloths to remove any odors.

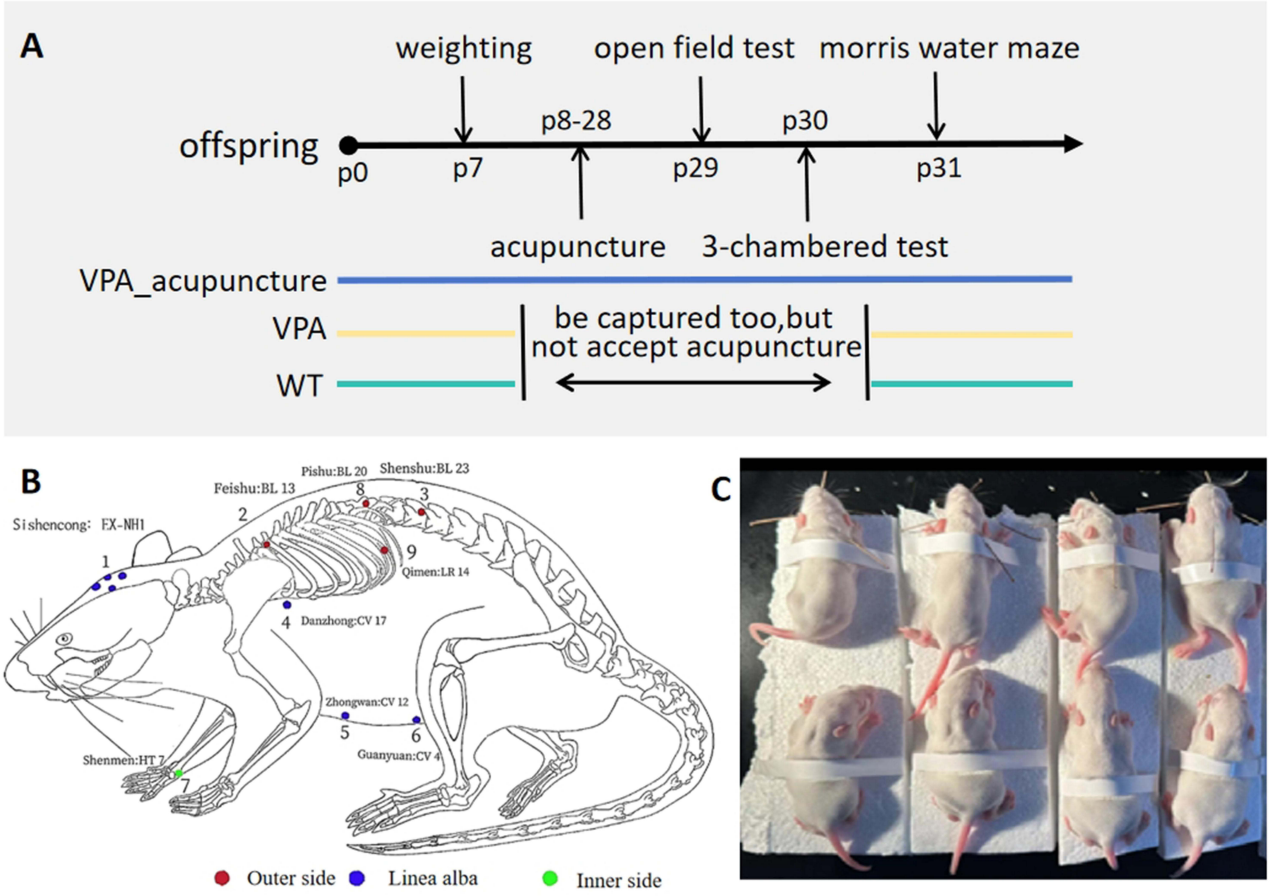


Figure 2 Experimental diagram of acupuncture.
Notes: (**A**) Time axis; (**B**) Acupuncture point selection chart; (**C**) Needle rat.

Morris Water Maze Test

On PND 31, the Morris water maze (MWM) test was conducted to assess the rats' spatial learning and memory abilities. The diameter of the water maze was 160 cm, and a circular platform with a diameter of 8 cm was placed 35 cm from the pool wall at the center of the third quadrant. A camera visible within the experimental area was used to track the rat's movements. The rats were placed on the platform and allowed to search for the platform until they successfully found it, and their movement trajectories were recorded.

Tissue Preparation

Twenty-four hours after completion of behavioral testing, the experimental rats were anesthetized using a gas anesthesia machine (R520IP, RWD, China) in an induction chamber. The initial anesthesia parameters were set at 5% isoflurane mixed gas at a flow rate of 0.2 L/min. After confirming deep anesthesia following a 5-minute induction period, cervical dislocation was performed for euthanasia. The whole brain was immediately extracted and rinsed with physiological saline to remove blood residues. After drying the surface with filter paper, the brain was placed in a pre-chilled Petri dish on ice. Coronal sections were used to sample the prefrontal cortex (within 2 mm anterior to bregma), and hippocampal tissue was carefully isolated. Portions of the tissue were aliquoted into 2 mL sterile Eppendorf tubes and stored at −80 °C for subsequent analyses.

Transmission Electron Microscope

The remaining hippocampal tissue was prepared for transmission electron microscopy (TEM) to observe ultrastructural features. Tissue blocks were trimmed to 1 mm × 1 mm and sectioned at a thickness of 100–110 nm. Sections were stained

with 1% toluidine blue for 5 minutes, followed by rinsing. Ultrastructural observations of cellular morphology, organelles, and synaptic structures were conducted under TEM.

Non-Targeted Genomics Analysis

For transcriptome analysis, mRNA-Seq was performed on the prefrontal cortex and hippocampus of rats from the control, model, and acupuncture groups to investigate differential gene expression. FastQC was used to assess sequence quality, HISAT2 was used for aligning sequences to the rat reference genome, and DESeq2 was employed for gene expression quantification and comparison across groups.

Expression of SFRP5, β-Catenin, and GSK-3β Proteins in Hippocampus

Hippocampal tissues stored at −80 °C were used for total protein extraction. Equal amounts of protein (30 μg) were separated by SDS-PAGE and transferred to membranes for Western blotting. Bands were visualized using a gel imaging system, and grayscale values were quantified using ImageJ software, with β-actin serving as the internal control.

Statistical Analysis

Data analysis was conducted using MATLAB. Results are presented as mean ± standard deviation (±SD). One-way ANOVA was used to determine statistical significance among groups, followed by Bonferroni post hoc tests. A p-value < 0.05 was considered statistically significant.

Results

Behavioral Results Analysis

Acupuncture Improved Spontaneous Activity in a Rat Model of VPA

The open field test (OFT), three-chamber social interaction test, and Morris water maze (MWM) are established paradigms for evaluating animal behavior and cognitive function.

In the OFT, representative locomotor trajectories of VPA_acupuncture and SD groups are shown in Figure 3A. Significant differences were observed among groups in both total distance traveled and average velocity ($F = 23.12$ and 23.11, respectively; $P < 0.001$ for both). Specifically, the VPA group exhibited significantly reduced total distance (9061.94 ± 2820.45 mm) compared to the SD group (23869.46 ± 4000.54 mm, $P < 0.01$), whereas the VPA_acupuncture group (23904.55 ± 4656.57 mm) showed a significant increase relative to the VPA group ($P < 0.001$), with no significant difference compared to the SD group ($P > 0.05$). Similarly, in terms of average speed, the VPA group (15.10 ± 4.70 mm/s) was significantly slower than the SD group (39.78 ± 6.67 mm/s, $P < 0.01$), while the VPA_acupuncture group (39.84 ± 7.76 mm/s) showed significantly higher speeds than the VPA group ($P < 0.01$), again showing no significant difference from the SD group ($P > 0.05$) (See Table 1). Figure 3B and C indicate that acupuncture effectively improved locomotor activity in the model rats.

Acupuncture Improved Abnormal Social Activity in a VPA Rat Model

In the three-chamber social interaction test, the VPA_acupuncture group displayed normal patterns of social interest and memory, with significant improvements compared to the VPA group ($P < 0.001$). Representative movement trajectories during the social novelty preference test are shown in Figure 4A. The VPA group exhibited markedly fewer exploratory movements than the SD group, while the VPA_acupuncture group's trajectories were concentrated around the unfamiliar and novel rats. In both the social preference and social novelty tests, significant differences in interaction time were observed among groups ($P < 0.001$). The VPA_acupuncture group showed significantly increased time interacting with both social and novel rats compared to the VPA group ($P < 0.01$), with no significant differences compared to the SD group ($P > 0.05$) (Figures 4B and C) (Table 2).

Acupuncture Alleviated Impaired Learning and Memory in the VPA Rat Model

In the Morris water maze test, the model group showed fluctuating latency times that were significantly longer than those of the control group, whereas the acupuncture group demonstrated latency patterns closer to those of the control group,

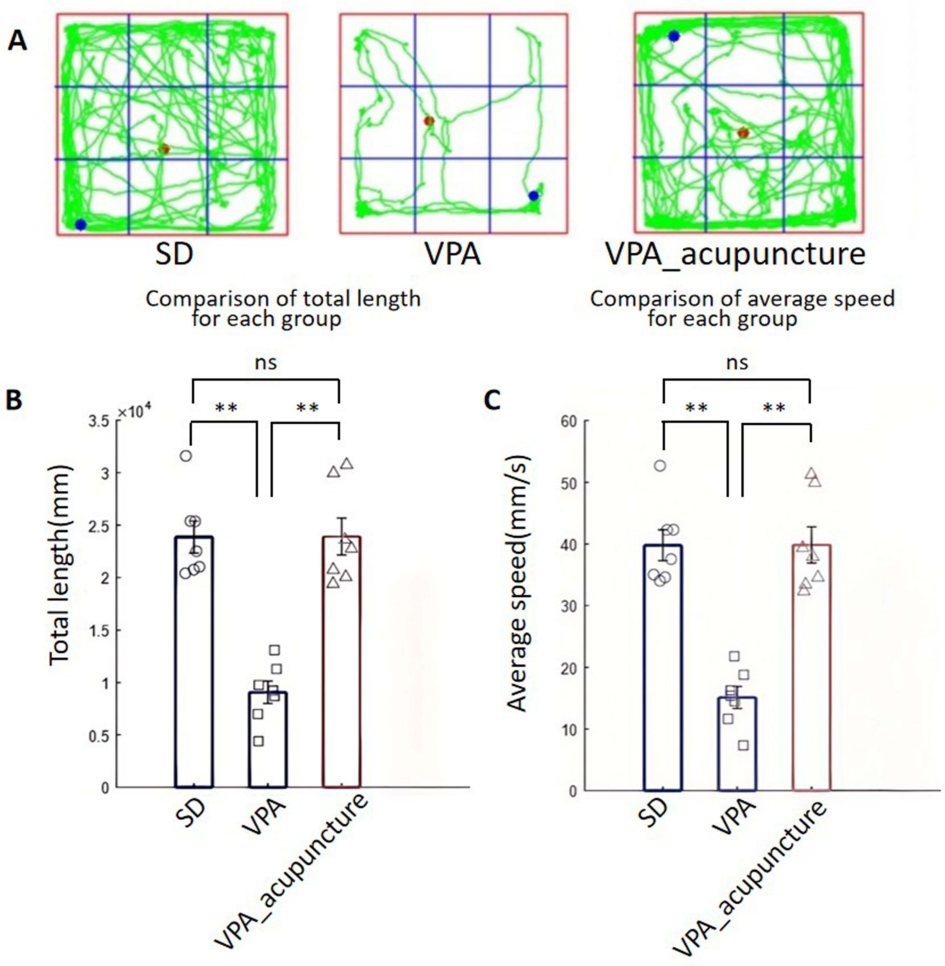


Figure 3 Results of open field test.
Notes: Representative movement trajectories (red is the starting point, blue is the endpoint); (**B**) Total distance traveled by the three groups of rats; (**C**) Average speed of three groups of rats; ** $p < 0.01$; ns $p > 0.05$.

with no significant difference between the two ($P > 0.05$) and notably reduced error margins compared to the model group. The number of successful platform landings was significantly higher in the acupuncture group than in the model group ($P < 0.01$), suggesting that acupuncture reduced behavioral variability and improved spatial memory in ASD model rats. Representative trajectories for each group are shown in Figure 5A. The SD group exhibited concentrated movements within the target quadrant and shorter search paths, whereas the VPA group showed stereotyped behavior along the pool perimeter. Figures 5B–E show group differences in performance, indicating that the "Yu-mu-Tiao-shen" acupuncture protocol effectively mitigated spatial learning and memory impairments in the VPA-induced ASD model.

Table 1 Comparison of Open Field Test Results Across Groups

Group	Total Distance (mm)	Average Speed (mm/s)
SD	23869.46±4000.54	39.78±6.67
VPA	9061.94±2820.45[a]	15.10±4.70[a]
VPA_Acupuncture	23904.55±4656.57[b]	39.84±7.76[b]
F-Value	23.12	23.11
P-value	<0.001	<0.001

Notes: [a]indicates a significant difference compared to the SD group*(P < 0.05)*; [b]indicates a significant difference compared to the VPA group*(P<0.05)*.

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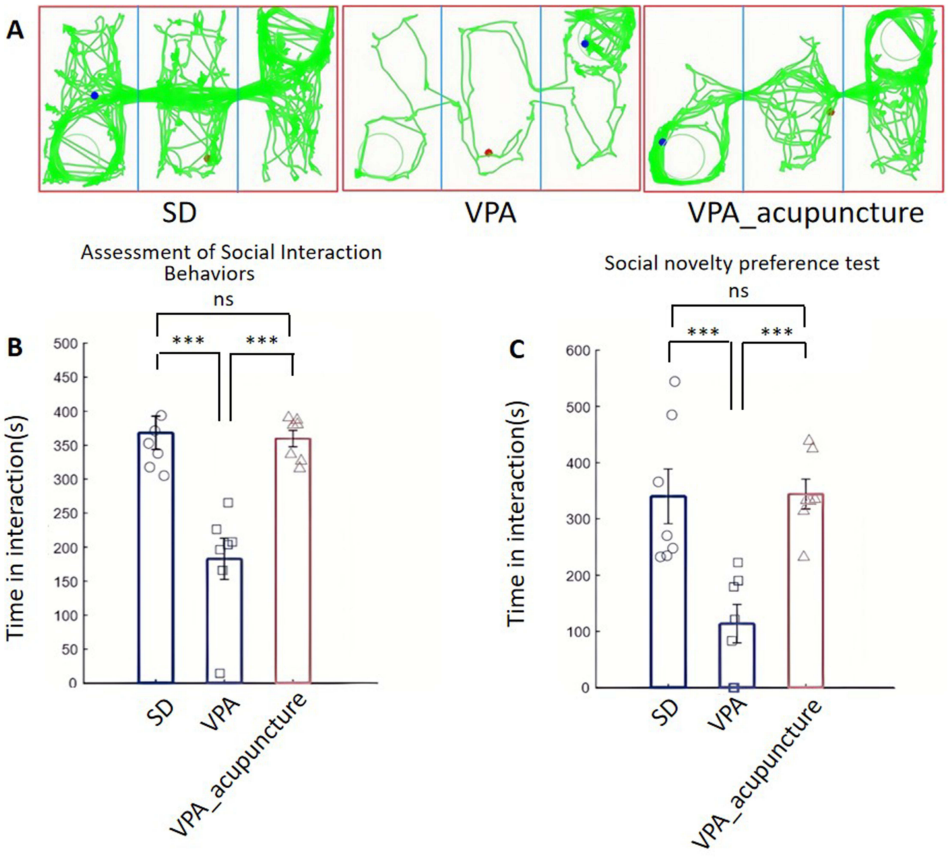

Figure 4 Results of Three-chamber social test.
Notes: (**A**) Representative movement trajectories in Assessment of Social Interaction Behaviors (red is the starting point, blue is the endpoint); (**B**) The contact time of the three groups of rats with unfamiliar rats; (**C**) The contact time between the three groups of rats and the new rats; *** $p < 0.001$; ns $p > 0.05$.

Transmission Electron Microscopy Analysis

In this study, we also analyzed the effects of acupuncture on the autism model rats. The control group showed relatively mild synaptic damage, with the mitochondrial structure still intact, the pre- and post-synaptic membrane structures continuous, an appropriate number of synaptic vesicles, and minimal damage. The synaptic gap was clear, with an appropriate width. In the acupuncture group, the mitochondria appeared slightly swollen, the pre- and post-synaptic membrane structures were clear but slightly shorter, the number of synaptic vesicles was moderate, with a small amount of damage, and the synaptic gap appeared somewhat blurred. The ASD group showed the most severe damage, with slight mitochondrial swelling, the pre- and post-synaptic membranes slightly blurred or broken, a reduced number of synaptic

Table 2 Time Spent in Each Zone During the Three-Chamber Social Test for Each Group [(±s), n=7]

	Experiment 1: Assessment of Social Interaction Behaviors	Experiment 2: Social Novelty Preference Test
Group	**Time Spent Visiting Rat (s)**	**Time Spent Visiting Novel Rat (s)**
SD	368.09 ± 60.13	340.10 ± 119.17
VPA	182.80 ± 74.15[a]	113.80 ± 83.52[a]
VPA_Acupuncture	345.18 ± 40.01[a]	350.39 ± 90.54[a]
F-Value	16.88	10.68
P-value	<0.001	<0.001

Notes: [a]indicates a significant difference compared to the SD group*(P < 0.05)*.

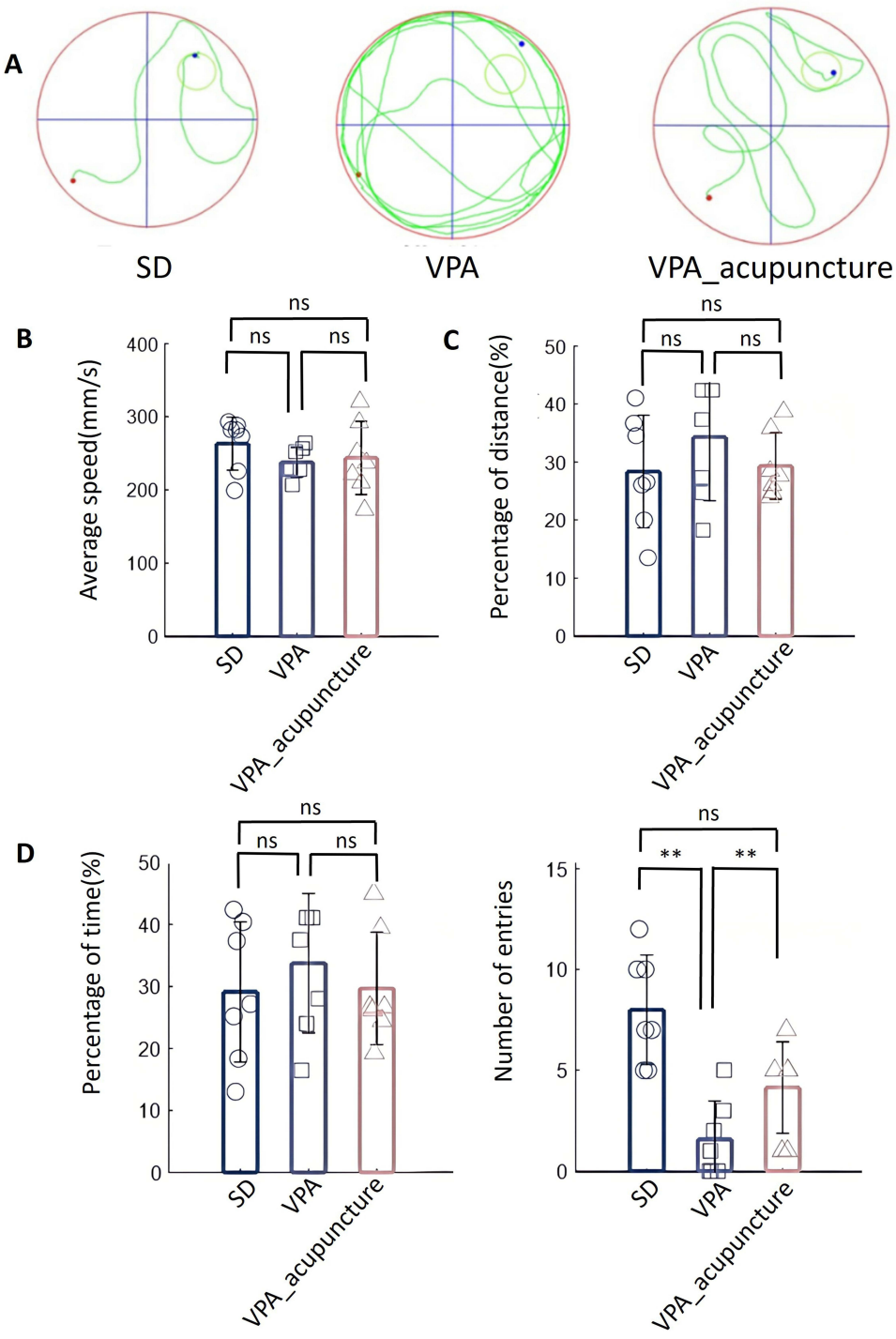

Figure 5 Results of Morris Water Maze Experiment.
Notes: (**A**) Representative movement trajectories in Morris Water Maze Experiment (red is the starting point, blue is the endpoint); (**B**) Average speed of three groups of rats; (**C**) Percentage of distance in the target quadrant of three groups of rats; (**D**) Percentage of time in the target quadrant of three groups of rats; (**E**) Number of platform entries of three groups of rats; ** $p < 0.01$; ns $p > 0.05$.

vesicles, most of them damaged, and a synaptic gap that was blurred and slightly narrow (Figure 6). In the control group, some mitochondria were approximately round in shape, smaller in size, with intact membranes, and minimal matrix and crista dissolution; in the acupuncture group, the mitochondria were oval, slightly larger, with mostly intact membranes,

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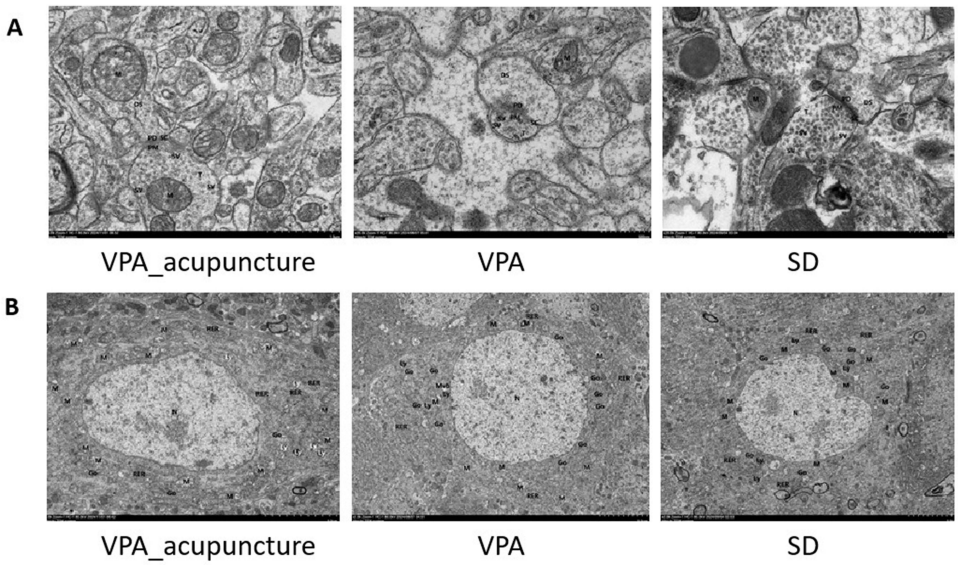

Figure 6 Results of transmission electron microscopy.
Notes: (**A**) Pathological electron microscopy of synapses of three groups of rats; (**B**) Pathological electron microscopy of hippocampal neurons of three groups of rats;

some sparse matrix, and partial cristae fragmentation; in the ASD group, mitochondrial damage was more evident, with swelling and enlargement, membrane bulging, extensive dissolution and disappearance of the matrix and cristae (Figure 6).

Untargeted Genomic Analysis of Biological Samples

Due to its cysteine-rich domain and high similarity to the Wnt binding site on curling proteins, Sfrp5 has the ability to regulate core processes such as cell proliferation, differentiation, and migration.[25,26] Lindner K et al found that Sfrp5 can reduce the viability, migration, and invasion of non-small cell lung cancer cells by inhibiting the Wnt/β-catenin pathway, thereby promoting cell apoptosis.[27]

In this study, we also analyzed the effect of acupuncture on gene expression in autism model rats. Log1 transformation of the data revealed that there were 111 differentially expressed genes (DEGs) in the hippocampus between the acupuncture and model groups (Figure 7A), and 599 DEGs in the prefrontal cortex (Figure 7B). GO and KEGG enrichment analyses were performed to predict the potential functions and pathways of the differentially expressed genes. KEGG enrichment analysis of the differential genes in the hippocampus primarily showed enrichment in the Wnt signaling pathway, platelet activation pathway, parathyroid hormone synthesis, secretion, and action, and olfactory signal transduction pathway ($P < 0.05$) (Figure 7C). Among these, DEGs between the acupuncture and VPA groups were enriched in major biological processes such as central nervous system development (Zic1, Zic2, and Otx2), cell differentiation (Sfrp5, Zic2, and Otx2), regulation of the Wnt signaling pathway (Sost, Six3, Sost, and Sfrp5), and forebrain development (Zic1 and Otx2). GO enrichment analysis of the prefrontal cortex mainly focused on extracellular matrix tissue ($P < 0.05$) (Figure 7D), while KEGG enrichment analysis showed significant enrichment in pathways such as spliceosome, protein digestion and absorption, and non-coding RNAs ($P < 0.05$).

Western Blot (WB)

Western blotting results showed that in the hippocampus, the expression levels of β-catenin and GSK-3β in the model group were significantly lower than those in the normal and acupuncture groups ($P < 0.05$) (Figure 8), with no significant difference between the normal and acupuncture groups ($P > 0.05$). The expression of SFRP5 protein in the model group was significantly increased compared to the normal group, while the acupuncture group showed significantly lower SFRP5 protein expression than the model group ($P < 0.05$) (Figure 9). This suggests that SFRP5 protein expression is increased in ASD rats, and

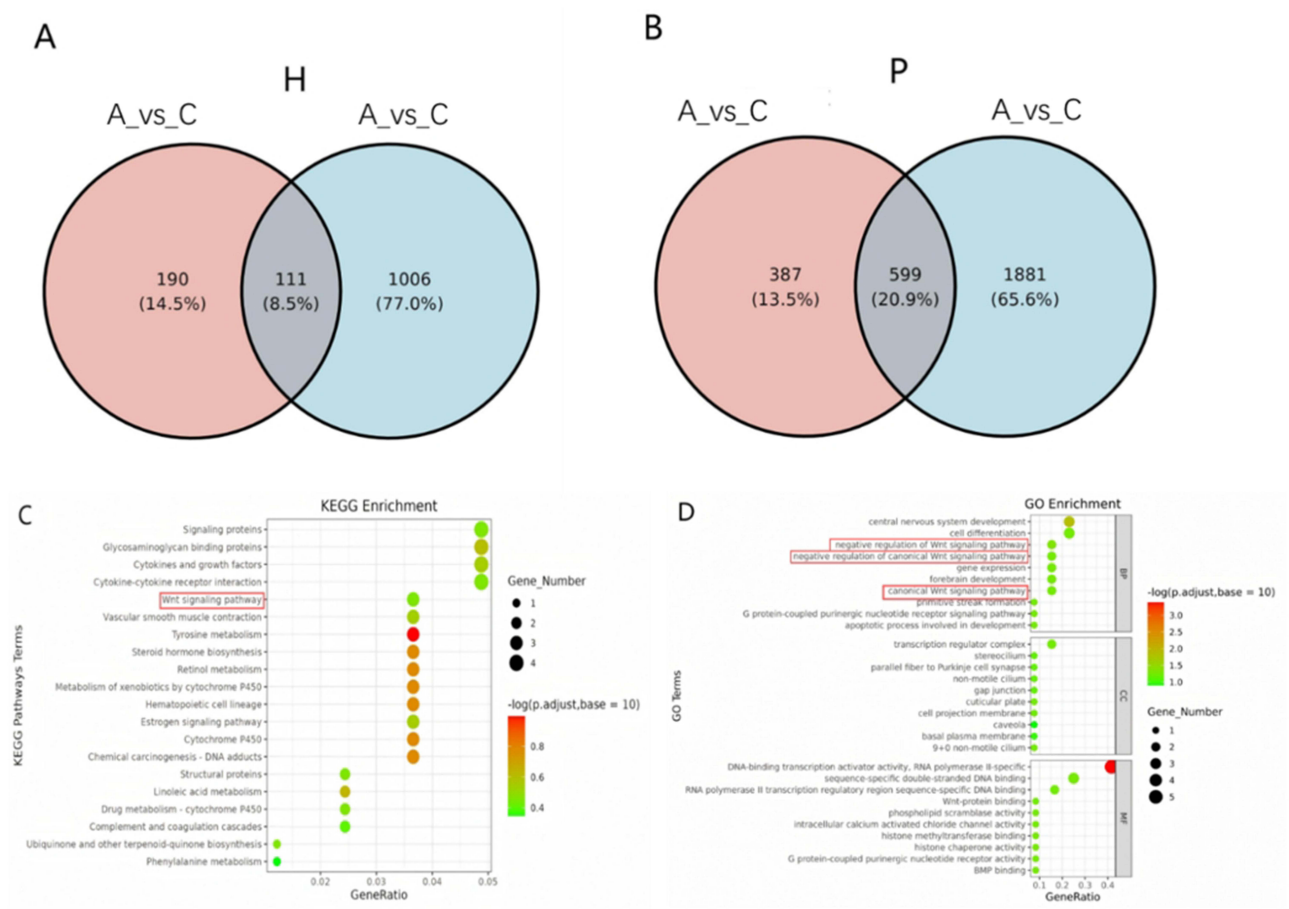

Figure 7 RNA result analysis.
Notes: (**A**) Number of differentially expressed genes in hippocampal tissue among the three groups; (**B**) Number of differentially expressed genes in prefrontal cortex tissue among the three groups; (A represents the acupuncture group from days 8–28, C represents the 28-day SD group. A vs C represents the differentially expressed genes between the acupuncture group and the model group); (**C**) KEGG enrichment analysis of differentially expressed genes in hippocampal tissue; (**D**) GO enrichment analysis of differentially expressed genes in hippocampal tissue; (X-axis: enrichment factor, Y-axis: enriched pathways, color: degree of difference, dot size: number of genes).

acupuncture treatment reduced the difference in protein expression between the two groups. There was no significant difference in the expression of this protein in the prefrontal cortex among the three groups ($P > 0.05$).

Discussion

The prenatal valproic acid exposure model is an important animal model in autism spectrum disorder research, as it effectively replicates the core behavioral and neuropathological features of ASD and is widely used.[28] This conclusion is supported by epidemiological studies, which have shown an association between maternal use of VPA and an increased risk of ASD in offspring.[29] Furthermore, rodents exposed to VPA during pregnancy also exhibit behavior phenotypes similar to those of human ASD, including defects in social interaction, repetitive behaviors, and abnormal changes in cortical neural connections. Our findings indicate that the VPA-induced rat model exhibits impaired social, cognitive, and sensory functions, consistent with previous research.

In the context of the rising diagnosis rates of autism in children, finding effective intervention methods has become particularly important. Acupuncture, originating from traditional Chinese medicine, is one of the treasures of Chinese culture that has been passed down and developed over thousands of years. This paper proposes a multi-point combination acupuncture treatment plan that integrates the theory of acupoint selection from Traditional Chinese Medicine with the "regulation of the mind" concept, combining them into the "Yumu Acupuncture". The effectiveness of acupuncture in treating autism has been clinically confirmed, but research into its potential related neurobiological biomarkers and

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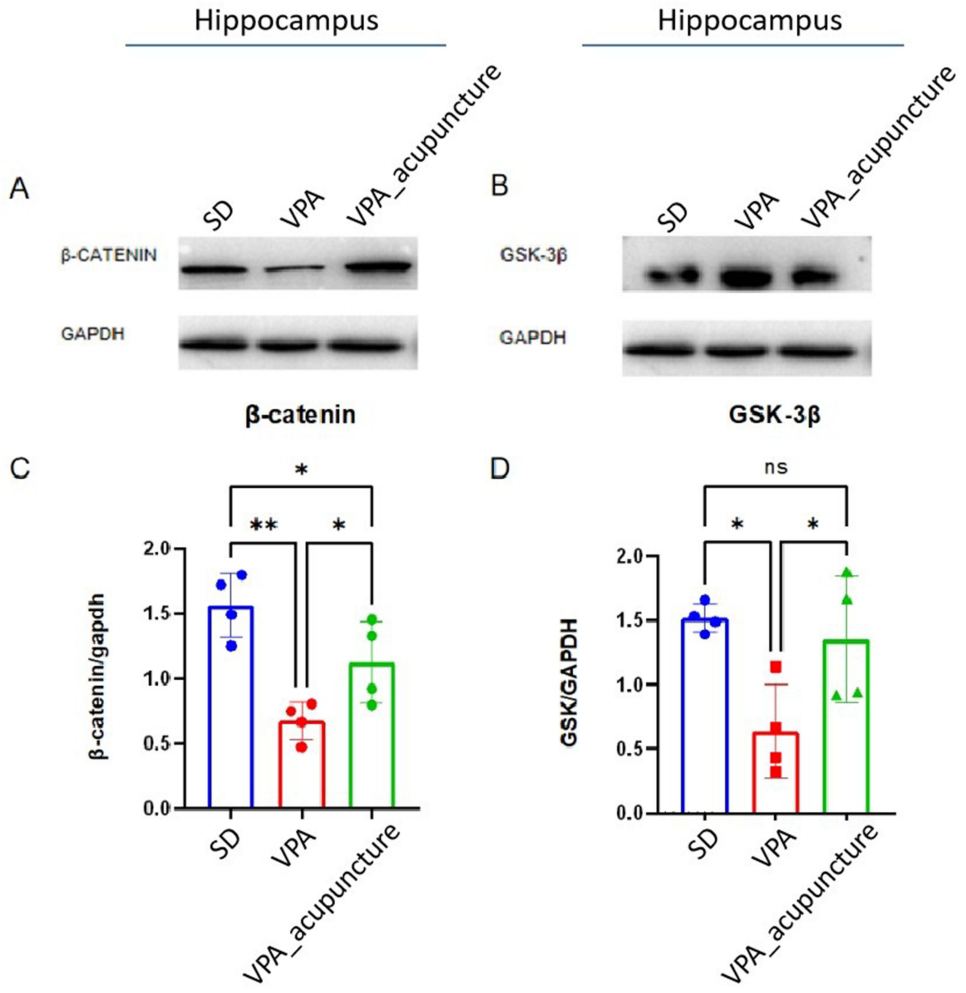

Figure 8 Results of immunoimprinting of β-catenin and GSK-3β protein.
Notes: (**A**) β-catenin results in hippocampal tissue of the three rat groups; (**B**) GSK-3β results in hippocampal tissue of the three rat groups; (**C**) Quantitative analysis of β-catenin in hippocampal tissue of the three rat groups; (**D**) Quantitative analysis of GSK-3β in hippocampal tissue of the three rat groups; * $p < 0.05$; ** $p < 0.01$; ns $p > 0.05$.

neural circuits is also crucial. In previous ASD animal studies, acupuncture interventions were typically administered after postnatal day 23 (PND23),[22] but in our study, we chose PND8 to PND28 as the treatment period. This is because PND8 to PND28 roughly corresponds to the age of 1–2 years in children. According to the recommendations of the Centers for Disease Control and Prevention (CDC), early intervention can significantly improve the development of children with ASD, and this period aligns with the CDC's recommendations for early intervention in ASD.[30]

We found that in the open field test, the VPA-induced model group showed a greater tendency to stay in the peripheral area compared to the SD and VPA_acupuncture groups, indicating an elevated baseline anxiety level in the model rats. Acupuncture significantly alleviated this anxiety. Additionally, the VPA_acupuncture group exhibited a significant increase in both total distance traveled and average speed compared to the VPA group, reaching levels comparable to the SD group, suggesting that acupuncture helped restore motor function to near-normal levels. These results indicate that the Yu-Mu-Tiao-Shen Acupuncture method significantly improves motor ability in ASD model rats, which is consistent with the findings of He J et al using electroacupuncture treatment.[31]

Neurons, as the basic functional units of the central nervous system, process information through their electrical excitability and dendrites. Synapses, specialized structures for communication between neurons, exhibit plasticity, which forms the cellular basis for cognitive functions. Mitochondria produce ATP through oxidative phosphorylation and regulate calcium homeostasis and reactive oxygen species (ROS) levels, providing metabolic support for the energy-demanding neuronal activities and synaptic transmission. Transmission electron microscopy results show that synaptic damage in the

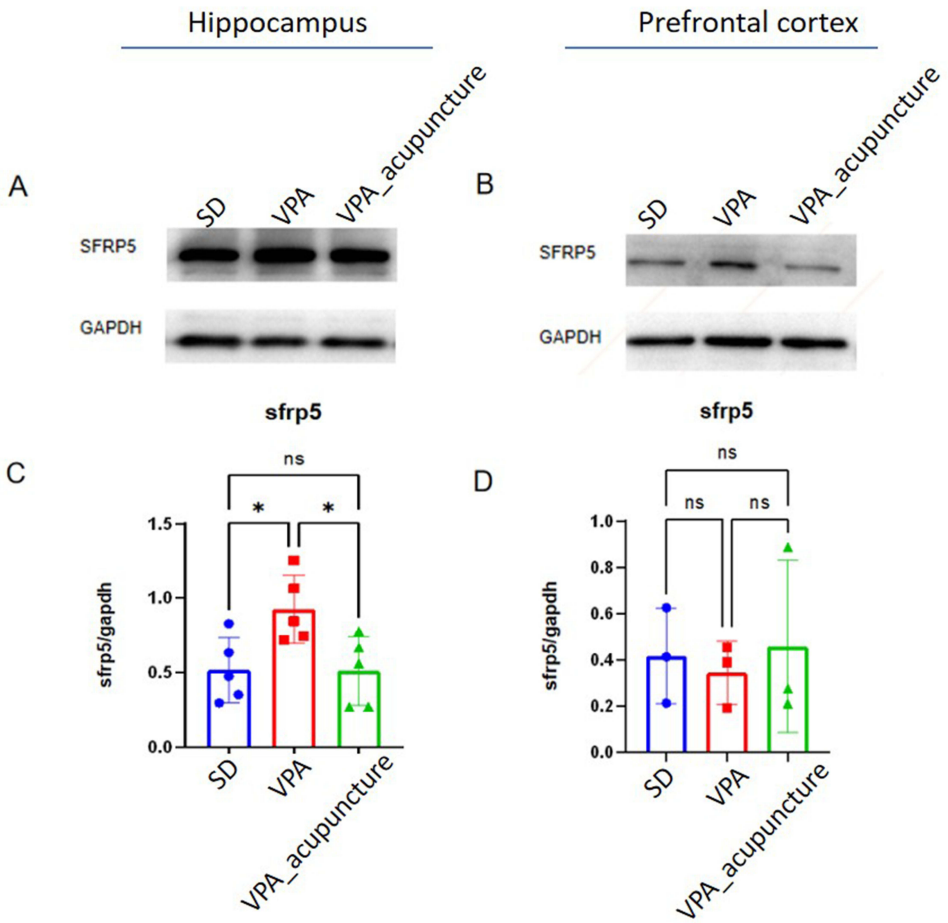

Figure 9 Results of immunoimprinting of SFRP5 protein.
Notes: (**A**) SFRP5 results in hippocampal tissue of the three rat groups; (**B**) SFRP5 results in prefrontal cortex tissue of the three rat groups; (**C**) Quantitative analysis of SFRP5 in hippocampal tissue of the three rat groups; (**D**) Quantitative analysis of SFRP5 in prefrontal cortex tissue of the three rat groups; * $p < 0.05$; ns $p > 0.05$.

VPA group of rats was relatively more severe, while in the VPA_acupuncture group, mitochondrial swelling was reduced compared to the VPA group, and the pre-and post-synaptic membrane structures were clearer and more abundant, with an increased number of synaptic vesicles. The neuronal cell membrane structure in the VPA _acupuncture group was more intact compared to the VPA group, with fewer dissolutions in the matrix and cristae. These findings suggest that the Yu-Mu-Tiao-Shen cupuncture method may be related to synaptic remodeling and could have a certain reparative effect. Furthermore, we observed that this result aligns with the trend observed in the transmission electron microscopy findings of Chen S et al, who used acupuncture at the "GV24" and bilateral "GB13" acupuncture points, both of which showed some improvement after treatment.[32]

Among the differentially expressed genes (DEGs), there were significant differences between the VPA_acupuncture group and the VPA group. KEGG pathway analysis revealed that the DEGs between these two groups were primarily enriched in biological processes such as central nervous system development, cell differentiation, and regulation of the Wnt signaling pathway. Abnormalities in cell differentiation, metabolism, and signaling pathways may contribute to the pathogenesis of ASD, and acupuncture may alleviate clinical manifestations and symptoms of ASD by reducing the number of these dysregulated pathways. Through clustering analysis and extensive literature review, the authors identified key DEGs closely associated with the pathogenesis of autism and the therapeutic mechanisms of acupuncture. Among these, the SFRP5 gene—currently a research hotspot—plays a crucial regulatory role in the Wnt signaling pathway. Due to the high structural similarity between its cysteine-rich domain and the Wnt-binding site of Frizzled receptors, SFRP5 is capable of modulating essential processes such as cell proliferation, differentiation, and migration.

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The SFRP5/Wnt signaling pathway has been reported to induce the secretion of pro-inflammatory cytokines and chemokines in neurodegenerative diseases. Previous studies have highlighted the pivotal role of SFRP5 in various pathological conditions, suggesting that it may participate in the pathogenesis of ASD through interactions between metabolic and neural mechanisms.

The results of the above study suggest that the Yu-Mu-Tiao-Shen acupuncture technique can significantly improve the motor, social cognitive, and spatial memory abilities of rats. This indicates that the therapeutic effect of acupuncture is not solely due to the mechanical stimulation at the acupuncture sites, but may involve more complex biological effects. In the three-chamber social interaction test, there were significant differences in the time spent by each group in the different sections, but the overall trend did not affect the assessment of the effect of the Yu-Mu Tiao-Shen acupuncture technique on the rats' social behavior.

However, despite achieving some positive results, this study has certain limitations, such as the lack of a placebo acupuncture group and the potential individual differences among the experimental rats. More autism models are needed to validate our further findings. Additionally, the interactions between the various differentially expressed genes, synaptic structural and functional changes, and how they work together in the autistic rat model need to be explored in future research. These issues are crucial for further understanding the pathogenesis, particularly for identifying drug targets and improving the prevention and treatment of autism.

In conclusion, this study designed and validated the role of the Yu-Mu-Tiao-Shen acupuncture technique in improving social behavior at the behavioral level in ASD rat models. It provides guidance for future in-depth exploration of its objective mechanisms in genomics and metabolomics, offering a more effective and standardized reference for acupuncture treatment of ASD.

Conclusion

In conclusion, this study designs and verifies the effect of Yu-mu-Tiao-Shen acupuncture on the improvement of social behavior in ASD model rats at the behavioral level, providing guidance for further in-depth research on its mechanisms in genomics and metabolomics. It also offers a more effective and standardized reference for acupuncture treatment of ASD.

Data Sharing Statement

The datasets created and examined in this study can be obtained from the corresponding author upon reasonable request.

Ethics

All experiments followed the National Standards for Laboratory Animal Management. This study was approved by the Animal Ethics Committee of Henan University of Chinese Medicine (IACUC-202404016).

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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