CHINESE ACUPUNCTURE: A POTENTIAL TREATMENT FOR AUTISM RAT MODEL VIA IMPROVING SYNAPTIC FUNCTION

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Abstract:-

Purpose: Autistic symptom improvement can be observed in children treated with acupuncture, but the mechanism is still being explored. In the present study, we used scalp acupuncture to treat autism rat model, and then their improvement in the abnormal behaviors and specific mechanisms behind were revealed by detecting animal behaviors, analyzing the RNA sequencing of the prefrontal cortex (PFC), and observing the ultrastructure of PFC neurons under the transmission electron microscope.
Methods: On gestational day 12.5, Wistar rats were given valproic acid (VPA) by intraperitoneal injection, and their offspring were considered to be reliable rat models of autism. They were randomized to VPA or VPA-acupuncture group (n=8). Offspring of Wistar pregnant rats that were simultaneously injected with saline were randomly selected as the wild-type group (WT). VPA_acupuncture group rats received acupuncture intervention at 23 days of age for 4 weeks, and the other two groups followed without intervention. After the intervention, all experimental rats underwent behavioral tests. Immediately afterward, they were euthanized by cervical dislocation, and their prefrontal cortex was isolated for RNA sequencing and transmission electron microscopy.
Results: The main results are as follows: 1. Animal behavioural tests: VPA group rats showed more anxiety-like behaviour and repetitive, stereotyped behaviour than WT group rats. While VPA group rats showed less spatial exploration ability, activity level, social interaction, and social novelty preference than WT group rats. It was gratifying to observe that acupuncture indeed improved these abnormal behaviors of autism rat model. 2. RNA-sequencing: The three groups of rats differed in the expression and enrichment pathways of multiple genes related to synaptic function, neural signal transduction, immune-inflammatory responses and circadian rhythm regulation. Our experiments indicated that acupuncture can alleviate the major symptoms of ASD by improving these neurological abnormalities. 3. Under the transmission electron microscopy, several lysosomes and mitochondrial structural abnormalities were observed in the prefrontal neurons of VPA group rats, which were manifested as atrophy of the mitochondrial membrane, blurring or disappearance of the mitochondrial cristae, and even vacuolization. Moreover, the number of synapses and synaptic vesicles was relatively small. Conversely, the mitochondrial structure of rats in the WT group and VPA_acupuncture was normal, and the number of synapses and synaptic vesicles was relatively large.
Conclusion: Acupuncture effectively improved the abnormal behaviors of autism rat model and the ultrastructure of the PFC neurons, which might worked by improving their abnormal synaptic function, synaptic plasticity promoting neuronal signal transduction and regulating immune-inflammatory responses.

Keywords - Autism spectrum disorder, Acupuncture, Animal behavior, RNA sequencing, Transmission electron microscope.

I. INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurobehavioral disorder. Social impairments, repetitive stereotypical behaviors and limited interests characterize the disease. The ASD incidence has been reported continuously increased globally[1]. However, the etiology of ASD is still poorly defined. Genetics, neurology and immunology factors are believed to be associated with the pathogenesis of ASD in modern medicine, while traditional Chinese medicine (TCM) believes that ASD has manifestations similar to "mental disorder", such as blurred vision, poor eye contact, inability to initiate communication, disobedience to instructions, and even manic symptoms. So far, ASD cannot be cured. To some extent, special education, behavioral intervention, family intervention can relieve symptoms of ASD, but it requires significant resources in terms of time and money, as well as family and social resources. And pharmacologic interventions are limited to treating symptoms and do not target social deficits[2, 3]. As a consequence, clinical treatments for children with ASD are limited. This has prompted us to actively seek alternative treatments. Traditional Chinese medicine, for example, consists mainly of acupuncture, massage and Chinese herbal medicine[4]. Acupuncture has been developed for thousands of years to treat multi-system diseases[5, 6]. In particular, scalp acupuncture can effectively improve brain dysfunction, such as ASD, Parkinson's disease (PD), Alzheimer's disease (AD), children's cerebral palsy, stroke and other neurological developmental disorder or degenerative diseases[7, 8, 9, 10] without fatal side effects[11, 12]. Extensive evidence suggests that the rodent VPA maternal challenge is an excellent animal model of autism, and that the VPA model is more representative of many cases of idiopathic autism caused by environmental insults than other models caused by single-gene or chromosomal disorders[13, 14]. Acupuncture has been applied to the study of various animal models, especially those related to brain function-related diseases, such as ASD, AD, vascular dementia[15, 16, 17, 18, 19, 20, 21]. Needling at “GV24” and bilateral “GB13” were selected for the treatment in this paper. “GV24” and

"GB13" are located on the forehead, which enters the brain and belongs to the Governor (Du) Vessel. They can activate blood and nourish the brain, regulate and calm the mind[22]. Previous studies have shown that cytokines and glial activation are present in the central nervous system of autism patients, suggesting that the pathogenesis of ASD may be related to the excessive immune-inflammatory response in the central nervous system[23, 24]. Despite acupuncture has clinical utility in treating autistic children, its action mechanism is still obscure, several studies have shown that acupuncture may improve clinical symptoms of many diseases by modulating immune-inflammatory functions[25, 26]. The PFC is considered to play a significant role in cognition, social behavior, memory, planning and execution activities. Previous studies found that the PFC dysfunction was closely associated with the onset of ASD [27]. In addition, neuronal synapses have been identified as key targets for neurological diseases such as ASD, schizophrenia, depression, etc. [28, 29].

Based on previous experiments [20, 30], we proposed the following hypothesis: acupuncture at GV24 and bilateral GB13 points can improve abnormal behaviors in autism rat model. Which might be linked to changes in frontal cortical function and ultrastructure of neuronal synapses and worked through specific pathways and genes. Therefore, autism rat model was constructed and acupuncture intervention was carried out on rats in the VPA_acupuncture group. After intervention, 3 groups of experimental rats were tested for behaviour, and the prefrontal cortex was taken for RNA sequencing or observation for their ultrastructure of neurons. By comparing the behavioural differences among the three groups of rats, the neuronal ultrastructural differences in the prefrontal cortex, and the genes and enriched pathways identified by RNA sequencing, the potential mechanism of acupuncture improving ASD was further explored.

II. PROCEDURE FOR PAPER SUBMISSION

A. Review Stage

Submit your manuscript electronically for review.

B. Final Stage

When you submit your final version, after your paper has been accepted, prepare it in two-column format, including figures and tables.

C. Figures

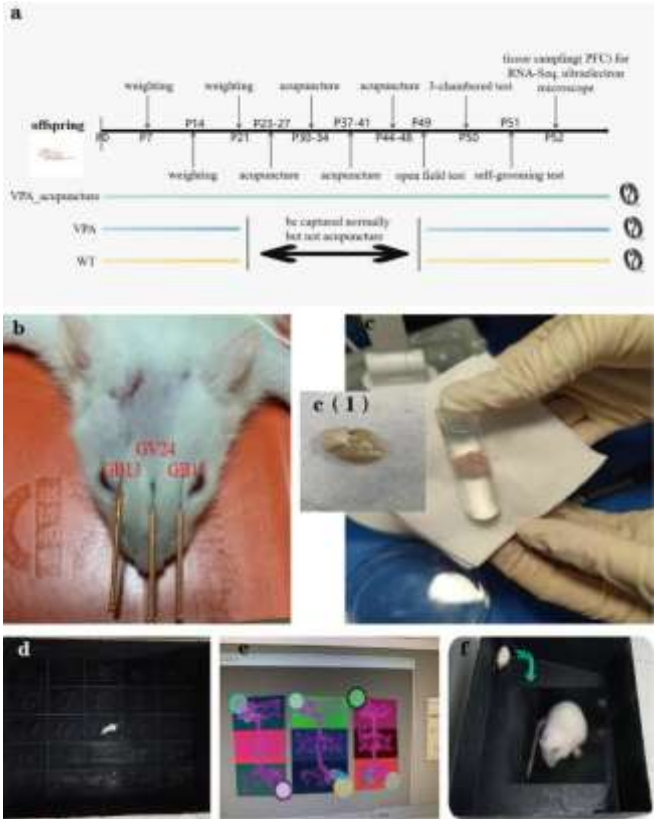


Figure 1 Experimental diagram of acupuncture and behavior tests.

Notes: (a) Time axis; (b) Acupoint localization; (c) Tissue sampling; (d)Open field test; (e)Social interaction test; (f) Self-grooming test.

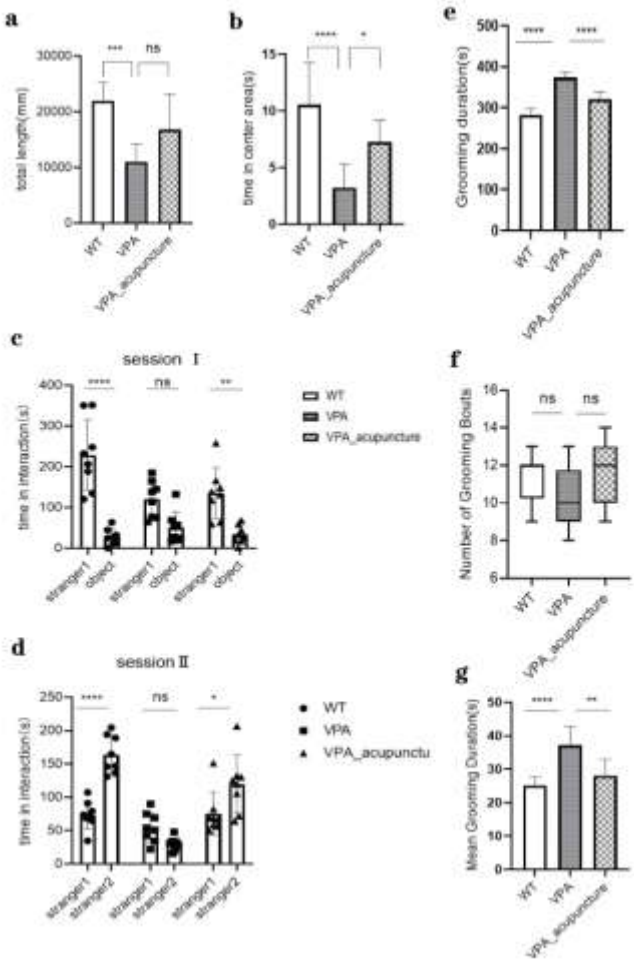


Figure 2 Results of behavioral test.
Notes: * p < 0.05; ** p < 0.01; ***p < 0.001; ****p < 0.0001; ns p > 0.05

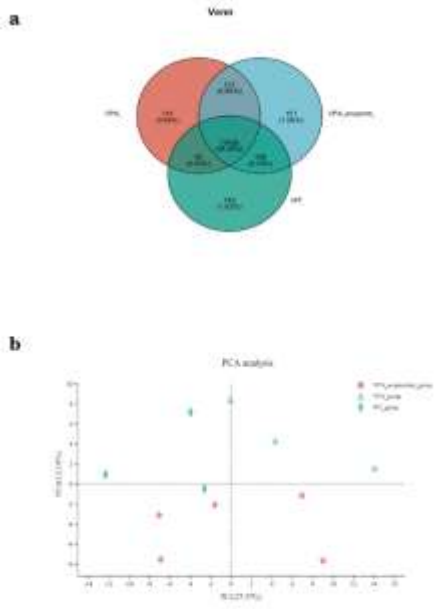


Figure 3 Gene expression analysis.
Notes: (a) Venn diagram; (b) PCA analysis

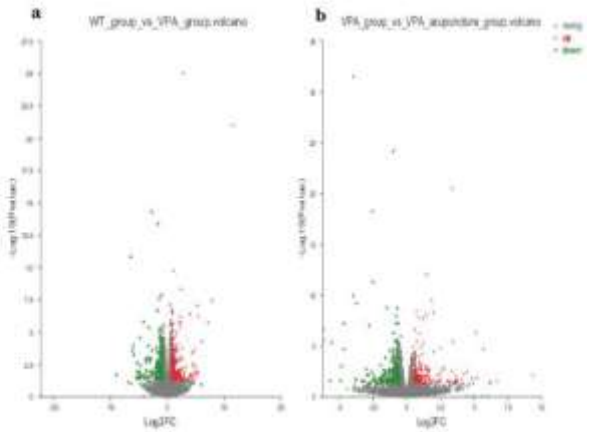


Figure 4 volcano plot

Notes: (a) Genes differentially expressed between WT and VPA groups; (b) Genes differentially expressed between VPA group and VPA_acupuncture group (red indicates up-regulated genes, blue indicates down-regulated genes)

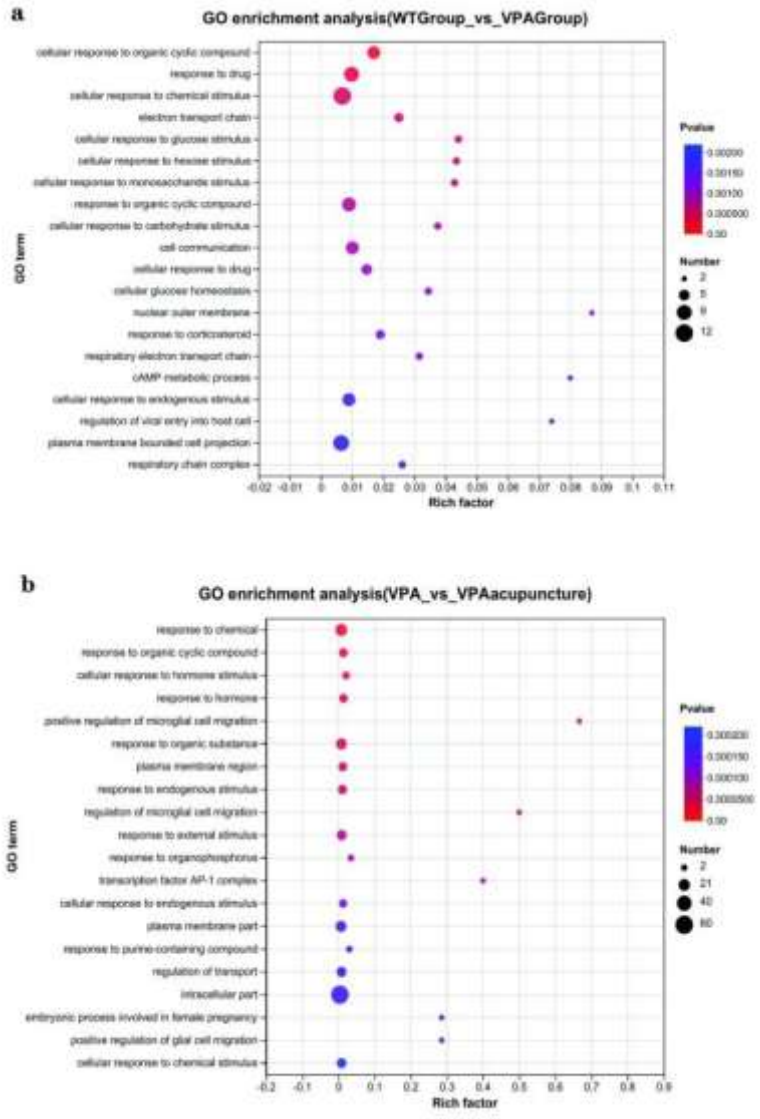


Figure 5 GO enrichment analysis

Notes: (a) GO enrichment analysis of differentially expressed genes between WT and VPA group. (b) GO enrichment analysis of differentially expressed genes between VPA and VPA_acupuncture group. The vertical axis represents GO Term, and the horizontal axis represents Rich factor.

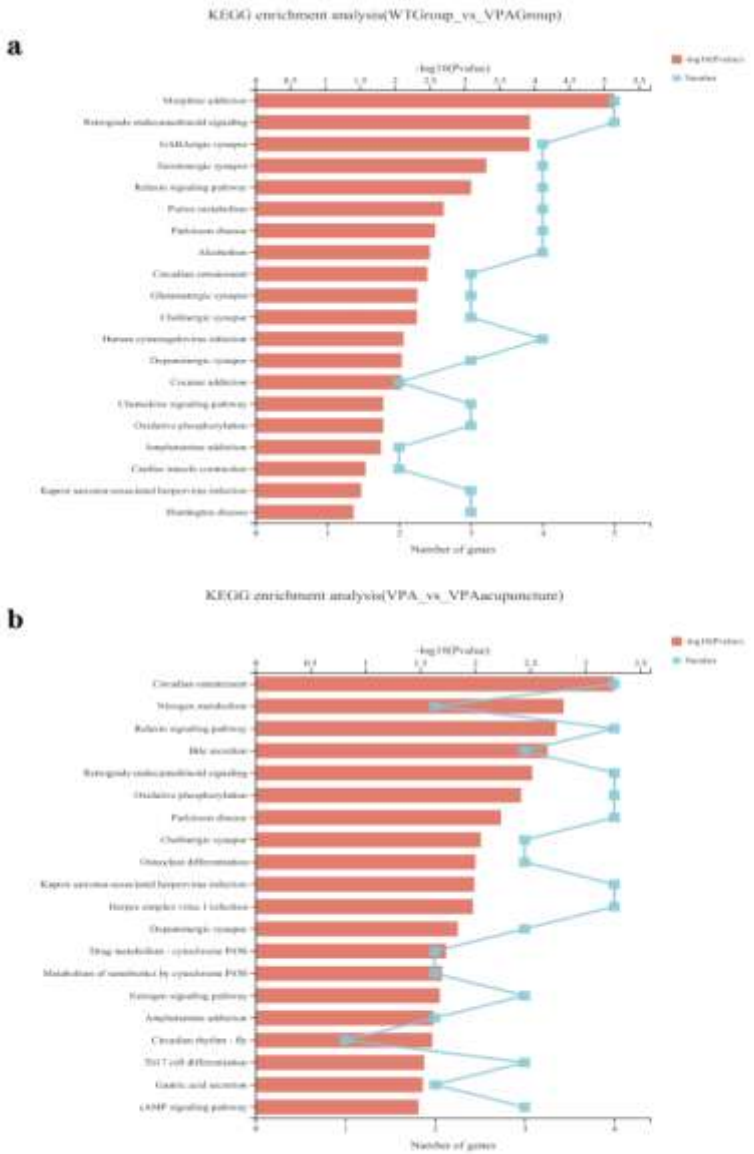
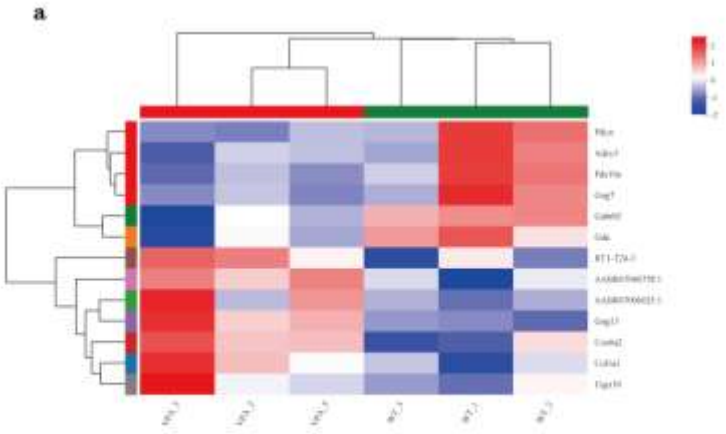


Figure 6 KEGG enrichment analysis

Notes: (a) KWGG enrichment analysis of differentially expressed genes between WT and VPA group. (b) KEGG enrichment analysis of differentially expressed genes between VPA and VPA_acupuncture group. The vertical axis represents the KEGG pathway, and the horizontal axis represents the significance level of enrichment.



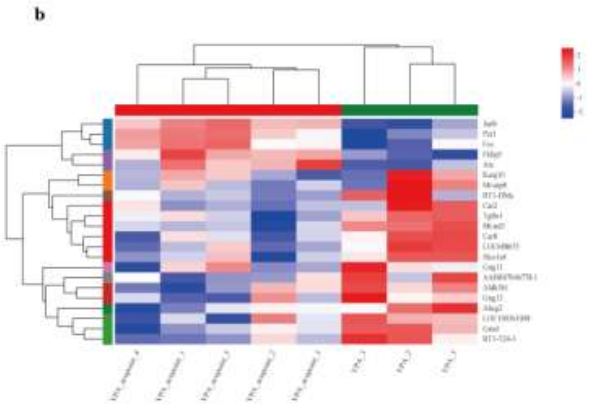


Figure 7 Cluster analysis

Notes: (a) Cluster analysis of differentially expressed genes between WT and VPA group. (b) Cluster analysis of differentially expressed genes between VPA and VPA_acupuncture group. Each column in the graph represents a sample, and each row represents a gene. The colors in the graph represent the standardized expression levels of the gene in each sample, with red representing a higher expression level of the gene in the sample and blue representing a lower expression level.

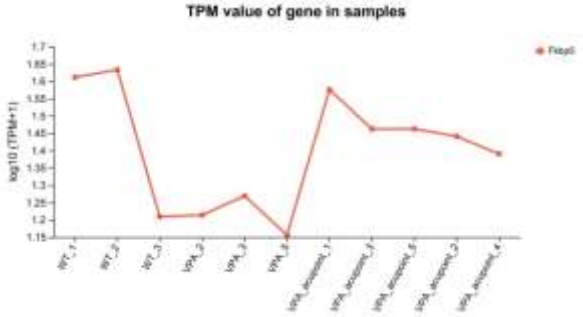


Figure 8 The expression level of Fkbp5 gene

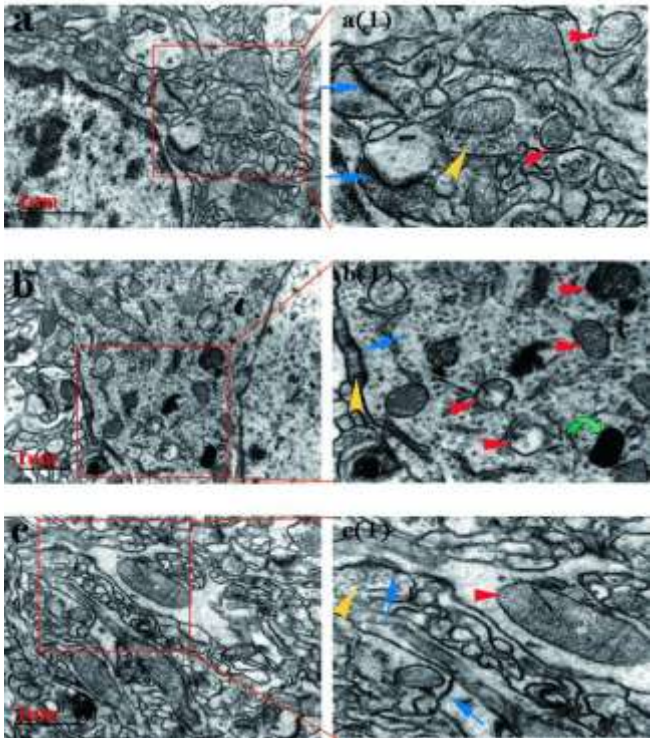


Figure 9 Transmission electron microscopy image of PFC neurons

Note: (a) wild type group; a (1), local enlarged image of a; (b) VPA group; b (1), local enlarged image of b; (c) VPA_acupuncture group; c (1),local enlarged image of c. Blue arrow represents synaptic structure, yellow arrow represents synaptic vesicle, red arrow represents Mitochondria and green arrow represents lysosome.

III. MATH

If you are using Word, use either the Microsoft Equation Editor or the MathType add-on (http://www.mathtype.com) for equations in your paper (Insert | Object | Create New | Microsoft Equation or MathType Equation). "Float over text" should not be selected.

IV. UNITS

Use either SI (MKS) or CGS as primary units. (SI units are strongly encouraged.) English units may be used as secondary units (in parentheses). This applies to papers in data storage. For example, write "15 Gb/cm^2 (100 Gb/in^2)." An exception is when English units are used as identifiers in trade, such as "3½ in disk drive." Avoid combining SI and CGS units, such as current in amperes and magnetic field in oversteps. This often leads to confusion because equations do not balance dimensionally. If you must use mixed units, clearly state the units for each quantity in an equation.

The SI unit for magnetic field strength H is A/m. However, if you wish to use units of T, either refer to magnetic flux density B or magnetic field strength symbolized as $\mu_0 H$. Use the center dot to separate compound units, e.g., "A·m^2."

V. HELPFUL HINTS

A. Figures and Tables

Because the final formatting of your paper is limited in scale, you need to position figures and tables at the top and bottom of each column. Large figures and tables may span both columns. Place figure captions below the figures; place table titles above the tables. If your figure has two parts, include the labels "(a)" and "(b)" as part of the artwork. Please verify that the figures and tables you mention in the text actually exist. Do not put borders around the outside of your figures. Use the abbreviation "Fig." even at the beginning of a sentence. Do not abbreviate "Table." Tables are numbered with Roman numerals. Include a note with your final paper indicating that you request color printing. Do not use color unless it is necessary for the proper interpretation of your figures. There is an additional charge for color printing. Figure axis labels are often a source of confusion. Use words rather than symbols. As an example, write the quantity "Magnetization," or "Magnetization M," not just "M." Put units in parentheses. Do not label axes only with units. As in Fig. 1, for example, write "Magnetization (A/m)" or "Magnetization (A·m^{-1})," not just "A/m." Do not label axes with a ratio of quantities and units. For example, write "Temperature (K)," not "Temperature/K." Multipliers can be especially confusing. Write "Magnetization (kA/m)" or "Magnetization (10^3 A/m)." Do not write "Magnetization (A/m) × 1000" because the reader would not know whether the top axis label in Fig. 1 meant 16000 A/m or 0.016 A/m. Figure labels should be legible, approximately 8 to 12 point type.

B. References

Number citations consecutively in square brackets [1]. The sentence punctuation follows the brackets [2]. Multiple references [2], [3] are each numbered with separate brackets [1]–[3]. When citing a section in a book, please give the relevant page numbers [2]. In sentences, refer simply to the reference number, as in [3]. Do not use "Ref. [3]" or "reference [3]" except at the beginning of a sentence: "Reference [3] shows ... ." Number footnotes separately in superscripts (Insert | Footnote).[1] Place the actual footnote at the bottom of the column in which it is cited; do not put footnotes in the reference list (endnotes). Use letters for table footnotes (see Table I).

Please note that the references at the end of this document are in the preferred referencing style. Give all authors' names; do not use "et al." unless there are six authors or more. Use a space after authors' initials. Papers that have not been published should be cited as "unpublished" [4]. Papers that have been submitted for publication should be cited as "submitted for publication" [5]. Papers that have been accepted for publication, but not yet specified for an issue should be cited as "to be published" [6]. Please give affiliations and addresses for private communications [7].

C. Abbreviations and Acronyms

VPA(valproic acid), PFC (the prefrontal cortex), WT (wild-type group), ASD(Autism Spectrum Disorder), TCM(traditional Chinese medicine), PD(Parkinson's disease), AD (Alzheimer's disease), DEGs(Differentially Expressed Genes)

D. Equations

Number equations consecutively with equation numbers in parentheses flush with the right margin, as in (1). First use the equation editor to create the equation. Then select the "Equation" markup style. Press the tab key and write the equation number in parentheses. To make your equations more compact, you may use the solidus (/), the exp function, or appropriate exponents. Use parentheses to avoid ambiguities in denominators. Punctuate equations when they are part of a sentence, as in

$$\int_0^{r_2} F(r,\varphi)\, dr\, d\varphi = [\sigma r_2 / (2\mu_0)] \cdot \int_0^{\infty} \exp(-\lambda\,|z_j - z_i|)\, \lambda^{-1}\, J_1(\lambda r_2)\, J_0(\lambda r_i)\, d\lambda . \quad (1)$$

Be sure that the symbols in your equation have been defined before the equation appears or immediately following. Italicize symbols (T might refer to temperature, but T is the unit tesla). Refer to "(1)," not "Eq. (1)" or "equation (1)," except at the beginning of a sentence: "Equation (1) is ... ."

E. Other Recommendations

Use one space after periods and colons. Hyphenate complex modifiers: "zero-field-cooled magnetization." Avoid dangling participles, such as, "Using (1), the potential was calculated." [It is not clear who or what used (1).] Write instead, "The potential was calculated by using (1)," or "Using (1), we calculated the potential."

Use a zero before decimal points: "0.25," not ".25." Use "cm^3," not "cc." Indicate sample dimensions as "0.1 cm × 0.2 cm," not "0.1 × 0.2 cm^2." The abbreviation for "seconds" is "s," not "sec." Do not mix complete spellings and abbreviations of units: use "Wb/m^2" or "webers per square meter," not "webers/m^2." When expressing a range of values, write "7 to 9" or "7-9," not "7~9."

A parenthetical statement at the end of a sentence is punctuated outside of the closing parenthesis (like this). (A parenthetical sentence is punctuated within the parentheses.) In American English, periods and commas are within quotation marks, like "this period." Other punctuation is "outside"! Avoid contractions; for example, write "do not" instead of "don't." The serial comma is preferred: "A, B, and C" instead of "A, B and C."

If you wish, you may write in the first person singular or plural and use the active voice ("I observed that ..." or "We observed that ..." instead of "It was observed that ..."). Remember to check spelling. If your native language is not English, please get a native English-speaking colleague to proofread your paper.

VI. SOME COMMON MISTAKES

The word "data" is plural, not singular. The subscript for the permeability of vacuum μ_0 is zero, not a lowercase letter "o." The term for residual magnetization is "remanence"; the adjective is "remanent"; do not write "remnance" or "remnant." Use the word "micrometer" instead of "micron." A graph within a graph is an "inset," not an "insert." The word "alternatively" is preferred to the word "alternately" (unless you really mean something that alternates). Use the word "whereas" instead of "while" (unless you are referring to simultaneous events). Do not use the word "essentially" to mean "approximately" or "effectively." Do not use the word "issue" as a euphemism for "problem." When compositions are not specified, separate chemical symbols by en-dashes; for example, "NiMn" indicates the intermetallic compound $Ni_{0.5}Mn_{0.5}$ whereas "Ni–Mn" indicates an alloy of some composition Ni_xMn_{1-x}.

Be aware of the different meanings of the homophones "affect" (usually a verb) and "effect" (usually a noun), "complement" and "compliment," "discreet" and "discrete," "principal" (e.g., "principal investigator") and "principle" (e.g., "principle of measurement"). Do not confuse "imply" and "infer."

Prefixes such as "non," "sub," "micro," "multi," and ""ultra" are not independent words; they should be joined to the words they modify, usually without a hyphen. There is no period after the "et" in the Latin abbreviation "et al." (it is also italicized). The abbreviation "i.e.," means "that is," and the abbreviation "e.g.," means "for example" (these abbreviations are not italicized).

An excellent style manual and source of information for science writers is [9].

VII. EDITORIAL POLICY

The submitting author is responsible for obtaining agreement of all coauthors and any consent required from sponsors before submitting a paper. It is the obligation of the authors to cite relevant prior work. Authors of rejected papers may revise and resubmit them to the journal again.

VIII. PUBLICATION PRINCIPLES

The contents of the journal are peer-reviewed and archival. The journal INTERNATIONAL JOURNAL OF ENGINEERING AND INNOVATIVE TECHNOLOGY (IJEIT) publishes scholarly articles of archival value as well as tutorial expositions and critical reviews of classical subjects and topics of current interest.

Authors should consider the following points:

1. Technical papers submitted for publication must advance the state of knowledge and must cite relevant prior work.
2. The length of a submitted paper should be commensurate with the importance, or appropriate to the complexity, of the work. For example, an obvious extension of previously published work might not be appropriate for publication or might be adequately treated in just a few pages.
3. Authors must convince both peer reviewers and the editors of the scientific and technical merit of a paper; the standards of proof are higher when extraordinary or unexpected results are reported.
4. Because replication is required for scientific

progress, papers submitted for publication must provide sufficient information to allow readers to perform similar experiments or calculations and use the reported results. Although not everything need be disclosed, a paper must contain new, useable, and fully described information. For example, a specimen's chemical composition need not be reported if the main purpose of a paper is to introduce a new measurement technique. Authors should expect to be challenged by reviewers if the results are not supported by adequate data and critical details.

IX. CONCLUSION

Acupuncture therapy can effectively improve the abnormal behavior of ASD rat model and the ultrastructure of prefrontal cortex neurons, which may work by improving their abnormal synaptic function and synaptic plasticity, promoting their neuronal signal transduction, and regulating immune-inflammatory responses.

This paper further revealed the possible mechanism of acupuncture therapy for ASD through animal behavior, RNA sequencing analysis, and observation of neuronal ultrastructure under electron microscopy, which provided a theoretical basis for the clinical treatment of ASD.

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