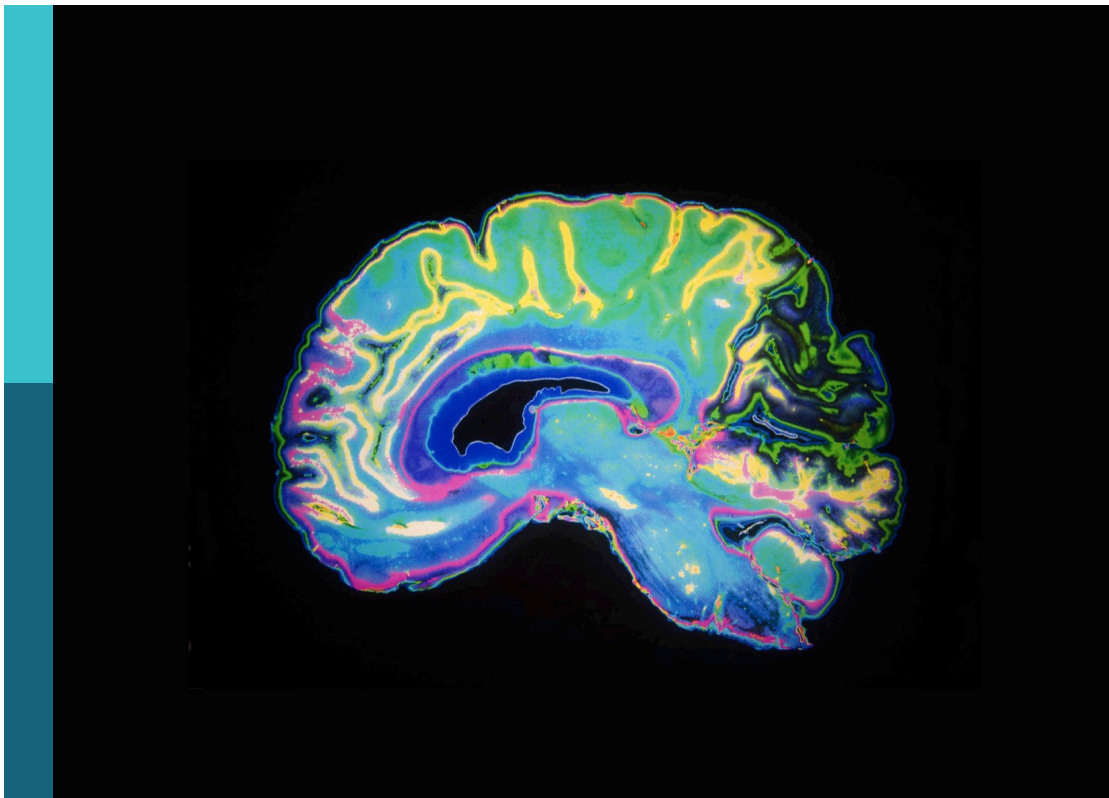
frontiers | Research Topics

Using novel technologies and models to identify biomarkers and explore therapeutic strategies for neurological disorders

Edited by
Dongdong Qin, Chengbiao Wu, Jiaojian Wang and Sheng Wei

Published in
Frontiers in Behavioral Neuroscience



ISSN 1664-8714
ISBN 978-2-83252-112-0
DOI 10.3389/978-2-83252-112-0

About Frontiers

Frontiers is more than just an open access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers journal series

The Frontiers journal series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the *Frontiers journal series* operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews. Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the *Frontiers journals series*: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area.

Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers editorial office: frontiersin.org/about/contact

Using novel technologies and models to identify biomarkers and explore therapeutic strategies for neurological disorders

Topic editors

Dongdong Qin — Yunnan University of Chinese Medicine, China
Chengbiao Wu — California College San Diego, United States
Jiaojian Wang — Kunming University of Science and Technology, China
Sheng Wei — Shandong University of Traditional Chinese Medicine, China

Citation

Qin, D., Wu, C., Wang, J., Wei, S., eds. (2023). *Using novel technologies and models to identify biomarkers and explore therapeutic strategies for neurological disorders*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83252-112-0

Table of contents

frontiers | Frontiers in Behavioral Neuroscience

TYPE Editorial
PUBLISHED 23 March 2023
DOI 10.3389/fnbeh.2023.1151667

OPEN ACCESS

EDITED AND REVIEWED BY
Xiaoming Du,
University of Maryland, United States

*CORRESPONDENCE
Dongdong Qin
✉ qindong108@163.com
Kai Yuan
✉ 190876072@qq.com

SPECIALTY SECTION
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

RECEIVED 26 January 2023
ACCEPTED 10 March 2023
PUBLISHED 23 March 2023

CITATION
Xing L, Wu C, Wang J, Wei S, Yuan K and Qin D (2023) Editorial: Using novel technologies and models to identify biomarkers and explore therapeutic strategies for neurological disorders. *Front. Behav. Neurosci.* 17:1151667. doi: 10.3389/fnbeh.2023.1151667

Editorial: Using novel technologies and models to identify biomarkers and explore therapeutic strategies for neurological disorders

Liwei Xing[1], Chengbiao Wu[2], Jiaojian Wang[3], Sheng Wei[4], Kai Yuan[5]* and Dongdong Qin[1]*

[1]School of Basic Medical Sciences, Yunnan University of Chinese Medicine, Kunming, Yunnan, China, [2]Department of Neurosciences, University of California, San Diego, San Diego, CA, United States, [3]State Key Laboratory of Primate Biomedical Research, Institute of Primate Translational Medicine, Kunming University of Science and Technology, Kunming, Yunnan, China, [4]Experimental Center, Shandong University of Traditional Chinese Medicine, Jinan, China, [5]The Second Clinical Medical School, Yunnan University of Chinese Medicine, Kunming, Yunnan, China

KEYWORDS

neurological disorders, biomarker, therapeutic strategy, animal model, novel technology

Editorial on the Research Topic
Using novel technologies and models to identify biomarkers and explore therapeutic strategies for neurological disorders

Neurological disorders are a group of diseases that affect the structure or function of the central and peripheral nervous systems. In addition to stroke and diseases of the blood vessels supplying the brain, representative central nervous system diseases also include neurodevelopmental disorders brought on by faulty genes, risky environmental factors, or gene-environment interactions, such as autism spectrum disorders (ASD), neurodegenerative disorders where nerve cells are damaged or die, such as Parkinson's disease (PD) and Alzheimer's disease (AD), as well as major depressive and bipolar disorders (Pena et al., 2020). More than 600 neurological disorders afflict the nervous system (Matilla-Dueñas et al., 2017). The absolute number of deaths brought by neurological disorders has climbed by 39%, and disability-adjusted life years have increased by 15% over the past 30 years (Feigin et al., 2020). According to a systematic analysis for the Global Burden of Disease Study 2015, neurological diseases are the second most common cause of death (GBD 2015 Disease Injury Incidence Prevalence Collaborators, 2016). Neurological disorders have attracted increasing attention from around the world and the resulting burden has become more widely acknowledged as a global public health concern in the coming decades.

Although these neurological disorders place a significant burden on society and individuals, the pathogenesis and biomarkers of these diseases are not yet fully understood. For example, ASD is a complex neurodevelopmental disorder probably caused by several pathological factors, such as neurochemical alterations including changes of gamma aminobutyric acid, glutamate, serotonin, dopamine, N-acetyl aspartate, oxytocin, arginine-vasopressin, melatonin, vitamin D, orexin, endogenous opioids, and acetylcholine (Marotta et al., 2020), abnormal brain structure and development (Gibbard et al., 2018; Lee et al., 2020; Thompson et al., 2020), as well as immunity dysregulation (Kim et al., 2017; Robinson-Agramonte et al., 2022). Studies have also shown that more

than 1,000 genes are related to the pathogenesis of ASD (Famitafreshi and Karimian, 2018; Qin et al., 2021). At the same time, environmental factors, including nutrition, medications, toxic substances, and maternal infections during pregnancy, have been extensively studied and found to be associated with ASD (Wang et al., 2016). The etiology and pathogenesis of depression are reported to be highly associated with synaptic remodeling, transcription factors and epigenetics, immunity and inflammation, as well as astrocyte function (Ménard et al., 2016). However, the understanding of the mechanisms and biomarkers underlying these neurological disorders has been still far from complete.

Based on public health trends and epidemiology patterns, the World Health Organization (WHO) has proposed a recommended formulary for high-priority diseases, serving as a guide for countries, particularly low- and lower-middle-income countries, to develop their own national essential medicines list. However, drugs that target neurologic disorders are poorly represented on the WHO model list (Rimmer et al., 2017). Besides, behavioral therapy, neurostimulation, and dietary interventions are also recommended in therapies and preventive measures of neurological disorders. New targeted drugs and novel therapeutic strategies concerning different pathogenesis are still depending on sufficient clinical and pre-clinical trials.

In view of the above realization, this special issue was organized to advance the understanding of the pathogenesis and biomarkers of neurological disorders, as well the most recent and advanced work on novel technologies identifying or preventing neurological disorders. Furthermore, novel therapeutic strategies are also discussed. This will serve as a foundation and perhaps provide valuable hints for clinical therapies and pharmacological development. For this Research Topic, twelve manuscripts have been submitted. After 7 months of critical peer review, ten papers have been accepted.

In the original research titled "*Brain somatic variant in Ras-like small GTPase RALA causes focal cortical dysplasia type II*", Xu et al. performed deep whole-exome sequencing and targeted amplicon sequencing in the postoperative brain tissue of epilepsy patients with focal cortical dysplasia type II (FCD II). In their study, HEK293T cells were transfected *in vitro*, with wild-type and mutant RALA plasmids were transfected into the local cortex of mice using *in utero* electroporation to evaluate the effect of RALA c.G482A on neuronal migration. The results demonstrated that the somatic gain-of-function variant of RALA activated the mTOR pathway and led to neuronal migration disorders in the brain, facilitating the development of FCD II.

In the case report titled "*Case report: Identification and clinical phenotypic analysis of novel mutation of the PPP1CB gene in NSLH2 syndrome*", He X. et al. screened and analyzed the genetic mutations in a patient with Noonan syndrome with loose anagen hair-2 (NSLH2) in Yunnan Province, China. The clinical manifestations of NSLH2 included prominent forehead, yellowish hair, slightly wide eye distance, sparse eyebrows, bilateral auricle deformity, reduced muscle tension, as well as cardiac and visual abnormalities. This article identified a novel mutation of PPP1CB, which enriched the mutation spectrum of the PPP1CB gene and provided a basis for the diagnosis of NSLH2.

In the methods article titled "*Development and validation of a system for the prediction of challenging behaviors of people with autism spectrum disorder based on a smart wearable shirt: A mixed-methods design*", Zwilling et al. developed an ML algorithm, which was capable of predicting immediate challenging behavior (CB) occurrence based on physiological parameter variations. An efficient proof of concept (POC) was also carried out to identify the strengths and weaknesses of the developed system. The results demonstrated the developed algorithm could be used to predict CBs that were about to occur in the upcoming 1 min.

In the mini review titled "*Research progress on transcranial magnetic stimulation for post-stroke dysphagia*", Li Y. et al. discussed the effectiveness, mechanisms, potential limitations, and prospects of transcranial magnetic stimulation (TMS) for clinical application in post-stroke dysphagia rehabilitation. This has introduced a safe and non-invasive technology of nerve stimulation that can be used to directly manipulate post-stroke dysphagia.

In the review titled "*Transcranial direct current stimulation of the dorsolateral prefrontal cortex for treatment of neuropsychiatric disorders*", Li Q. et al. performed searches on PubMed to collect clinical and preclinical studies that using transcranial direct current stimulation (tDCS) as neuromodulation technique, dorsolateral prefrontal cortex (DLPFC) as the stimulation target in treating neuropsychiatric disorders. The results indicated that tDCS stimulation of DLPFC could alleviate the clinical symptoms of schizophrenia, depression, drug addiction, attention deficit hyperactivity disorder and other mental disorders.

In the original research titled "*Safety and effects of transcranial direct current stimulation on hand function in preschool children with hemiplegic cerebral palsy: A pilot study*", He W. et al. designed a crossover, single-blind, sham-controlled study in 30 preschool children with hemiplegic cerebral palsy (HCP). Transcranial direct current stimulation (tDCS) on the primary motor cortex of the affected hemisphere was given with a 24-h interval between the two sessions. Box and Block Test, Selective Control of the Upper Extremity Scale, Modified Ashworth Scale, and Melbourne Assessment 2 were conducted at baseline, immediately, and 90 min after each session. The results supported the safety and efficacy of a single anodal tDCS on improving the manual dexterity of the hemiplegic hand for preschool children with HCP.

In the mini review titled "*Application of cognitive bias testing in neuropsychiatric disorders: a mini-review based on animal studies*", Zhang et al. summarized the application of cognitive bias tests in animal models of neuropsychiatric disorders such as depression, anxiety, bipolar disorder, and pain. They also discussed its critical value in the identification of neuropsychiatric disorders and the validation of therapeutic approaches.

In the review titled "*Research progress on the role of vitamin D in autism spectrum disorder*", Wang et al. reviewed the correlation between vitamin D level and ASD, the effects of vitamin D supplementation on ASD, the possible mechanism of vitamin D involved in ASD, and insights from ASD animal models. This can help to open-up a simple, cheap, and safe strategy for the prevention and treatment of ASD.

In the original research titled "*The effect of constraint-induced movement therapy combined with repetitive transcranial magnetic stimulation on hand function in preschool children with unilateral*

cerebral palsy: A randomized controlled preliminary study", Wu et al. designed a prospective, assessor-blinded, randomized controlled study. In their study, 40 preschool children (aged 2.5–6 years) with unilateral cerebral palsy (UCP) were randomized to receive 10 days of constraint-induced movement therapy (CIMT) combined with active or sham rTMS (repetitive transcranial magnetic stimulation). Upper limb extremity, social life ability, and perceived changes by parents and motor-evoked potentials were assessed. The CIMT plus active rTMS had greater gains in the affected hand function (range of motion, accuracy, and fluency) than the CIMT plus sham rTMS group, but there was no significant difference in muscular tone, social life ability, and perceived changes by parents between the two groups. This demonstrates that the treatment of CIMT combined with rTMS is safe and feasible for preschool children with UCP.

In the review titled "*Research progress on rheumatoid arthritis-associated depression*", Liu et al. provided an overview of the etiology and pathological mechanisms of rheumatoid arthritis-associated depression. They also reviewed recent advances in treatment with biologics, which would facilitate the development of new and effective prevention and treatment strategies.

Overall, these studies have systematically explored the pathophysiology and biomarkers of neurological disorders. They also covered the most recent and cutting-edge research on technologies for diagnosing or preventing neurological disorders, as well as novel therapy approaches. Future large-scale multi-center randomized controlled trials and in-depth mechanistic analysis are still required to further clarify the pathophysiological mechanisms underlying neurological disorders, thereby promoting translational medicine and drug development.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

Funding

This study was supported by the National Natural Science Foundation of China (31960178 and 82160923); Applied Basic Research Programs of Science and Technology Commission Foundation of Yunnan Province (2019FA007); Key Laboratory of Traditional Chinese Medicine for Prevention and Treatment of Neuropsychiatric Diseases, Yunnan Provincial Department of Education; Scientific Research Projects for High-level Talents of Yunnan University of Chinese Medicine (2019YZG01); Young Top-Notch Talent in 10,000 Talent Program of Yunnan Province (YNWR-QNBJ-2019-235); National Science and Technology Innovation 2030 Major Program (2021ZD0200900); Yunnan Key Research and Development Program (202103AC100005); and Yunnan Province Fabao Gao Expert Workstation Construction Project (202105AF150037).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Famitafreshi, H., and Karimian, M. (2018). Overview of the recent advances in pathophysiology and treatment for autism. *CNS Neurol. Disord. Drug Targets* 17, 590–594. doi: 10.2174/1871527317666180706141654

Feigin, V. L., Vos, T., Nichols, E., Owolabi, M. O., Carroll, W. M., Dichgans, M., et al. (2020). The global burden of neurological disorders: translating evidence into policy. *Lancet Neurol.* 19, 255–265. doi: 10.1016/S1474-4422(19)30411-9

GBD 2015 Disease and Injury Incidence and Prevalence Collaborators (2016). Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 388, 1545–1602. doi: 10.1016/S0140-6736(16)31678-6

Gibbard, C. R., Ren, J., Skuse, D. H., Clayden, J. D., and Clark, C. A. (2018). Structural connectivity of the amygdala in young adults with autism spectrum disorder. *Hum. Brain Mapp.* 39, 1270–1282. doi: 10.1002/hbm.23915

Kim, S., Kim, H., Yim, Y. S., Ha, S., Atarashi, K., Tan, T. G., et al. (2017). Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* 549, 528–532. doi: 10.1038/nature23910

Lee, J. K., Amaral, D. G., Solomon, M., Rogers, S. J., Ozonoff, S., and Nordahl, C. W. (2020). Sex differences in the amygdala resting-state connectome of children with autism spectrum disorder. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 5, 320–329. doi: 10.1016/j.bpsc.2019.08.004

Marotta, R., Risoleo, M. C., Messina, G., Parisi, L., Carotenuto, M., Vetri, L., et al. (2020). The neurochemistry of autism. *Brain Sci.* 10, 163. doi: 10.3390/brainsci10030163

Matilla-Dueñas, A., Corral-Juan, M., Rodríguez-Palmero Seuma, A., Vilas, D., Ispierto, L., Morais, S., et al. (2017). Rare neurodegenerative diseases: clinical and genetic update. *Adv. Exp. Med. Biol.* 1031, 443–496. doi: 10.1007/978-3-319-67144-4_25

Ménard, C., Hodes, G. E., and Russo, S. J. (2016). Pathogenesis of depression: Insights from human and rodent studies. *Neuroscience* 321, 138–162. doi: 10.1016/j.neuroscience.2015.05.053

Pena, S. A., Iyengar, R., Eshraghi, R. S., Bencie, N., Mittal, J., Aljohani, A., et al. (2020). Gene therapy for neurological disorders: challenges and recent advancements. *J. Drug Target.* 28, 111–128. doi: 10.1080/1061186X.2019.1630415

Qin, D. D., Zhou, J. K., He, X. C., Shen, X. Y., Li, C., Chen, H. Z., et al. (2021). Depletion of giant ANK2 in monkeys causes drastic brain volume loss. *Cell Discov.* 7, 113. doi: 10.1038/s41421-021-00336-4

Rimmer, K., Shah, H., and Thakur, K. (2017). Expanding medicines for neurologic disorders on the WHO Model List. *Neurology* 88, e87–e91. doi: 10.1212/WNL.0000000000003691

Robinson-Agramonte, M. L. A., Noris García, E., Fraga Guerra, J., Vega Hurtado, Y., Antonucci, N., Semprún-Hernández, N., et al. (2022). Immune dysregulation in autism spectrum disorder: What do we know about it? *Int. J. Mol. Sci.* 23, 3033. doi: 10.3390/ijms23063033

Thompson, A., Shahidiani, A., Fritz, A., O'Muircheartaigh, J., Walker, L., D'Almeida, V., et al. (2020). Age-related differences in white matter diffusion measures in autism spectrum condition. *Mol. Autism* 11, 36. doi: 10.1186/s13229-020-00325-6

Wang, T., Shan, L., Du, L., Feng, J., Xu, Z., Staal, W. G., et al. (2016). Serum concentration of 25-hydroxyvitamin D in autism spectrum disorder: a systematic review and meta-analysis. *Eur. Child Adolesc. Psychiatry* 25, 341–350. doi: 10.1007/s00787-015-0786-1

frontiers | Frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 05 April 2022
doi: 10.3389/fnbeh.2022.876567

The Effect of Constraint-Induced Movement Therapy Combined With Repetitive Transcranial Magnetic Stimulation on Hand Function in Preschool Children With Unilateral Cerebral Palsy: A Randomized Controlled Preliminary Study

Qianwen Wu[1†], Tingting Peng[1†], Liru Liu[1], Peishan Zeng[1], Yunxian Xu[1], Xubo Yang[1], Yiting Zhao[1], Chaoqiong Fu[1], Shiya Huang[1], Yuan Huang[1,2], Hongyu Zhou[1], Yun Liu[3], Hongmei Tang[1], Lu He[1] and Kaishou Xu[1*]*

[1] Department of Rehabilitation, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China, [2] School of Medicine, South China University of Technology, Guangzhou, China, [3] Department of Rehabilitation, Kunming Children's Hospital, Kunming, China

Constraint-induced movement therapy (CIMT) combined with repetitive transcranial magnetic stimulation (rTMS) have shown great potential in improving function in schoolchildren with unilateral cerebral palsy attributed to perinatal stroke. However, the prospect of application in preschool children with unilateral cerebral palsy (UCP) attributed to various brain disorders remains unclear. In this prospective, assessor-blinded, randomized controlled study, 40 preschool children with UCP (aged 2.5–6 years) were randomized to receive 10 days of CIMT combined with active or sham rTMS. Assessments were performed at baseline, 2 weeks, and 6 months post-intervention to investigate upper limb extremity, social life ability, and perceived changes by parents and motor-evoked potentials. Overall, 35 participants completed the trial. The CIMT plus active stimulation group had greater gains in the affected hand function (range of motion, accuracy, and fluency) than the CIMT plus sham stimulation group ($P < 0.05$), but there was no significant difference in muscular tone, social life ability, and perceived changes by parents between the two groups ($P > 0.05$). In addition, there was no significant difference in hand function between children with and without motor-evoked potential ($P > 0.05$). No participants reported severe adverse events during the study session. In short, the treatment of CIMT combined with rTMS is safe and feasible for preschool children with UCP attributed to various brain disorders. Randomized controlled studies with large samples and long-term effects are warranted.

Keywords: constraint-induced movement therapy, repetitive transcranial magnetic stimulation, preschool children, unilateral cerebral palsy, hand function

OPEN ACCESS

Edited by:
Dongdong Qin,
Yunnan University of Chinese Medicine, China

Reviewed by:
Dinesh Bhatia,
North Eastern Hill University, India
Qin Gu,
Children's Hospital of Soochow University, China
Peng Zhao,
Tianjin Children's Hospital, China

***Correspondence:**
Kaishou Xu
xksyi@126.com
orcid.org/0000-0002-0639-3488
Lu He
kittyhelu@126.com

[†]These authors share first authorship

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *15 February 2022*
Accepted: *02 March 2022*
Published: *05 April 2022*

Citation:
Wu Q, Peng T, Liu L, Zeng P, Xu Y, Yang X, Zhao Y, Fu C, Huang S, Huang Y, Zhou H, Liu Y, Tang H, He L and Xu K (2022) The Effect of Constraint-Induced Movement Therapy Combined With Repetitive Transcranial Magnetic Stimulation on Hand Function in Preschool Children With Unilateral Cerebral Palsy: A Randomized Controlled Preliminary Study. Front. Behav. Neurosci. 16:876567. doi: 10.3389/fnbeh.2022.876567

INTRODUCTION

Cerebral palsy is the most common physical disability in childhood, occurring in 2.5–3.5 per 1,000 live births and with complicated etiology (Bax et al., 2005; Li et al., 2021). Unilateral cerebral palsy (UCP), which mainly affects the function of children's lateral extremity, accounts for 44% of the cases (Stavsky et al., 2017). The main manifestations in children are motor impairments and may encompass heterogeneous clinical performance including impairment of communication, cognition, or sensation (especially tactile sensation), the difficulty of daily task performance, and quality of life (Senst, 2014).

Over the last decade, studies on interventions for children with UCP have grown exponentially. It was indicated that the effect of most treatments for upper limb function in hemiplegic patients is induced by the principles of task- and context-specific motor learning and repetition (Veerbeek et al., 2014). One of the most popular treatments among clinicians and researchers is constraint-induced movement therapy (CIMT), with an emphasis on constraining the unaffected extremity and coupling task-related practice with the affected upper extremity, and increasing evidence proved the effect of CIMT in children with UCP (Reedman et al., 2017; Hoare et al., 2019; Ilieva and Ilieva, 2020; Simon-Martinez et al., 2020). What is more, it was proved that CIMT might promote neural remodeling and thereby improve motor function (Liu et al., 2021). However, the findings on the effect of CIMT on improving bimanual coordination are controversial (Reid et al., 2015; Hoare et al., 2019). In addition, it has not been proven to have much effect on improving decreasing muscle tone (Reid et al., 2015). Studies have shown that neuromodulation technology such as repetitive transcranial magnetic stimulation (rTMS), which acts directly on the central nervous system, may yield a great impact on the overall motor ability and decrease the muscle tone of children with cerebral palsy (Boddington and Reynolds, 2017; Gupta and Bhatia, 2018; Parvin et al., 2018; Rajak et al., 2019).

Children with UCP demonstrate atypical patterns of corticospinal tract development and organization, which leads to an imbalance in excitability between the affected and unaffected hemispheres (Berweck et al., 2008; Chen et al., 2016). These neural changes may underlie the limitations in upper extremity function and social life ability (Holmström et al., 2010). Given that rTMS depolarizes neurons by means of strong, short magnetic pulses, aiming to suppress or facilitate cortical excitability depending on electrode polarity, it may make up the shortcomings of CIMT (Klomjai et al., 2015; Lefaucheur et al., 2020). Indeed, the effect of CIMT combined with rTMS has been proven in improving behavioral function and neurophysiologic responses in school-aged children with UCP attributed to perinatal strokes (Kirton et al., 2016).

Furthermore, emerging evidence suggested that the CIMT was more effective during the early developmental period (Reid et al., 2015; Boddington and Reynolds, 2017). To our best of knowledge, there are few studies to evaluate the effect of CIMT combined with rTMS on the treatment response in young children (Novak et al., 2020). Due to the immature pattern of hand function and poor self-control, preschool children with UCP, who are often affected by joint reaction and mirror movements, i.e., involuntary imitations of unilateral voluntary movements, can easily be affected by the motor pattern of the affected side. This period may be critical for more effective rehabilitation. On the other hand, most studies focused on perinatal strokes, although UCP has complicated pathogenic factors.

To fill this gap, we carried out a randomized controlled study to evaluate the effect of CIMT combined with rTMS in preschool children with UCP attributed to various brain disorders.

METHODS

The design of this study was a prospective, assessor-blind, and randomized controlled trial, which was registered at chictr.org (ChiCTR1900021924). The institutional research ethics board approval was obtained from Guangzhou Women and Children's medical center, and written informed consent was obtained from the legal representative of each participant before enrollment.

Participants

Eighty-four preschool children with UCP were recruited through the goal-directed, peer-supported CIMT camp program. Recruitment occurred from March 25, 2019 to August 31, 2019. Inclusion criteria were as follows: (i) aged 2.5–6 years; (ii) Manual Ability Classification System levels I-II or Mini-Manual Ability Classification System levels I-II; (iii) $\geq 20^{\circ}$ wrist active extension and $\geq 10^{\circ}$ metacarpophalangeal active extension from full flexion; (iv) a 20–80% difference of global rating scale scores between the affected and unaffected hands; and (v) written informed consent. Participants were excluded if they met any of these criteria: (i) other neurological diagnosis; (ii) uncontrolled seizures; (iii) severe sensory impairment or visual problems; (iv) contraindication for rTMS (Wassermann, 1998; Kirton et al., 2008); (v) upper limb surgery; or (vi) botulinum toxin treatment within 6 months. A total of 40 children met the inclusion criteria. Thirty-five children completed the study in the end (with 17 children in the CIMT plus active stimulation group). The flow of patients is summarized in **Figure 1**.

Design

Participants were randomly assigned to CIMT plus active or sham stimulation groups (1:1) in an unbiased manner using a random number table produced by Statistical Product and Service Solutions for Windows (release 25.0, SPSS). Assessments and administration of the functional scales and questionnaires were performed by two independent assessors who had received training and certification of the study measures. CIMT assignment was concealed from the assessors. Assessors were blind to rTMS assignment. Endpoints were assessed at the baseline visit, 2 weeks, and 6 months postintervention.

Interventions

All the involved children participated in the 10 consecutive days of goal-directed CIMT camp, active or sham rTMS was applied independently in a separate room before daily CIMT therapy. During the rTMS stimulation, participants were seated in a chair

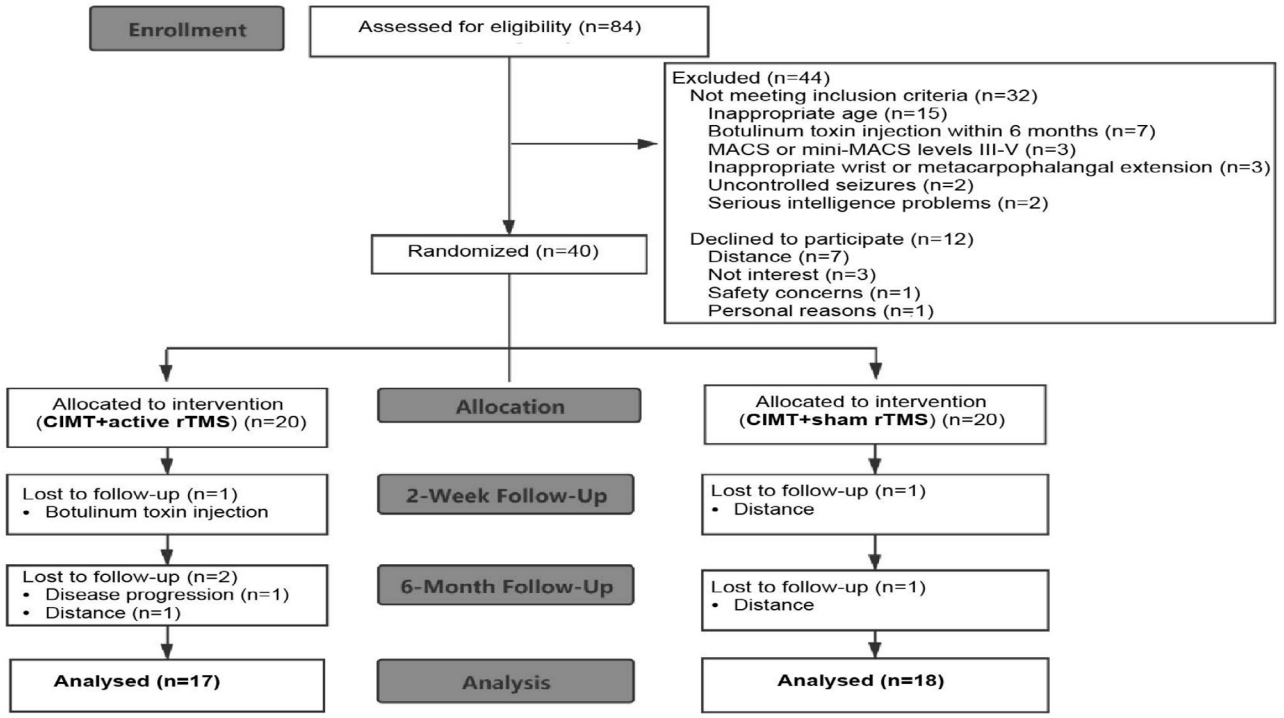


FIGURE 1 | Study flow diagram.

in a comfortably static position and wore a cap for marking stimulation points. An eight-shaped circular coil connected to a Yiruide CCY-1 stimulator (Yiruide Company Limited, Wuhan, China) was positioned on the hotspot area pressing to the scalp. A single pulse of transcranial magnetic stimulation was delivered to detect the motor-evoked potential (MEP) by electromyographic monitoring from the affected first dorsal interosseous muscle. The minimum stimulation intensity was considered as the resting motor threshold when the collected amplitude was $>50\ \mu V$ in at least 5 out of 10 trials.

After the determination of the resting motor threshold, participants received priming rTMS for the unaffected primary motor cortex. A therapist orientated the handle pointing at a 45° angle to the sagittal line for the CIMT plus active stimulation group or a 90° angle for the CIMT plus sham stimulation group. Parameters for rTMS were as follows: intensity 90% resting motor threshold multiplied by 1 T, frequency 1 Hz for 20 min. For children with absent resting motor thresholds, the fixed resting motor threshold was set as 40% machine output for the consideration of the rough mean resting motor threshold in studies with different groups of people (Delvaux et al., 2003; Ciechanski et al., 2017). That is to say, the stimulation intensity was set as 40% × 90% × 1 T for the participants with absent resting thresholds.

After the active or sham rTMS intervention, a tailor-made restrictive glove was fitted and applied to all the participants on the unaffected hand and forearm (from fingertips to middle forearm) for more than 6 h each day. The restricted hand retained the ability to support or prevent falls (Xu et al., 2012). Motor learning for the affected upper extremity totaled 3 h each day. The participant/therapist ratio of group activities was 3:1 to secure individual guidance. Group activities were age-appropriate and play-based daily living activities to improve children's desire to participate (e.g., tug-of-war, shooting contest, balloon transmission, and desktop cleaning). After the 3-h hospital-centered CIMT training, participants continued family-centered training for 3 h with an exercise program set by therapists to practice with the affected upper extremity under the guidance of caregivers. Telephone follow-up and rehabilitation guidance were conducted every 2 weeks. Daily caregiver-supervised records were followed-up.

Outcome Assessment

Assessments based on the dimensions of the international classification of functioning, disability, and health (ICF) were performed at the baseline visit, 2 weeks, and 6 months postintervention (Cieza et al., 2019; Angeli et al., 2021). The MEPs and adverse events were assessed to investigate corticospinal excitability changes and safety. Safety was assessed through the self-reporting of symptoms, updating medical records, and physician review.

The manual abilities were classified by the Manual Ability Classification System (for children aged over 4 years old) or Mini-Manual Ability Classification System (for children aged 1–4 years old), the evidence-based standard for upper extremity functional levels (Eliasson et al., 2017; Palisano et al., 2018). The

Melbourne Assessment 2 (MA2), a validated tool to evaluate the unaffected upper limb function, was the main outcome measure in this study (Wang et al., 2017). The modified Ashworth scale was performed for the description muscle tone (Meseguer-Henarejos et al., 2018; Zurawski et al., 2019). Bimanual hand performance was assessed by the selective control of the upper extremity scale (Wagner et al., 2016). Perceived changes by caregivers were evaluated by global rating scale and social life ability was evaluated by social life ability scale for Chinese infant–junior school students, which comprised six domains: independent living, athletic abilities, operational abilities, communicative abilities, participation in collective activities, and self-management abilities, with excellent reliability and validity (Zhang et al., 1995). The MEPs in the unaffected motor cortices were measured in the first dorsal interosseous muscles by single-pulse TMS.

Adverse events related to CIMT or rTMS were assessed during the whole study period. A summary of the transient minor adverse events was summarized in prior publications (Gillick et al., 2018).

Statistical Analysis

The data were analyzed using SPSS version 25.0. For continuous variables, an independent sample *t*-test was performed to compare the baseline data between the two groups which accorded to normal distribution. The ranked variables or variables that did not conform to normal distribution were analyzed by 2 independent samples such as Wilcoxon signed-rank sum test. For categorical variables, the chi-squared test was analyzed. Repeated measures analyses of variance and simple effect analysis were performed for the within-group and between-group differences of upper extremity function, social life ability, perceived changes by parents, and MEP data. Analysis of covariance was used to compute mean differences between the two groups adjusting for baseline. Level information was expressed by frequency and percentage. For every analysis, the significance level was set at $P < 0.05$.

RESULTS

There were no significant differences in baseline demographic characteristics or functional performance between the two groups (**Table 1**), with the independent sample *t*-test or Wilcoxon signed-rank sum test ($P > 0.05$).

Improvement of Affected Upper Extremity Function

Most participants had significantly increased MA2 subscale scores (range of motion, accuracy, dexterity, and fluency) at both 2 weeks and 6 months post-intervention compared with the baseline in the two groups ($P < 0.05$, **Table 2**). The CIMT plus active stimulation group was associated with larger gains in the subscales of accuracy, fluency, and range of motion than the CIMT plus sham stimulation group ($P < 0.05$). Just as important, the difference of average change value of MA2 subscales between groups exceeded the minimum clinically important difference (MCID) of MA2 subscales that has been established (the MCID of MA2 subscales are 2.35, 3.20, 2.09, and 2.22, respectively) (Wang et al., 2017). No significant diffidence was reported between the two groups in the subscale of dexterity ($P > 0.05$).

For muscular tone, no treatment-related change emerged in the modified Ashworth scale (forearm, wrist, thumb, and fingers) in the two groups ($P > 0.05$, **Figure 2**).

TABLE 1 | Baseline participant characteristics by the group.

	CIMT + rTMS $^{(+)}$ ($n = 17$)	CIMT + rTMS $^{(-)}$ ($n = 18$)	*P*-value
Age (m)	50.6 (10.5)	43.83 (12.6)	0.123
Gender, male/female	6/11	8/10	0.594
Left side of hemiparesis, *n* (%)	8 (47.1)	10 (55.6)	0.620
Gross motor function classification system, level I/II	15/2	13/5	0.249
Manual ability classification system, level I/II	11/6	8/10	0.236
The modified Ashworth scale, median (range)	1^{+} (1–3)	2 (1–3)	0.756
Melbourne assessment 2			
Range of motion	72.77 (17.37)	68.90 (19.05)	0.535
Accuracy	82.59 (15.16)	74.49 (19.20)	0.177
Dexterity	65.03 (15.34)	59.18 (15.52)	0.271
Fluency	70.31 (13.21)	62.15 (11.85)	0.052
SCUES of affected side	8.71 (2.4)	8.17 (3.6)	0.603
SCUES of the unaffected side	14.00 (2.3)	14.17 (1.7)	0.465
Global rating scale	4.76 (1.8)	4.11 (1.9)	0.304
Standard scores of social life ability scale	10 (1.2)	10 (1.4)	0.930
Magnetic resonance imaging (*n*)	PVL (6), ventricle broadening (3), cyst (1); normal (1), absence (6)	PVL (9); ventricle broadening (4); cyst (2); absence (3)	

P-value represents between-group differences. Data shown are means (SD) or n (%), unless otherwise stated.
CIMT, constraint-induced movement therapy; rTMS, repetitive transcranial magnetic stimulation; SCUES, selective control of upper extremity scale; PVL, periventricular leukomalacia.

TABLE 2 | Pre- and post-intervention changes in the Melbourne Assessment 2 in the 2 treatment groups.

Assessments	Intervention point	CIMT + rTMS $^{(+)}$ ($n = 17$)	CIMT + rTMS $^{(-)}$ ($n = 18$)	*P*-value
MA2-range of motion	Baseline	72.77 (17.4)	68.90 (19.0)	**0.021**
	2 Weeks	83.44 (13.7)$^{\#\#}$	75.36 (20.5)$^{\#}$	
	6 Months	81.25 (14.4)$^{\#}$	71.80 (16.1)	
MA2-accuracy	Baseline	82.59 (15.2)	74.44 (19.2)	**0.017**
	2 Weeks	90.35 (10.5)$^{\#\#}$	82.89 (19.1)$^{\#\#}$	
	6 Months	90.59 (10.6)$^{\#}$	80.4 (19.3)$^{\#}$	
MA2-dexterity	Baseline	65.03 (15.3)	59.18 (15.5)	0.356
	2 weeks	78.28 (14.2)$^{\#\#}$	64.06 (13.8)$^{\#}$	
	6 months	73.49 (13.4)$^{\#\#}$	65.25 (13.7)$^{\#\#}$	
MA2-fluency	Baseline	70.31 (13.2)	62.15 (11.8)	**0.020**
	2 Weeks	79.83 (11.1)$^{\#\#}$	74.60 (11.4)$^{\#\#}$	
	6 Months	79.27 (12.8)$^{\#\#}$	68.26 (11.0)$^{\#\#}$	
SCUES (affected)	Baseline	8.71 (2.4)	8.17 (3.6)	0.742
	2 Weeks	12.59 (2.5)$^{\#\#}$	11.28 (3.5)$^{\#\#}$	
	6 Months	10.76 (2.2)$^{\#\#}$	9.56 (2.5)	
SCUES (unaffected)	Baseline	14.00 (2.3)	14.17 (1.7)	0.451
	2 Weeks	13.24 (2.4)	12.94 (3.6)	
	6 Months	14.06 (1.5)	13.89 (1.2)	

P-value represents between-group differences (the bold values represent P < 0.05). Values are reported as mean (SD). Within-group and between-group differences were analyzed with repeated measures analyses of variance.

$^{\#}$Significantly different than baseline, P < 0.05.

$^{\#\#}$Significantly different than baseline, P < 0.01.

CIMT, constraint-induced movement therapy; rTMS, repetitive transcranial magnetic stimulation; MA2, the modified Melbourne assessment 2; SCUES, selective control of upper extremity scale.

Bimanual Performance

Although most participants had increased selective control of the affected upper extremity scale scores, there was no significant difference between the two groups ($P > 0.05$, **Table 2**). As for the unaffected upper extremity, there was no significant within-group and between-groups difference ($P > 0.05$).

Social Life Ability and Perceived Changes by Caregivers

For the social life ability scale, there were no significant within-group and between-group differences between the two groups ($P > 0.05$; **Figure 3A**). We found that the global rating scale scores achieved clinically significant gains at 2 weeks of post-intervention in both the groups ($P < 0.01$), even though there was no significant between-group difference ($P > 0.05$; **Figure 3B**).

Motor-Evoked Potential Outcomes

To investigate the correlations between MEP outcomes and hand function after the intervention of CIMT combined with active rTMS stimulation, we compared MA2 outcomes between children with ($n = 7$) and without ($n = 10$) MEPs in the lesioned hemisphere at 2 weeks of post-intervention. No significant difference emerged between the groups ($P > 0.05$, **Table 3**).

Safety

Headache occurred in one participant, which was relieved after several minutes. No participants reported severe adverse events such as epileptic seizures or behavioral problems during the study session.

DISCUSSION

We examined the effect of the intervention of CIMT combined with rTMS on preschool children with UCP and found that the addition of rTMS exaggerated the effect on the affected upper extremity function induced by CIMT. No serious adverse events occurred during the study period, only one participant reported a self-limiting headache.

In this study, most participants experienced improvements in the affected upper extremity function after 2 weeks and 6 months post-intervention. Greater improvement in accuracy, fluency, and range of motion in the CIMT plus active stimulation group, suggested a greater impact of CIMT combined with rTMS than CIMT alone, which is consistent with the previous study in school-age children (Kirton et al., 2016). Young children with UCP are often affected by joint reaction and mirror movements (Ismail et al., 2017). Hence, it is still necessary to carry out effect-oriented trials of CIMT combined with rTMS in younger children, and our results complemented this evidence in preschool children with UCP. Even though no significant difference in dexterity and selective control of the upper extremity scale of the affected upper extremity were reported between the two groups, participants who received CIMT combined with active stimulation had more favorable mean scores 2 weeks postintervention. Notably, improvements

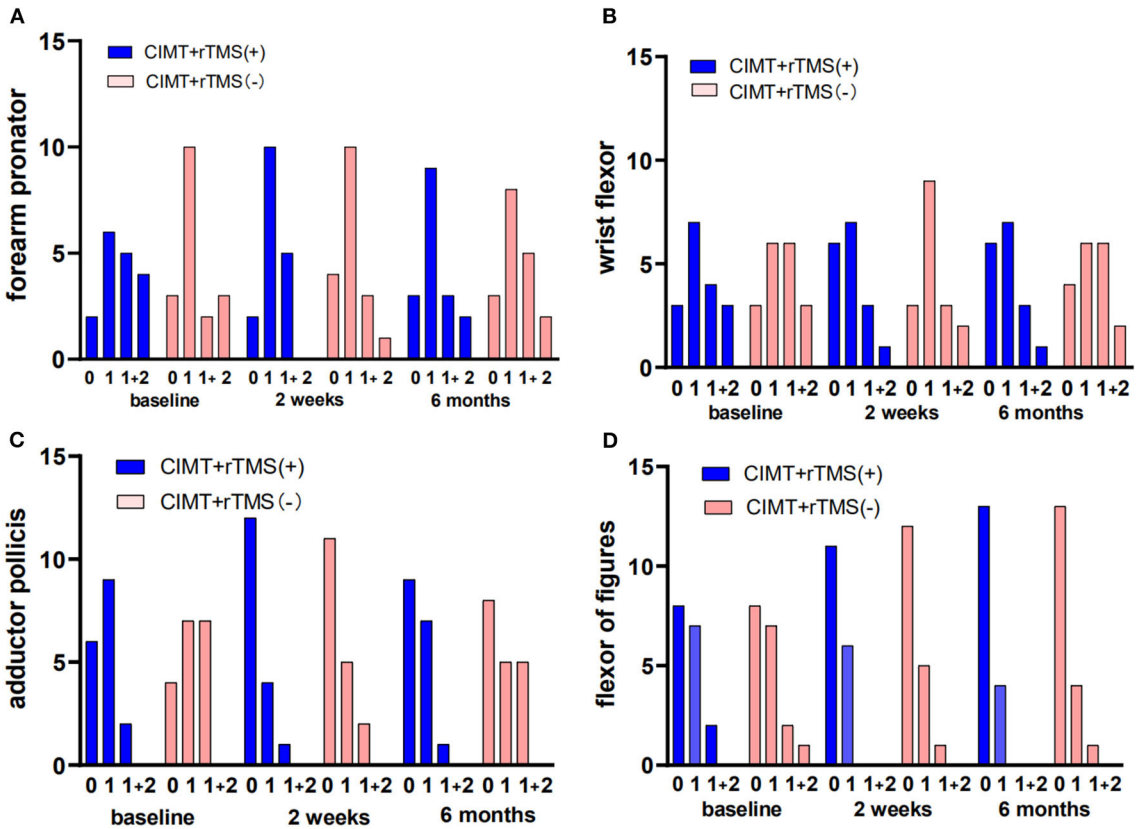


FIGURE 2 | Changes of muscle tone of the affected upper extremity in the constraint-induced movement therapy (CIMT) plus active stimulation group and sham stimulation group. **(A)** Muscle tone of forearm. **(B)** Muscle tone of wrist. **(C)** Muscle tone of thumb. **(D)** Muscle tone of the other fingers. rTMS, repetitive transcranial magnetic stimulation.

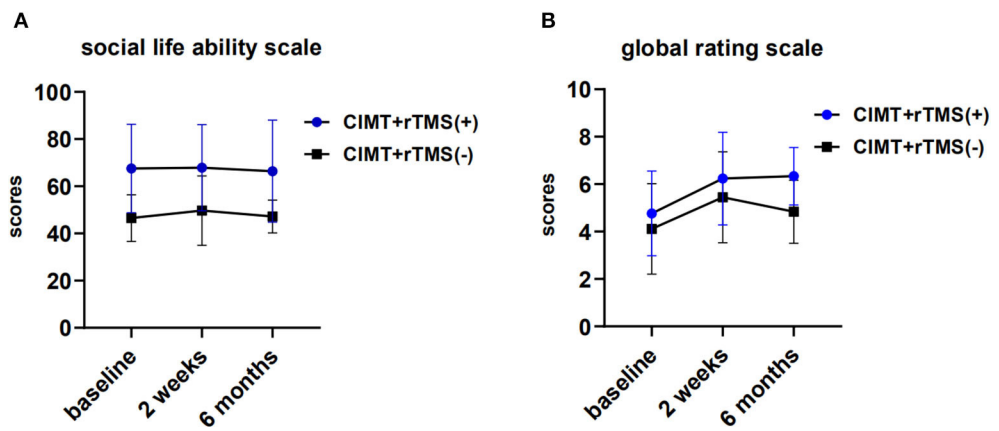


FIGURE 3 | Perceived changes by caregivers and changes of activity of daily living in the constraint induced movement therapy (CIMT) plus active stimulation group and sham stimulation group. **(A)** Changes of global rating scale. **(B)** Changes of social life ability scale for Chinese infant-junior school student. rTMS, repetitive transcranial magnetic stimulation.

measured with MA2 sustained for 6 months in this study may reflect long-term depression of 1-Hz rTMS in corticospinal excitability. The maintained after-effect, which may be relevant to a complex scenario (e.g., gene activation/regulation, *de-novo* protein expression, and postsynaptic excitability state), is the rationale for rTMS applications as a clinical tool (Cirillo et al., 2017; Baur et al., 2020).

The muscle tone was not reported with significant differences between groups. The previous study has indicated the positive effect of 10-Hz rTMS on muscle tone of children with cerebral

TABLE 3 | Comparison of upper extremity function between the groups with or without MEPs at 2 weeks of postintervention.

Measurements	Intervention point	With MEP ($n = 7$)	Without MEP ($n = 10$)	Between-group comparison (P)
MA2-range of motion	Baseline	80.42 (4.90)	78.27 (8.51)	0.826
	2 Weeks	87.83 (4.41)	82.54 (6.40)	
MA2-accuracy	Baseline	76.57 (18.82)	86.86 (13.41)	0.426
	2 Weeks	88.57 (13.35)	90.29 (9.48)	
MA2-dexterity	Baseline	58.64 (18.59)	74.47(8.24)	0.381
	2 Weeks	78.59 (19.39)	82.57 (8.42)	
MA2-fluency	Baseline	69.38 (17.14)	75.51 (9.29)	0.329
	2 Weeks	74.53 (19.48)	80.03 (6.40)	
SCUES (affected)	Baseline	7.71 (2.36)	10.00 (1.91)	0.648
	2 Weeks	12.71 (2.87)	13.43 (2.15)	
SCUES (unaffected)	Baseline	14.29 (1.89)	15.00 (0.00)	0.288
	2 Weeks	12.71 (2.87)	13.86 (2.27)	

Values are reported as mean (SD).
MEP, motor-evoked potential; CIMT, constraint-induced movement therapy; rTMS, repetitive transcranial magnetic stimulation.

palsy (Rajak et al., 2019). In light of the proven safety of low-frequency rTMS, we adhered to established principles of 1-Hz rTMS applied to the unaffected motor cortex (Emara et al., 2010; Gillick et al., 2014a). Rossi et al. had compared the safety between high-frequency and low-frequency rTMS and found that induction of seizures was with 1.4% and crude risk estimate in epileptic patients and <1% under high-frequency stimulation in patients without the history of seizures, yet was hardly reported in studies with low-frequency stimulation (Gillick et al., 2014a). In line with the evidence of low-frequency rTMS, no serious adverse event was reported in this study. For developing brains, safety deserves to be handled with the utmost seriousness, and more studies of low-frequency rTMS on this group are warranted.

A previous study reported improvements in quality-of-life measures in children older than six (Gillick et al., 2014b; Kirton et al., 2016; Rich et al., 2016). However, we did not find any significant differences in social life ability scale scores and perceived changes by caregivers between the two groups. One of the potential factors to consider was the educational environment in China. Many Chinese caregivers, especially grandparents, usually overprotect their kids and are used to reducing the opportunities of their children to complete the tasks in life by themselves, which may limit the improvements to the children's social abilities to a certain extent. What is more, the optimal timing of follow-up for clinically relevant change of CIMT combined with rTMS is not well understood in young children. A longer follow-up period and more follow-up time points may be important for the understanding of clinically relevant change.

It was shown that MEPs were detected only from some participants. We wondered if children with absent MEP on the affected side do worse than the others after the intervention of CIMT combined with active rTMS. Interestingly, we did not find significant differences in upper extremity function between the groups with or without MEP, which provides a train of thought to search for an optimal fixed motor threshold for young children with absent MEP. On the other hand, the reason for MEP absence in young children is not well understood yet. We presumed that the high level of motor cortex excitability and the difficulty for young children to maintain relaxed muscles may be important resources. An increased understanding of the developmental neurophysiological processes in preschool children with cerebral palsy is essential for the establishment of neuromodulation principles. Considering the difficulty of measuring the MEPs for preschool children, our study may be a beneficial exploration of the rTMS parameters for this group.

In addition, studies reported that the integrity of underlying brain anatomy and various brain disorders could potentially influence the distribution of current across the scalp, which may contribute to the variable efficacy of rTMS in children with brain disorders (Rossi et al., 2009; Klomjai et al., 2015). Importantly, a large number of studies have focused on UCP attributed to perinatal stroke, although complicated factors may play an important role in cerebral palsy (e.g., leukomalacia and intracranial hemorrhage in infants). Participants in the study were represented with various brain disorders, expanding the chance of variable efficacy of rTMS. Furthermore, consistent with the adult stroke model, current models considered interhemispheric balance in young children as a spectrum, rather than a dichotomy. Pino et al. (2014) demonstrated that the cerebral structural reserve (preservation of neural pathways and connections) was important to cerebral plasticity. The chance is that the treatment effect is related to interhemispheric balance rather than the simple interhemispheric competition model. In this context, the determination of brain damage is important to the rTMS effect.

Limitations of this study embodied the modest sample size, the insufficient follow-up time points, and lack of subgroups for lesion location of brain and age. Still, there was no formal assessment of potential complications and the impact of parental education and social background on treatment. Different requirements and expectations of the parent may lead to bias in some subjective indicators.

Concerns about the deeper influence of age and lesion location of the brain on CIMT combined with rTMS warrant further investigation in studies. With the combination of neuroimaging techniques, we can observe the changes of cerebral blood flow and molecular biology in the course of rTMS action, thus providing more help for studying the mechanism of rTMS and the best treatment parameters.

CONCLUSION

The rTMS combined with CIMT has a superimposed therapeutic effect on the affected hand function in preschool children with UCP attributed to various brain disorders, which is safe and worthy of promotion among this group of children.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Guangzhou Women and Children's Medical Center Research Ethics Committee (Approved No. 14300). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KX conceived this study, contributed to the study design, and attributed to project management and fund procurement. QW and TP wrote this manuscript and performed data collection. LL and YX generated the figures and tables. PZ contributed to guidance on English writing. XY and YZ performed data analysis. CF, SH, YH, and HZ carried out the literature search. YL, HT, and LH contributed to participant recruitment. KX and LH revised the manuscript. All authors have read and approved the content of the manuscript.

FUNDING

This study was supported by the National Natural Science Foundation of China (81902309 and 81672253), the Natural Science Foundation of Guangdong Province (2021A1515011303, 2021A1515012543, and 2019A1515010420), the Basic and Applied Basic Foundation of Guangdong Province (2020A1515110435), funds from the Guangzhou Municipal Science and Technology Project (202102010205 and 202102020581), and the Featured Clinical Technique of Guangzhou (2019TS55). The funders played no role in the design, conduct, or reporting of this study.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the extraordinary generosity of the study participants and the CIMT camp staff for delivering professional intervention and measurements.

REFERENCES

Angeli, J. M., Schwab, S. M., Huijs, L., Sheehan, A., and Harpster, K. (2021). ICF-inspired goal-setting in developmental rehabilitation: an innovative framework for pediatric therapists. *Physiother. Theory Pract.* 37, 1167–1176. doi: 10.1080/09593985.2019.1692392

Baur, D., Galevska, D., Hussain, S., Cohen, L. G., Ziemann, U., and Zrenner, C. (2020). Induction of LTD-like corticospinal plasticity by low-frequency rTMS depends on pre-stimulus phase of sensorimotor μ-rhythm. *Brain Stimul.* 13, 1580–1587. doi: 10.1016/j.brs.2020.09.005

Bax, M., Goldstein, M., Rosenbaum, P., Leviton, A., Paneth, N., and Dan, B. (2005). Proposed definition and classification of cerebral palsy, April 2005. *Dev. Med. Child Neurol.* 7, 571–576. doi: 10.1017/S001216220500112X

Berweck, S., Walther, M., Brodbeck, V., Wagner, N., Koerte, I., and Henschel, V. (2008). Abnormal motor cortex excitability in congenital stroke. *Pediatr. Res.* 63, 84–88. doi: 10.1203/PDR.0b013e31815b88f1

Boddington, L. J., and Reynolds, J. N. J. (2017). Targeting interhemispheric inhibition with neuromodulation to enhance stroke rehabilitation. *Brain Stimul.* 10, 214–222. doi: 10.1016/j.brs.2017.01.006

Chen, C. Y., Rich, T. L., Cassidy, J. M., and Gillick, B. T. (2016). Corticospinal excitability in children with congenital hemiparesis. *Brain Sci.* 6, 49. doi: 10.3390/brainsci6040049

Ciechanski, P., Zewdie, E., and Kirton, A. (2017). Developmental profile of motor cortex transcallosal inhibition in children and adolescents. *J. Neurophysiol.* 118, 140–148. doi: 10.1152/jn.00076.2017

Cieza, A., Fayed, N., Bickenbach, J., and Prodinger, B. (2019). Refinements of the ICF Linking Rules to strengthen their potential for establishing comparability of health information. *Disabil. Rehabil.* 41, 574–583. doi: 10.3109/09638288.2016.1145258

Cirillo, G., Di Pino, G., Capone, F., Ranieri, F., Florio, L., Todisco, V., et al. (2017). Neurobiological after-effects of non-invasive brain stimulation. *Brain Stimul.* 10, 1–18. doi: 10.1016/j.brs.2016.11.009

Delvaux, V., Alagona, G., Gérard, P., De Pasqua, V., Pennisi, G., de Noordhout, A. M., et al. (2003). Post-stroke reorganization of hand motor area: a 1-year prospective follow-up with focal transcranial magnetic stimulation. *Clin. Neurophysiol.* 114, 1217–1225. doi: 10.1016/S1388-2457(03)00070-1

Eliasson, A. C., Ullenhag, A., Wahlström, U., and Krumlinde-Sundholm, L. (2017). Mini-MACS: development of the Manual Ability Classification System for children younger than 4 years of age with signs of cerebral palsy. *Dev. Med. Child Neurol.* 59, 72–78. doi: 10.1111/dmcn.13162

Emara, T. H., and Moustafa, R. R., ElNahas, N. M., ElGanzoury, A. M., Abdo, T. A., Mohamed, S. A., et al. (2010). Repetitive transcranial magnetic stimulation at 1Hz and 5Hz produces sustained improvement in motor function and disability after ischaemic stroke. *Eur. J. Neurol.* 17, 1203–1209. doi: 10.1111/j.1468-1331.2010.03000.x

Gillick, B. T., Gordon, A. M., Feyma, T., Krach, L. E., Carmel, J., Rich, T. L., et al. (2018). Non-invasive brain stimulation in children with unilateral cerebral palsy: a protocol and risk mitigation guide. *Front. Pediatr.* 6, 56. doi: 10.3389/fped.2018.00056

Gillick, B. T., Krach, L. E., Feyma, T., Rich, T. L., Moberg, K., and Menk, J. (2014a). Safety of primed repetitive transcranial magnetic stimulation and modified constraint-induced movement therapy in a randomized controlled trial in pediatric hemiparesis. *Arch. Phys. Med. Rehabil.* 96(Suppl. 4), S104–113. doi: 10.1016/j.apmr.2014.09.012

Gillick, B. T., Krach, L. E., Feyma, T., Rich, T. L., Moberg, K., Thomas, W., et al. (2014b). Primed low-frequency repetitive transcranial magnetic

stimulation and constraint-induced movement therapy in pediatric hemiparesis: a randomized controlled trial. *Dev. Med. Child Neurol.* 56, 44–52. doi: 10.1111/dmcn.12243

Gupta, M., and Bhatia, D. (2018). Evaluating the effect of repetitive transcranial magnetic stimulation in cerebral palsy children by employing electroencephalogram signals. *Ann Indian Acad Neurol* 21, 280–284. doi: 10.4103/aian.AIAN_413_17

Hoare, B. J., Wallen, M. A., Thorley, M. N., Jackman, M. L., Carey, L. M., and Imms, C. (2019). Constraint-induced movement therapy in children with unilateral cerebral palsy. *Cochrane Database Syst. Rev.* 4, CD004149. doi: 10.1002/14651858.CD004149.pub3

Holmström, L., Vollmer, B., Tedroff, K., Islam, M., Persson, J. K., and Kits, A. (2010). Hand function in relation to brain lesions and corticomotor-projection pattern in children with unilateral cerebral palsy. *Dev. Med. Child Neurol.* 52, 145–152. doi: 10.1111/j.1469-8749.2009.03496.x

Ilieva, E., and Ilieva, A. (2020). What is the effect of constraint-induced movement therapy on children with unilateral cerebral palsy? A cochrane review summary with commentary. *Dev. Med. Child Neurol.* 62, 1236–1238. doi: 10.1111/dmcn.14676

Ismail, F. Y., Fatemi, A., and Johnston, M. V. (2017). Cerebral plasticity: windows of opportunity in the developing brain. *Eur. J. Paediatr. Neurol.* 21, 23–48. doi: 10.1016/j.ejpn.2016.07.007

Kirton, A., Andersen, J., Herrero, M., Nettel-Aguirre, A., Carsolio, L., and Damji, O. (2016). Brain stimulation and constraint for perinatal stroke hemiparesis: the PLASTIC CHAMPS Trial. *Neurology* 86, 1659–1667. doi: 10.1212/WNL.0000000000002646

Kirton, A., Chen, R., Friefeld, S., Gunraj, C., Pontigon, A. M., and Deveber, G. (2008). Contralesional repetitive transcranial magnetic stimulation for chronic hemiparesis in subcortical paediatric stroke: a randomised trial. *Lancet Neurol.* 7, 507–513. doi: 10.1016/S1474-4422(08)70096-6

Klomjai, W., Katz, R., and Lackmy-Vallée, A. (2015). Basic principles of transcranial magnetic stimulation (TMS) and repetitive TMS (rTMS). *Ann. Phys. Rehabil. Med.* 58, 208–213. doi: 10.1016/j.rehab.2015.05.005

Lefaucheur, J. P., Aleman, A., Baeken, C., Benninger, D. H., Brunelin, J., Lazzaro, D. V. et al. (2020). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): an update (2014-2018). *Clin. Neurophysiol.* 131, 474–528. doi: 10.1016/j.clinph.2020.02.003

Li, N., Zhou, P., Tang, P., He, H., Fang, L., Zhao, X., et al. (2021). In-depth analysis reveals complex molecular aetiology in a cohort of idiopathic cerebral palsy. *Brain awab*209.

Liu, L. R., Wang, Y. X., He, L., Xu, Y. X., Huang, J. Y., and Peng, T. T. (2021). Constraint-induced movement therapy promotes neural remodeling and functional reorganization by overcoming nogo-A/NgR/RhoA/ROCK signals in hemiplegic cerebral palsy mice. *Neurorehabil. Neural. Repair* 35, 145–157. doi: 10.1177/1545968320981962

Meseguer-Henarejos, A. B., Sánchez-Meca, J., López-Pina, J. A., and Carles-Hernández, R. (2018). Inter- and intra-rater reliability of the Modified Ashworth Scale: a systematic review and meta-analysis. *Eur. J. Phys. Rehabil. Med.* 54, 576–590. doi: 10.23736/S1973-9087.17.04796-7

Novak, I., Morgan, C., Fahey, M., Finch-Edmondson, M., Galea, C., and Hines, A. (2020). State of the evidence traffic lights 2019: systematic review of interventions for preventing and treating children with cerebral palsy. *Curr. Neurol. Neurosci. Rep.* 20, 3. doi: 10.1007/s11910-020-1022-z

Palisano, R. J., Avery, L., Gorter, J. W., and Galuppi, B., McCoy, S. W. (2018). Stability of the gross motor function classification system, manual ability classification system, and communication function classification system. *Dev. Med. Child Neurol.* 60, 1026–1032. doi: 10.1111/dmcn.13903

Parvin, S., Shahrokhi, A., Tafakhori, A., Irani, A., Rasteh, M., and Mirbagheri, M. M. (2018). Therapeutic effects of repetitive transcranial magnetic stimulation on corticospinal tract activities and neuromuscular properties in children with cerebral palsy. *Annu. Int. Conf. IEEE Eng. Med. Biol. Soc.* 2018, 2218–2221. doi: 10.1109/EMBC.2018.8512805

Pino, D. I., Pellegrino, G., and Assenza, G., Capone, F., Ferreri, F., Formica, D., et al. (2014). Modulation of brain plasticity in stroke: a novel model for neurorehabilitation. *Nat. Rev. Neurol.* 10, 597–608. doi: 10.1038/nrneurol.2014.162

Rajak, B. L., Gupta, M., Bhatia, D., and Mukherjee, A. (2019). Increasing number of therapy sessions of repetitive transcranial magnetic stimulation improves motor development by reducing muscle spasticity in cerebral palsy children. *Ann. Indian Acad. Neurol.* 22, 302–307. doi: 10.4103/aian.AIAN_102_18

Reedman, S., Boyd, R. N., and Sakzewski, L. (2017). The efficacy of interventions to increase physical activity participation of children with cerebral palsy: a systematic review and meta-analysis. *Dev. Med. Child Neurol.* 59, 1011–1018. doi: 10.1111/dmcn.13413

Reid, L. B., Rose, S. E., and Boyd, R. N. (2015). Rehabilitation and neuroplasticity in children with unilateral cerebral palsy. *Nat. Rev. Neurol.* 11, 390–400. doi: 10.1038/nrneurol.2015.97

Rich, T. L., Menk, J., Krach, L. E., Feyma, T., and Gillick, B. T. (2016). Repetitive transcranial magnetic stimulation/behavioral intervention clinical trial: long-term follow-up of outcomes in congenital hemiparesis. *J. Child Adolesc. Psychopharmacol.* 26, 598–605. doi: 10.1089/cap.2015.0157

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2009). Safety of TMS Consensus Group. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Senst, S. (2014). Unilaterale spastische Zerebralparese (Hemiparese) [Unilateral spastic cerebral palsy (hemiparesis)]. *Orthopade* 43, 649–655. doi: 10.1007/s00132-013-2219-5

Simon-Martinez, C., Mailleux, L., Jaspers, E., Ortibus, E., Desloovere, K., and Klingels, K. (2020). Effects of combining constraint-induced movement therapy and action-observation training on upper limb kinematics in children with unilateral cerebral palsy: a randomized controlled trial. *Sci. Rep.* 26, 10421. doi: 10.1038/s41598-020-67427-2

Stavsky, M., Mor, O., Mastrolia, S. A., Greenbaum, S., Than, N. G., and Erez, O. (2017). Cerebral palsy-trends in epidemiology and recent development in prenatal mechanisms of disease, treatment, and prevention. *Front. Pediatr.* 5, 21. doi: 10.3389/fped.2017.00021

Veerbeek, J. M., van Wegen, E., van Peppen, R., van der Wees, P. J., and Hendriks, E. (2014). What is the evidence for physical therapy poststroke? A systematic review and meta-analysis. *PLoS ONE* 9, e87987. doi: 10.1371/journal.pone.0087987

Wagner, L. V., Davids, J. R., and Hardin, J. W. (2016). Selective control of the upper extremity scale: validation of a clinical assessment tool for children with hemiplegic cerebral palsy. *Dev. Med. Child Neurol.* 58, 612–617. doi: 10.1111/dmcn.12949

Wang, T. N., Liang, K. J., Liu, Y. C., Shieh, J. Y., and Chen, H. L. (2017). Psychometric and clinimetric properties of the melbourne assessment 2 in children with cerebral palsy. *Arch. Phys. Med. Rehabil.* 98, 1836–1841. doi: 10.1016/j.apmr.2017.01.024

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr. Clin. Neurophysiol.* 108, 1–16. doi: 10.1016/S0168-5597(97)00096-8

Xu, K., Wang, L., Mai, J., and He, L. (2012). Efficacy of constraint-induced movement therapy and electrical stimulation on hand function of children with hemiplegic cerebral palsy: a controlled clinical trial. *Disabil. Rehabil.* 34, 337–346. doi: 10.3109/09638288.2011.607213

Zhang, Z. X., Zuo, Q. H., and Lei, Z. W. (1995). Re-standardization of social life ability scale for Chinese infant-junior school student. *Chin. J. Clin. Psychol.* 3, 12–15.

Zurawski, E., Behm, K., Dunlap, C., Koo, J., Ismail, F., and Boulias, C. (2019). Interrater reliability of the modified

ashworth scale with standardized movement speeds: a pilot study. *Physiother. Can.* 71, 348–354. doi: 10.3138/ptc-2018-0086

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Behavioral Neuroscience

REVIEW
published: 10 May 2022
doi: 10.3389/fnbeh.2022.859151

Research Progress on the Role of Vitamin D in Autism Spectrum Disorder

Jing Wang[1†], Haoyu Huang[1†], Chunming Liu[1], Yangping Zhang[1], Wenjuan Wang[1], Zhuo Zou[1], Lei Yang[1], Xuemei He[1], Jinting Wu[1], Jing Ma[2] and Yun Liu[1*]*

[1] Department of Rehabilitation, Kunming Children's Hospital, Kunming Medical University, Yunnan, China, [2] Department of Otolaryngology, Head and Neck Surgery, Kunming Children's Hospital, Kunming Medical University, Yunnan, China

OPEN ACCESS

Edited by:
Dongdong Qin,
Yunnan University of Chinese Medicine, China

Reviewed by:
Kaishou Xu,
Guangzhou Medical University, China
Wu Shi Hao,
Kunming Institute of Zoology (CAS), China

***Correspondence:**
Jing Ma
majing@etyy.cn
Yun Liu
liuyun@etyy.cn

[†]These authors share first authorship

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: 21 January 2022
Accepted: 08 March 2022
Published: 10 May 2022

Citation:
Wang J, Huang H, Liu C, Zhang Y, Wang W, Zou Z, Yang L, He X, Wu J, Ma J and Liu Y (2022) Research Progress on the Role of Vitamin D in Autism Spectrum Disorder. Front. Behav. Neurosci. 16:859151. doi: 10.3389/fnbeh.2022.859151

Autism spectrum disorder (ASD) is a neurodevelopmental disorder that can lead to severe social behavioral difficulties, which mainly manifests as social communication and interaction disorders; narrow interests; and repetitive, stereotyped behaviors. In recent years, the prevalence of ASD has increased annually, and it has evolved from a rare disease to one with a high incidence among childhood developmental disorders. The pathogenesis of ASD is considered to be the interaction of genetic and environmental factors. There is increasing evidence that vitamin D deficiency in pregnancy and early childhood can lead to the occurrence of ASD. Studies have demonstrated that vitamin D intervention can significantly improve the symptoms of ASD, but the underlying mechanism is still unclear. Therefore, exploring the neuroprotective mechanism of vitamin D against ASD is a huge challenge currently being worked on by current basic and clinical researchers, a task which is of great significance for the clinical promotion and optimization of vitamin D in the treatment of ASD. To further clarify the relationship between vitamin D and ASD, this review summarizes the correlation between vitamin D level and ASD, the effects of vitamin D supplementation on ASD, the possible mechanism of vitamin D involved in ASD, and insights from ASD animal models.

Keywords: autism spectrum disorder, vitamin D, neurodevelopmental disorder, pathogenesis, research progress

INTRODUCTION

Autism spectrum disorder (ASD) is a group of neurodevelopmental disorders characterized by impaired social interaction and communication, repetitive and stereotyped behaviors, limited interests, and abnormalities in sensory processing, generally occurring in early childhood (Lord et al., 2018). The American Psychiatric Association's *Diagnostic and Statistical Manual of Mental Disorders*, fifth edition, refers to childhood autism, uncategorized generalized developmental disorder, and Asperger's syndrome collectively as ASD (Battle, 2013). The etiology and pathogenesis of ASD are unknown, although the interaction of genetic and environmental factors is believed to play a role in the occurrence of ASD. The specific pathogenesis still requires further study (Lord et al., 2020). In recent years, it was determined that vitamin D, besides regulating calcium and

phosphorus metabolism, also has a significant role in fetal and early postnatal brain development. Mounting evidence suggests that vitamin D likely participates in the pathogenesis of ASD, and vitamin D deficiency may be one of the causes of ASD (Eissa et al., 2018). Meanwhile, some studies have illustrated the fact that vitamin D can improve the core symptoms of ASD in children (Wang et al., 2020). This review discusses the correlation between vitamin D level and ASD, the treatment effects of vitamin D on the symptoms of ASD, the possible mechanism of vitamin D's involvement in ASD, and the current insights available from ASD animal models.

AUTISM SPECTRUM DISORDER

The Epidemiology of Autism Spectrum Disorder

ASD was first reported in 1943 by Leo Kanner, an American physician, who referred to it as "early infantile autism" (Harris, 2018). ASD was once considered an infrequent disease; nevertheless, the prevalence of ASD has risen dramatically in the last several decades in various countries (Zablotsky et al., 2015). The World Health Organization pointed out that ASD has become one of the most rapidly growing severe diseases in the world, and it is a major public concern that seriously affects the health of children (Lai et al., 2014; Sandin et al., 2017). The U.S. Centers for Disease Control and Prevention reported that the prevalence of autism among 8-year-old children in the United States in 2016 was 1/54, with a 4.3:1 ratio of males to females (Maenner et al., 2020). Surveys in other countries have shown that the prevalence of ASD has increased over time (Lai et al., 2019). Although there are no national investigation data on the epidemic of ASD in China, a multicenter study reported that the prevalence of ASD among those 6–10 years of age is about 1% in China, suggesting a rising tendency (Sun et al., 2019). However, the reason for such a jump has not been fully recognized. ASD is more common in urban than rural areas, and urban areas are associated more closely with cloudy and rainy weather, less ultraviolet B (UVB) exposure, and greater air pollution (Cannell, 2017). These factors reduce ultraviolet radiation at the surface and vitamin D production in the skin, consistent with the etiological hypothesis that vitamin D deficiency might contribute to ASD (Cannell, 2008; Wai et al., 2015).

The Etiology and Pathogenesis of Autism Spectrum Disorder

The etiology and pathogenesis of ASD are currently unclear. Since the 1980s, research on autism has entered a novel stage, and researchers began to abandon the hypothesis of the cause being so-called "improper parental care." Medical workers first tried to identify the cause of ASD in the field of biology (Campisi et al., 2018). With the deepening of investigations on ASD, however, we have come to know that ASD is a complex neurodevelopmental disorder caused by the interaction of genetic and environmental factors (Steinman, 2020). Studies have shown that > 1,000 genes are related to ASD. The comorbidity rate of ASD in identical twins is also significantly higher than that in fraternal twins, and some immediate family members of ASD patients have clinical symptoms similar to ASD even if they themselves have not been diagnosed with ASD, such as social and communication disorders, stereotyped behaviors, etc. These findings all suggest that genetic factors play an important role in the pathogenesis of ASD (Famitafreshi and Karimian, 2018; Bölte et al., 2019). However, only 25–30% of ASD children have detectable ASD-related genes, and nearly 70% of cases have a cause that does not involve genetics (Famitafreshi and Karimian, 2018). Therefore, the role of environmental factors in the pathogenesis of ASD cannot be overlooked (Modabbernia et al., 2017; Uçar et al., 2020). Lifestyle and environmental factors, such as nutrition (Bala et al., 2016), medications (Bromley, 2016; Kaplan et al., 2016), toxic substances (Kardas et al., 2016; Skalny et al., 2016), maternal infections during pregnancy (Jiang et al., 2016; Bilbo et al., 2018), stress, and vaccine immunization, have been extensively studied and found to be associated with ASD (Wang et al., 2016). About 1/3 of ASD children have significantly increased serotonin (5-hydroxytryptamine [5-HT]) levels in peripheral blood, and 5-HT reuptake inhibitors can improve the emotional symptoms and repetitive, stereotyped behaviors of ASD patients (Abdulamir et al., 2018). The rise in dopamine levels in the hypothalamus can induce stereotyped behaviors in ASD children, while dopamine-blocking drugs can reduce the stereotyping of ASD children (Kirsten and Bernardi, 2017). Meanwhile, ASD children have abnormal electroencephalographs, brain structure, and brain function (Gibbard et al., 2018). Studies have found that the frontal, parietal, and occipital cortices of ASD patients are thinner. ASD patients also have brain network–connection disorders, and the functional connections between the frontal and temporal cortices of the brain and other brain regions are reduced, resulting in less information transmission (Naaijen et al., 2018; Pagnozzi et al., 2018). Immunological studies have illustrated that the numbers of white blood cells and $CD38^+$ B-cells and the levels of HLA-DR (the cell-activation marker of $CD8^+$ and $CD4^+$ T-cells) are increased, suggesting that there is an imbalance in immunity, in ASD children (Kim et al., 2017). The currently applied maternal immune-activation (MIA) model is a type of ASD model that activates the immune system during pregnancy and leads to disease in the offspring. Pregnant rats were injected with double-stranded polyinosine:cytosine at 9.5 days of gestation, and their offspring showed ASD-like behaviors (Meltzer and Van de Water, 2017). Cannell's epidemiological survey results revealed that the prevalence of ASD was higher in areas with greater air pollution, urban areas, and those at higher altitudes. These areas have less UVB light, which results in a deficiency of vitamin D. Using these findings, combined with the impact of vitamin D on brain development, the etiological hypothesis that vitamin D deficiency might result in ASD was first proposed (Cannell, 2008). In particular, the factors that cause ASD—such as air pollution, cloudy weather, seasonal factors, migration of dark-skinned immigrants to poleward latitudes, birth order, gestational diabetes, preeclampsia, cesarean delivery, autoimmune disease in the family, and nutrition—are all associated with a deficiency of vitamin D (Alzghoul, 2019).

AUTISM SPECTRUM DISORDER AND VITAMIN D

Vitamin D

Vitamin D, a fat-soluble vitamin, is a general title for a collection of steroid-like substances, including ergocalciferol and cholecalciferol (D_3; Grant, 2016). Vitamin D is an essential nutrient for the human body. Vitamin D_3 is synthesized in the skin by the reaction of 7-dehydrocholesterol with UVB radiation (Bivona et al., 2019). It mainly comes from skin exposure to UVB radiation (Landel et al., 2018). Subsequently, it undergoes 2-step hydroxylation in the liver and kidneys to first form 1,25(OH)D, then $1,25(OH)_2D_3$, which binds to vitamin D receptors (VDRs) and exerts a biological effect. Because $1,25(OH)_2D_3$ synthesis is tightly regulated, 25-(OH)D in serum has been suggested to be the best single indicator of vitamin D status. It has been thought that the main role of vitamin D is to regulate calcium and phosphorus metabolism, thus affecting bone growth and development (García-Serna and Morales, 2020). Current studies have found that 1α-hydroxylase, the key enzyme for vitamin D synthesis, and VDRs are widely present in brain tissue, and vitamin D plays a crucial role in brain development (Zhou et al., 2018). Vitamin D has important effects on brain development and function, including neuronal differentiation, proliferation, and apoptosis; regulates synaptic plasticity and the dopaminergic system; and reduces the oxidative burden (Karras et al., 2018). Studies have found that vitamin D_3 can also promote the development of regulatory T-cells and inhibit an excessive immune response and autoimmune reactions (Mak, 2018). In addition, vitamin D plays an important role in the regulation of gene expression. One study revealed that 223 ASD risk genes in the SFARI database were vitamin D_3–sensitive genes, which means that these ASD-relevant genes might be regulated by vitamin D (Trifonova et al., 2019).

The Correlation Between Vitamin D Level and Autism Spectrum Disorder

Peripheral Blood Vitamin D Levels in Children With Autism Spectrum Disorder

Since Cannell proposed the hypothesis that vitamin D deficiency may contribute to ASD, an increasing number of researchers have begun to assess changes in serum vitamin D levels among ASD children. A great number of studies investigating the vitamin D status of children and adolescents with ASD from different countries and races reported that ASD children and adolescents had lower vitamin D levels (Fahmy et al., 2016; Basheer et al., 2017; Cieśliñska et al., 2017; Desoky et al., 2017; Garipardic et al., 2017; Altun et al., 2018; Arastoo et al., 2018). Arastoo et al. analyzed the vitamin D levels of 31 ASD children and 31 healthy children and found that 96.8% of the ASD children were deficient in vitamin D (Arastoo et al., 2018). The level of 25-(OH)D in ASD children was significantly lower than that of the control group, and the Social Response Scale (SRS) scores of ASD children with vitamin D deficiency were significantly higher than those of ASD children with normal vitamin D levels (Dong et al., 2017; Guo et al., 2019). Compared to the healthy children, they found that the level of vitamin D in ASD children was lower than that in the control group, and the level of vitamin D was significantly negatively correlated with the total scores of the Autism Behavior Checklist (ABC), Childhood Auditing Scale (CARS), SRS, Autism Treatment Evaluation Checklist (ATEC), behavioral energy zone, and the ATEC social energy zone, indicating that the lower the vitamin D level, the more severe the core symptoms of ASD were. Recently, a meta-analysis of 24 case–control studies demonstrated that children and adolescents with ASD had significantly lower vitamin D concentrations than those of participants in the control group (mean difference, −7.46 ng/mL; 95% confidence interval, −10.26 to −4.66 ng/mL; $p < 0.0001$; $I^2 = 98\%$; Wang et al., 2020).

The Maternal Vitamin D Level During Pregnancy

A higher prenatal 25-(OH)D level exerts a positive influence on the cognitive development of the offspring, and the 25-(OH)D level in early pregnancy may have a stronger influence on the offspring's neurodevelopment relative to that in the late period (García-Serna and Morales, 2020). Diverse studies have investigated the impact of prenatal exposure to vitamin D on brain development. A systematic review and meta-analysis published in 2019 summarized evidence of the association between 25-(OH)D levels in maternal blood in pregnancy or newborn blood at birth and neurodevelopmental outcomes and found that children with a low prenatal 25-(OH)D (<20 ng/mL) level exhibited more ASD-related symptoms, greater behavioral difficulties, and less social skills at 5 years of age (López-Vicente et al., 2019). In a study of 4,229 children, researchers detected maternal vitamin D levels in the second trimester and at birth, then used SRS to assess ASD-like symptoms in the children until 6 years of age, ultimately finding that vitamin D deficiency during pregnancy increased the risk of ASD-like symptoms in childhood (Vinkhuyzen et al., 2018). Another study demonstrated that mothers in the ASD group had significantly lower maternal serum levels of 25-(OH)D than those in the neurotypical group, with 55.9 and 29.4% of mothers being vitamin D–deficient, respectively (Chen et al., 2016). Lower first-trimester maternal serum levels of 25-(OH)D are associated with a significantly increased risk of ASD development in offspring ($p < 0.001$; Vinkhuyzen et al., 2018). Researchers have also illustrated that decreased vitamin D levels during pregnancy and decreased exposure to solar UVB might increase the risk of ASD (Wang et al., 2016). In 2020, a systematic review found that low vitamin D levels might lead to the development of ASD (Famitafreshi and Karimian, 2018).

Vitamin D Level in the Peripheral Blood of Autism Spectrum Disorder Rats

Clinical studies have shown that the use of valproate acid (VPA) during pregnancy is a risk factor for children to suffer from ASD (Wood et al., 2015). The valproic acid (VPA) rat model is a common ASD animal model. The offspring of rats exposed to VPA *in utero* by an intraperitoneal injection of 600 mg/kg of VPA at 12.5 days of gestation showed symptoms consistent with those of ASD children. Researchers tested the level of 25-(OH)D in the peripheral blood of ASD rats treated by VPA at birth and

21 days after birth and recorded a persistent vitamin D deficiency from birth to 21 days after birth, consistent with measurements of the peripheral blood of ASD children (Kim et al., 2013; Selim and Al-Ayadhi, 2013; Ahn et al., 2014). However, this study could not prove that the symptoms of ASD rats were caused by vitamin D deficiency. Vitamin D deficiency in animals causes brain structural and functional alterations similar to those found in humans with ASD. A severe vitamin D deficiency during pregnancy in rats can cause pathological changes, such as brain volume enlargement and ventricular enlargement, and can affect neuron differentiation and axonal connection (Principi and Esposito, 2019). The behaviors of offspring born to vitamin D–deficient animals are similar to those of young children with ASD. An animal model of the development of vitamin D (DVD) deficiency has been proven to reproduce the phenotype related to ASD in the domain of neuroanatomy (Ali et al., 2019). Meanwhile, some studies have reported that vitamin D has preventive or therapeutic effects in ASD rats induced by propionic acid (Alfawaz et al., 2014).

THE IMPACT OF VITAMIN D ADMINISTRATION ON SYMPTOMS OF AUTISM SPECTRUM DISORDER

The Preventive Effects of Vitamin D on Autism Spectrum Disorder

A systematic review and meta-analysis published in 2019 summarized evidence of the association between 25-(OH)D levels in maternal blood during pregnancy or newborn blood at birth and neurodevelopmental consequences (García-Serna and Morales, 2020). The meta-analysis offered evidence that prenatal exposure to increased 25-(OH)D levels is associated with improved cognitive development and a reduced risk of attention-deficit/hyperactivity disorder and ASD-related traits later in life (Stubbs et al., 2016). Researchers enrolled mothers who had given birth to ASD children already and provided them with 5,000 IU of supplemental vitamin D daily during subsequent pregnancies. Then, after delivery, each mother was given 7,000 IU of supplemental vitamin D daily during lactation or 1,000 IU of supplemental vitamin D daily if their child was not breastfed until they reached 1 year of age. The study investigators found that the ASD prevalence of these children was reduced to 1/4 (5 vs. 20%) compared to reports in the literature. Nevertheless, some scholars believe that this result needs to be carefully considered because this study was uncontrolled and included an exceedingly low number of pregnant women with varied durations of vitamin D supplementation; they argue that the current data do not support the hypothesis that vitamin D supplementation during pregnancy can prevent the development of ASD (Principi and Esposito, 2019).

The Therapeutic Effect of Vitamin D on Autism Spectrum Disorder

Quite a few studies have also demonstrated that vitamin D can help to improve symptoms of ASD children. The first report of the use of vitamin D_3 for ASD treatment dates to 2014 wherein a 32-month-old boy with ASD and vitamin D deficiency was administered 150,000 IU of vitamin D_3 intramuscularly every month and 400 IU/day orally for 2 months and showed improvements in ASD core symptoms in a transitory amount of time (Jia et al., 2015). In 2015, a randomized controlled trial reported that the CARS scores and social intelligence quotients of ASD children were better than those in the control group after 3 months of supplementation with vitamin D (Azzam et al., 2015). A recent clinical study found that daily high-dose vitamin D (300 U/kg·d) supplementation significantly improved the core symptoms of ASD children as mainly reflected in the CARS score, stereotypes, and greater eye contact and attention duration (Saad et al., 2019). During a long-term follow-up study of vitamin D treatment for ASD children, in 37 children with ASD [25-(OH)D $<$ 75 nm/L] who were supplemented with vitamin D for 3 months, ASD symptoms were significantly improved when assessed using the ABC and CARS scores (Feng et al., 2017). One study found that early intervention with vitamin D could improve the growth, development, and behavioral performance of ASD rats, and vitamin D had a therapeutic effect on ASD rats. On the contrary, vitamin D_3 supplementation was reported to have no effect in a double-blind, randomized, placebo-controlled trial in which 38 children (mean age, 7.1 years) were enrolled (Kerley et al., 2017). Among them, 18 were given vitamin D_3 (2,000 IU/day for 20 weeks) and 20 received placebo therapy. Serum 25-(OH)D_3 levels were measured before vitamin D_3 administration and at the end of the study period, and ASD symptoms were evaluated before and after supplementation by parents and clinicians using the ABC, SRS, and Developmental Disabilities—Children's Global Assessment Scale (DD-CGAS). Although the treatment group showed a significant increase in serum 25-(OH)D_3 concentrations (23.4 vs. 34.4 ng/mL) compared to patients receiving placebo therapy (20.7 vs. 20.2 ng/mL; $p = 0.0016$), no improvements in scores of the SRS, DD-CGAS, or 5 ABC subscales were recorded. Similarly, some scholars have found that oral 2,000 U/d of vitamin D_3 can increase vitamin D levels in children with ASD, but their symptoms of ASD did not improve. Researchers believe that, in order to determine whether ASD symptoms have improved due to vitamin D supplementation given to alleviate insufficient vitamin D levels, it is essential to further increase the vitamin D supplement dose (Principi and Esposito, 2019).

THE POSSIBLE MECHANISM OF VITAMIN D INVOLVED IN AUTISM SPECTRUM DISORDER

It has been hypothesized that ASD is a combination of both organ-specific physiologic and systematic abnormalities, such as gene mutations, oxidative stress, an impaired detoxification system, inflammation, immune dysregulation, abnormal neurotrophic factor and neurotransmitter levels, and seizures—at least, in a subset of individuals with ASD (Rossignol and Frye, 2014). Mounting evidence suggests that low vitamin D levels are involved in the etiology of the aforementioned abnormalities (Groves et al., 2014).

Vitamin D and Gene Mutations

ASD is partly genetically derived (Sandin et al., 2014). A study linking vitamin D metabolic gene variants to ASD risk illustrated that the risk for ASD was increased in children inheriting the AA genotype of the *GC* gene (vitamin D–binding protein), the GG genotype of the *CYP2R1* gene (a catalyst enzyme involved in the transformation of vitamin D to 25-(OH)D), and the paternal Taq l and Bsml genotypes of the *VDR* gene, highlighting the possible etiological role of low vitamin D levels in ASD (Schmidt et al., 2015). Current genetic studies on ASD have found that there are multiple neonatal mutations in affected children (De Rubeis et al., 2014). Vitamin D can perform DNA-repair and -maintenance functions through a variety of mechanisms. At present, it has been confirmed that > 5 vitamin D–dependent genes encode DNA-repair proteins as full-time DNA mutation–repair proteins (Fleet et al., 2012). In addition, studies have found that, when the vitamin D level is reduced, the DNA repair enzyme poly(adenosine diphosphate ribose) polymerase tends to overreact and damage neighboring DNA, and daily supplementation with minor doses of vitamin D_3 can increase Bax levels, promote apoptosis, and prevent gene mutations (Fedirko et al., 2009). Studies have found that Growth arrest and DNA-damage-inducible alpha, p53, RAD23 homolog B, Proliferating cell nuclear antigen, Poly(adenosine diphosphate ribose) and polymerase Death associated protein 1α (Trifonova et al., 2019), which are closely related to vitamin D, have the function of repairing DNA damage (Fedirko et al., 2009; Fleet et al., 2012; De Rubeis et al., 2014) (**Table 1**). Therefore, it is speculated that vitamin D deficiencies may cause novel gene mutations in children with ASD, and the multiple novel mutations currently found in children with ASD may be the result of vitamin D deficiencies rather than a pathogenic factor of ASD (Shan et al., 2016; Siracusano et al., 2020).

Vitamin D Deficiency Leads to Excessive Proliferation of Neuronal Cells

Early brain overgrowth is considered an important neuropathological feature of ASD. Magnetic resonance imaging studies have revealed that the brain volumes of children with ASD are greater than those of normal children. At the cellular level, the expansion of the ASD brain involves quite a few neurons in the anterior prefrontal and dorsolateral prefrontal cortices (Hazlett et al., 2011). Studies have found that vitamin D can inhibit cell proliferation by inducing the proliferation of the cyclin-dependent kinase inhibitors p21 and p27. It can also inhibit cell proliferation by inhibiting the expression of other proteins required for the cell cycle, such as proliferating cell nuclear antigen and cyclin D1 (Marini et al., 2010). An animal model of developmental vitamin D deficiency has been proven to reproduce the phenotype associated with ASD in the field of neuroanatomy (Ali et al., 2018). Therefore, when the vitamin D level is deficient, neuronal cells proliferate excessively, leading to overgrowth of the brain in the early stages of development, which may correlate with the occurrence of ASD.

Vitamin D and Neurotransmitters

Multiple lines of evidence suggest an involvement of dysregulated neurotransmitter systems (serotonergic, oxytocinergic, and dopaminergic systems) in ASD. These systems play key roles in neurotransmission, brain maturation, cortical organization, and behavior (including social and repetitive behaviors; Staal et al., 2012). Vitamin D-associated neurotransmitters regulates learning, memory, and emotions (Staal et al., 2012; Patrick and Ames, 2014; Pertile et al., 2018) (**Table 2**). A deficiency of the inhibitory neurotransmitter γ–aminobutyric acid (GABA) in the brain is associated with ASD. Studies have found that long-term treatment of rodents with vitamin D can promote the synthesis of GABA in brain tissues, such as the prefrontal cortex, anterior cingulate cortex, and hippocampus (Staal et al., 2012). Vitamin D influences the synthesis and metabolism of dopamine and the expression of glial cell line–derived neurotropor (GDNF). GDNF is crucial to the survival of dopaminergic neurons, and the absence of vitamin D may be involved in dopamine signal transduction (Pertile et al., 2018). Lower levels of plasma oxytocin and abnormal serotonin concentrations in the brain and tissues outside the blood–brain barrier have been reported in populations with ASD (Patrick and Ames, 2014). While the binding of brain serotonin transporter was significantly lower in high-functioning ASD adults than healthy controls, the binding of brain dopamine transporter was significantly higher in ASD patients (Nakamura et al., 2010). Studies have shown that the concentration of 5-HT in the brains of ASD patients is lower and that of 5-HT in the peripheral blood is higher (Patrick and Ames, 2014). Vitamin D can increase the expression of tyrosine hydroxylase, which is involved in the synthesis of dopamine; vitamin D can also increase the transcription of

TABLE 1 | Vitamin D and gene-mutation repair (Fedirko et al., 2009; Fleet et al., 2012; De Rubeis et al., 2014).

Repair proteins/enzymes	Function
Growth arrest and DNA-damage-inducible alpha	DNA-damage repair
p53	DNA-damage repair
RAD23 homolog B	DNA-damage repair
Proliferating cell nuclear antigen	DNA-damage repair
Death-associated protein 1α (Trifonova et al., 2019)	DNA-damage repair
Poly(adenosine diphosphate ribose) polymerase	DNA-damage repair
Bax	Promoting apoptosis and preventing gene mutations

TABLE 2 | Vitamin D–associated neurotransmitters (Staal et al., 2012; Patrick and Ames, 2014; Pertile et al., 2018).

Neurotransmitter	Function
5-hydroxytryptamine	Regulating emotions in social decision-making
Oxytocin	Improving social skills
Seronine	Promote pro-social behavior and assess emotions
Dopamine	Motor control, reward motivation, emotional regulation, and social interaction
γ-aminobutyric acid	Involved in brain cognition, learning, and memory

tryptophan hydroxylase 2, which promotes the synthesis of 5-HT synthetase. 5-HT is a monoamine neurotransmitter, which plays a significant role in regulating emotions in social decision-making. At the same time, a proper amount of vitamin D can inhibit the transcription of tryptophan hydroxylase 1 in peripheral tissues, thus explaining the serotonin paradox in ASD in which peripheral serotonin is increased but central serotonin is decreased (Patrick and Ames, 2014).

The Immunomodulatory Effects of Vitamin D

Studies have demonstrated that immune-activation may be a risk factor for ASD. A lack of vitamin D may alter the immune responses of patients with ASD, and vitamin D may prevent ASD-related behavior dilemmas induced by immune activation (Nakamura et al., 2010). Studies have shown that patients with ASD have higher levels of autoimmune markers, such as anti-nuclear antibodies, anti-ganglioside M1 antibodies, anti-MPB autoantibodies, and anti-nucleosome–specific antibodies. Some studies have shown that the levels of these markers are significantly positively correlated with the severity of ASD (Wang et al., 2016). Vitamin D exerts an immunomodulatory effect through helper T-cells and $CD4^+CD25^+$ regulatory T-cells, and regulatory T-cells prevent autoimmunity by inhibiting Th17 cells (Chambers and Hawrylowicz, 2011). Some scholars have found that vitamin D supplementation can increase the proportion of regulatory T-cells in the body, upregulate the production of dendritic cells, and upregulate interleukin (IL)-10, thereby reducing the intensity of autoimmune attacks, inhibiting damage to tissues by immune cells, and reducing the severity of autoimmune diseases (Saad et al., 2018). Mostafa et al. found that 70% of children with ASD had higher anti-myelin–associated glycoprotein (anti-MAG) levels, and research suggests that serum 25-(OH)D) levels are significantly negatively correlated with anti-MAG levels (Mostafa and Al-Ayadhi, 2012). As the level of anti-MAG correlates with the severity of ASD, this finding suggests that the 25-(OH)D) deficiency in some ASD children is likely a factor that promotes increased anti-MAG levels, and anti-MAG may play a role in brain damage in children with ASD. Vitamin D also modifies the expression of several genes involved in axogenesis and myelination (Ritterhouse et al., 2011). These findings suggest that vitamin D plays a crucial role in auto-antibody production and ASD pathogenesis, perhaps being similar in its manner to other autoimmune diseases like multiple sclerosis and systemic lupus erythematosus (Mazahery et al., 2016).

The Anti-inflammatory Effects of Vitamin D

Current studies have found that ASD is an inflammation-related disease (Basheer et al., 2017; Cieśliñska et al., 2017). Some studies contend that vitamin D has an immunomodulatory effect, which can enhance the protective immune response and reduce the inflammatory response (El-Sharkawy and Malki, 2020). Vitamin D has different anti-inflammatory effects on the brain, including decreasing harmful inflammatory cytokines and neuro-inflammation caused by oxidants and toxins. ASD individuals have immune function abnormalities similar to those of individuals afflicted by vitamin D deficiency, such as increased inflammatory cytokine levels (Cannell, 2017). Evidence suggests that children with ASD have elevated levels of pro-inflammatory cytokines, including IL-6, tumor necrosis factor alpha (TNF-α), and interferon-γ, in different tissues (Napolioni et al., 2013). When elevated, it is strongly associated with cognitive impairment in ASD (Krakowiak et al., 2017). Vitamin D metabolites have been shown to decrease the secretion of IL-6 and TNF-α, enhance the expression of anti-inflammatory cytokines such as interleukin 10 (IL-10) from activated B-cells, and direct dendritic cells toward a more tolerogenic state. The activation of vitamin D hormone (calcitriol) protects brain tissue by reducing inflammatory cytokine levels (Mazahery et al., 2016).

The Anti-oxidative Effects of Vitamin D

At present, there are several lines of evidence indicating oxidative stress and mitochondrial dysfunction are prevalent in ASD, and oxidative stress may be a general feature of ASD (Giulivi et al., 2010). Antioxidants, especially glutathione, are the key to early neural survival. Elevated levels of oxidative stress in the brain can damage or interfere with brain development and result in ASD-like symptoms (Jia et al., 2018). It has been found that the concentration of oxidized glutathione in the plasma of ASD children is increased, and the concentration of oxidative stress in these individuals is similarly increased as a result of ASD (James et al., 2006). Vitamin D has an antioxidant effect and can inhibit the synthesis of nitric oxide synthase, upregulate glutathione, reduce glial cell activation and neuro-inflammation, and play a significant role in neuroprotection and neuromodulation (DeLuca, 2016). It has also been reported that vitamin D can directly upregulate certain antioxidant-related genes (such as those that encode superoxide dismutase and thioredoxin reductase; Halicka et al., 2012). Existing evidence also reveals that 25-(OH)D concentrations correlate significantly positively with glutathione levels in healthy adult populations (Alvarez et al., 2014). Therefore, it is believed that vitamin D supplementation can reduce the level of oxidative stress and play a protective role in the brain.

INSIGHTS FROM AUTISM SPECTRUM DISORDER ANIMAL MODELS

Animal models provide advantages over human research due to their controllability, availability, and predictability. They play a crucial role in exploring the etiology and pathogenesis of diseases. The animal model of ASD has become a key platform by which to explore the relationship between vitamin D and ASD. At present, the understanding of ASD and its animal models in medical circles domestic and abroad is extremely limited, and existing ASD animal models may be categorized in three ways: genetic animal models (Südhof, 2017; Dadalko and Travers, 2018; Nakai et al., 2018), VPA-exposure models (Barrett et al., 2017), and MIA models (Kim et al., 2017). Although researchers have reached a consensus on the close relationship between genetic factors and ASD, definite genetic evidence

has been found in only 25–30% of ASD cases using existing technical methods. In such cases, the factors at play usually involve chromosome rearrangement and gene copy number variations or point mutation (De Rubeis et al., 2014). The gene models currently in use domestically and abroad include a neuroligins gene model, neurexins gene model, SH3 and multiple ankyrin repeat domains protein 3 gene model, methyl-CpG–binding protein 2 gene model, fragile X mental retardation 1 gene model, and tuberous sclerosis complex 1/2 gene model (Südhof, 2017; Dadalko and Travers, 2018; Nakai et al., 2018). These genes are all found in ASD individuals and have been verified by gene-knockout animal models. Strong evidence of the genetic heritability of ASD is also a key gene for studying gene target regulation. Gene-abnormality models have been created for specific gene deletions and research purposes, and they have specific applications. Regulated environmental factor ASD models include the VPA-exposure model (Barrett et al., 2017), MIA model (Kim et al., 2017), and maternal auto-antibody model (Martínez-Cerdeño et al., 2016). Offspring mice exposed to VPA during pregnancy exhibit typical behavioral performances similar to those of children with ASD, consistent with the current research results of brain structure and function damage and changes in brain transmitters. This model is also the most commonly employed animal model in China (Caspers et al., 2014). To induce the MIA model, pregnant female mice are exposed to polyinosine:cytosine, lipid polysaccharides, simulated viruses, bacterial infections, and other environments to activate the maternal immune system (Boksa, 2010). Studies have found that offspring in the MIA model have behaviors similar to those of children with clinical ASD, which mainly manifest as social impairment and increased repetitive, stereotyped behaviors (Wong and Hoeffer, 2018). At present, several mouse and rat strains have been selected worldwide through behavioral methods that can better simulate the core symptoms of ASD and may be considered to be models of idiopathic ASD; primary examples include the inbred line BTBR-T^{+}tf/J mouse model and the inbred line BALB/cByJ mouse model (Chang et al., 2018). Current animal studies of vitamin D and ASD mainly assess the level of vitamin D in the serum and the therapeutic and preventive effects of vitamin D on animal ASD models. Although studies have found lower levels of vitamin D in the peripheral blood of ASD rats, there is currently no animal model of ASD caused by vitamin D deficiency (Kim et al., 2013; Selim and Al-Ayadhi, 2013; Ahn et al., 2014). To further investigate the relationship between vitamin D deficiency and ASD, the behavior of offspring of vitamin D-deficient animals should be studied in the future (Ali et al., 2019). Research has found that serum vitamin D levels of ASD rats were significantly lower than those of normal rats, and the behavior of ASD ratwas aggravated with a reduction in serum vitamin D level in the later developmental stage. At the same time, it was found that a vitamin D intervention could promote the growth and development of ASD rats and improve their ASD-like behaviors. Currently, animals used for vitamin D deficiency disease research include pigs, dogs, rats, and mice. Research on diseases associated with vitamin D deficiency has shifted from rickets to immune, tumor, cardiovascular, and other diseases (Cannell, 2017) and has gradually progressed to cellular and molecular levels. A vitamin D deficiency model constructed by researchers suggests that maternal vitamin D deficiency is one of the important factors that causes embryonic development delay in mice after pregnancy. Nevertheless, whether this embryonic developmental delay can cause ASD in humans remains to be further studied.

OUTLOOK

The high incidence of ASD has made it a social problem that urgently requires solutions, but the cause of the disease is still unknown. ASD is mostly considered to be the result of a combination of genetic and environmental factors, but current findings concerning the etiology of genetic factors and environmental factors cannot reasonably explain the epidemiological characteristics of ASD, and clinical drug treatments based on various existing pathogenesis have not achieved recognized clinical efficacy. Therefore, it is necessary to explore the etiology and pathogenesis of ASD from a new perspective to provide novel ideas for the treatment of ASD. Low vitamin D levels *in utero*, postnatal, and in early childhood have been hypothesized to be a risk factor for neurodevelopmental disorders, particularly ASD. Animal and human cellular, biological, and physiologic studies have provided compelling evidence for numerous roles of vitamin D in various body processes, some of which are involved in the pathobiology of ASD. Some researchers have found that children with ASD and vitamin D deficiency experience improvements in their core symptoms with an increase in vitamin D levels; however, due to different methods, fewer interventional experiments, and inconsistent results, there is no consensus on the therapeutic effect of vitamin D in ASD, so it is necessary to further implement large-sample, randomized double-blind trials in the future. Progress in understanding the etiology and pathogenesis of vitamin D and ASD requires the conduct of a large number of rigorous scientific experiments. At the same time, we can observe vitamin D levels in ASD animal models and further study the preventive and therapeutic effects and mechanism of vitamin D in this context. If the relationship between vitamin D and ASD is clarified by further research, it would open up a simple, cheap, and safe new path for the prevention and treatment of ASD.

AUTHOR CONTRIBUTIONS

JWa, JM, and YL organized this study, reviewed data, and finalized this manuscript. JWa, HH, and ZZ performed the literature search and data analysis. JWu, WW, and CL performed literature management. JWu, YL, XH, and YZ performed literature review and drafted the manuscript. All authors read and approved the final manuscript.

FUNDING

This study was supported by the Kunming Medical University Joint Project-the Science and Technology Planning Project of the Yunnan Provincial Department of Science and Technology (No. 202101AY070001-215).

REFERENCES

Abdulamir, H. A., Abdul-Rasheed, O. F., and Abdulghani, E. A. (2018). Serotonin and serotonin transporter levels in autistic children. *Saudi Med. J.* 39, 487–494. doi: 10.15537/smj.2018.5.21751

Ahn, Y., Narous, M., Tobias, R., Rho, J. M., and Mychasiuk, R. (2014). The ketogenic diet modifies social and metabolic alterations identified in the prenatal valproic acid model of autism spectrum disorder. *Dev. Neurosci.* 36, 371–380. doi: 10.1159/000362645

Alfawaz, H. A., Bhat, R. S., Al-Ayadhi, L., and El-Ansary, A. K. (2014). Protective and restorative potency of vitamin D on persistent biochemical autistic features induced in propionic acid-intoxicated rat pups. *BMC Complement. Altern. Med.* 14:416. doi: 10.1186/1472-6882-14-416

Ali, A., Cui, X., and Eyles, D. (2018). Developmental vitamin D deficiency and autism: putative pathogenic mechanisms. *J. Steroid Biochem. Mol. Biol.* 175, 108–118. doi: 10.1016/j.jsbmb.2016.12.018

Ali, A., Vasileva, S., Langguth, M., Alexander, S., Cui, X., Whitehouse, A., et al. (2019). Developmental vitamin D deficiency produces behavioral phenotypes of relevance to autism in an animal model. *Nutrients* 11:1187. doi: 10.3390/nu11051187

Altun, H., Kurutaş, E. B., Şahin, N., Güngör, O., and Fındıklı, E. (2018). The levels of vitamin D, vitamin D receptor, homocysteine and complex B vitamin in children with autism spectrum disorders. *Clin. Psychopharmacol. Neurosci.* 16, 383–390. doi: 10.9758/cpn.2018.16.4.383

Alvarez, J. A., Chowdhury, R., Jones, D. P., Martin, G. S., Brigham, K. L., Binongo, J. N., et al. (2014). Vitamin D status is independently associated with plasma glutathione and cysteine thiol/disulphide redox status in adults. *Clin. Endocrinol.* 81, 458–466. doi: 10.1111/cen.12449

Alzghoul, L. (2019). Role of vitamin D in autism spectrum disorder. *Curr. Pharm. Des.* 25, 4357–4367. doi: 10.2174/1381612825666191122092215

Arastoo, A. A., Khojastehkia, H., Rahimi, Z., Khafaie, M. A., Hosseini, S. A., Mansouri, M. T., et al. (2018). Evaluation of serum 25-hydroxy vitamin D levels in children with autism spectrum disorder. *Ital. J. Pediatr.* 44:150. doi: 10.1186/s13052-018-0587-5

Azzam, H. M. E., Sayyah, H., Youssef, S., Lotfy, H., Abdelhamid, I. A., Abd Elhamed, H. A., et al. (2015). Autism and vitamin D. *Middle East Curr. Psychiatry* 22, 9–14. doi: 10.1097/01.Xme.0000457269.05570.78

Bala, K. A., Doğan, M., Kaba, S., Mutluer, T., Aslan, O., and Doğan, S. Z. (2016). Hormone disorder and vitamin deficiency in attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders (ASDs). *J. Pediatr. Endocrinol. Metab.* 29, 1077–1082. doi: 10.1515/jpem-2015-0473

Barrett, C. E., Hennessey, T. M., Gordon, K. M., Ryan, S. J., McNair, M. L., Ressler, K. J., et al. (2017). Developmental disruption of amygdala transcriptome and socioemotional behavior in rats exposed to valproic acid prenatally. *Mol. Autism* 8:42. doi: 10.1186/s13229-017-0160-x

Basheer, S., Natarajan, A., van Amelsvoort, T., Venkataswamy, M. M., Ravi, V., Srinath, S., et al. (2017). Vitamin D status of children with autism spectrum disorder: case-control study from India. *Asian J. Psychiatry* 30, 200–201. doi: 10.1016/j.ajp.2017.10.031

Battle, D. E. (2013). Diagnostic and statistical manual of mental disorders (DSM). *Codas* 25, 191–192. doi: 10.1590/s2317-17822013000200017

Bilbo, S. D., Block, C. L., Bolton, J. L., Hanamsagar, R., and Tran, P. K. (2018). Beyond infection - maternal immune activation by environmental factors, microglial development, and relevance for autism spectrum disorders. *Exp. Neurol.* 299(Pt A), 241–251. doi: 10.1016/j.expneurol.2017.07.002

Bivona, G., Gambino, C. M., Iacolino, G., and Ciaccio, M. (2019). Vitamin D and the nervous system. *Neurol. Res.* 41, 827–835. doi: 10.1080/01616412.2019.1622872

Boksa, P. (2010). Effects of prenatal infection on brain development and behavior: a review of findings from animal models. *Brain Behav. Immun.* 24, 881–897. doi: 10.1016/j.bbi.2010.03.005

Bölte, S., Girdler, S., and Marschik, P. B. (2019). The contribution of environmental exposure to the etiology of autism spectrum disorder. *Cell. Mol. Life Sci.* 76, 1275–1297. doi: 10.1007/s00018-018-2988-4

Bromley, R. (2016). The treatment of epilepsy in pregnancy: the neurodevelopmental risks associated with exposure to antiepileptic drugs. *Reprod. Toxicol.* 64, 203–210. doi: 10.1016/j.reprotox.2016.06.007

Campisi, L., Imran, N., Nazeer, A., Skokauskas, N., and Azeem, M. W. (2018). Autism spectrum disorder. *Br. Med. Bull.* 127, 91–100. doi: 10.1093/bmb/ldy026

Cannell, J. J. (2008). Autism and vitamin D. *Med. Hypotheses* 70, 750–759. doi: 10.1016/j.mehy.2007.08.016

Cannell, J. J. (2017). Vitamin D and autism, what's new? *Rev. Endocr. Metab. Disord.* 18, 183–193. doi: 10.1007/s11154-017-9409-0

Caspers, J., Zilles, K., Amunts, K., Laird, A. R., Fox, P. T., and Eickhoff, S. B. (2014). Functional characterization and differential coactivation patterns of two cytoarchitectonic visual areas on the human posterior fusiform gyrus. *Hum. Brain Mapp.* 35, 2754–2767. doi: 10.1002/hbm.22364

Chambers, E. S., and Hawrylowicz, C. M. (2011). The impact of vitamin D on regulatory T cells. *Curr. Allergy Asthma Rep.* 11, 29–36. doi: 10.1007/s11882-010-0161-8

Chang, Y. C., Cole, T. B., and Costa, L. G. (2018). Prenatal and early-life diesel exhaust exposure causes autism-like behavioral changes in mice. *Part. Fibre Toxicol.* 15:18. doi: 10.1186/s12989-018-0254-4

Chen, J., Xin, K., Wei, J., Zhang, K., and Xiao, H. (2016). Lower maternal serum 25(OH) D in first trimester associated with higher autism risk in Chinese offspring. *J. Psychosom. Res.* 89, 98–101. doi: 10.1016/j.jpsychores.2016.08.013

Cieślińska, A., Kostyra, E., Chwała, B., Moszyńska-Dumara, M., Fiedorowicz, E., Teodorowicz, M., et al. (2017). Vitamin D receptor gene polymorphisms associated with childhood autism. *Brain Sci.* 7:115. doi: 10.3390/brainsci7090115

Dadalko, O. I., and Travers, B. G. (2018). Evidence for brainstem contributions to autism spectrum disorders. *Front. Integr. Neurosci.* 12:47. doi: 10.3389/fnint.2018.00047

De Rubeis, S., He, X., Goldberg, A. P., Poultney, C. S., Samocha, K., Cicek, A. E., et al. (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature* 515, 209–215. doi: 10.1038/nature13772

DeLuca, H. F. (2016). Vitamin D: historical overview. *Vitam. Horm.* 100, 1–20. doi: 10.1016/bs.vh.2015.11.001

Desoky, T., Hassan, M. H., Fayed, H. M., and Sakhr, H. M. (2017). Biochemical assessments of thyroid profile, serum 25-hydroxycholecalciferol and cluster of differentiation 5 expression levels among children with autism. *Neuropsychiatr. Dis. Treat.* 13, 2397–2403. doi: 10.2147/ndt.S146152

Dong, H. Y., Wang, B., Li, H. H., Shan, L., and Jia, F. Y. (2017). [Correlation between serum 25-hydroxyvitamin D level and core symptoms of autism spectrum disorder in children]. *Zhonghua Er Ke Za Zhi* 55, 916–919. doi: 10.3760/cma.j.issn.0578-1310.2017.12.010

Eissa, N., Al-Houqani, M., Sadeq, A., Ojha, S. K., Sasse, A., and Sadek, B. (2018). Current enlightenment about etiology and pharmacological treatment of autism spectrum disorder. *Front. Neurosci.* 12:304. doi: 10.3389/fnins.2018.00304

El-Sharkawy, A., and Malki, A. (2020). Vitamin D signaling in inflammation and cancer: molecular mechanisms and therapeutic implications. *Molecules* 25:3219. doi: 10.3390/molecules25143219

Fahmy, F., Sabri, N., El Hamamsy, M., El Sawi, M., and Zaki, O. (2016). Vitamin D intake and sun exposure in autistic children. *Int. J. Pharm. Sci. Res.* 7, 1043–1049. doi: 10.13040/IJPSR.0975-8232.7

Famitafreshi, H., and Karimian, M. (2018). Overview of the recent advances in pathophysiology and treatment for autism. *CNS Neurol. Disord. Drug Targets* 17, 590–594. doi: 10.2174/1871527317666180706141654

Fedirko, V., Bostick, R. M., Flanders, W. D., Long, Q., Shaukat, A., Rutherford, R. E., et al. (2009). Effects of vitamin D and calcium supplementation on markers of apoptosis in normal colon mucosa: a randomized, double-blind, placebo-controlled clinical trial. *Cancer Prev. Res.* 2, 213–223. doi: 10.1158/1940-6207.Capr-08-0157

Feng, J., Shan, L., Du, L., Wang, B., Li, H., Wang, W., et al. (2017). Clinical improvement following vitamin D3 supplementation in autism spectrum disorder. *Nutr. Neurosci.* 20, 284–290. doi: 10.1080/1028415x.2015.1123847

Fleet, J. C., DeSmet, M., Johnson, R., and Li, Y. (2012). Vitamin D and cancer: a review of molecular mechanisms. *Biochem. J.* 441, 61–76. doi: 10.1042/bj20110744

García-Serna, A. M., and Morales, E. (2020). Neurodevelopmental effects of prenatal vitamin D in humans: systematic review and meta-analysis. *Mol. Psychiatry* 25, 2468–2481. doi: 10.1038/s41380-019-0357-9

Garipardic, M., Doğan, M., Bala, K. A., Mutluer, T., Kaba, S., Aslan, O., et al. (2017). Association of attention deficit hyperactivity disorder and autism spectrum

disorders with mean platelet volume and vitamin D. *Med. Sci. Monit.* 23, 1378–1384. doi: 10.12659/msm.899976

Gibbard, C. R., Ren, J., Skuse, D. H., Clayden, J. D., and Clark, C. A. (2018). Structural connectivity of the amygdala in young adults with autism spectrum disorder. *Hum. Brain Mapp.* 39, 1270–1282. doi: 10.1002/hbm.23915

Giulivi, C., Zhang, Y. F., Omanska-Klusek, A., Ross-Inta, C., Wong, S., Hertz-Picciotto, I., et al. (2010). Mitochondrial dysfunction in autism. *JAMA* 304, 2389–2396. doi: 10.1001/jama.2010.1706

Grant, W. B. (2016). The role of geographical ecological studies in identifying diseases linked to UVB exposure and/or vitamin D. *Dermatoendocrinol.* 8:e1137400. doi: 10.1080/19381980.2015.1137400

Groves, N. J., McGrath, J. J., and Burne, T. H. (2014). Vitamin D as a neurosteroid affecting the developing and adult brain. *Annu. Rev. Nutr.* 34, 117–141. doi: 10.1146/annurev-nutr-071813-105557

Guo, M., Zhu, J., Yang, T., Lai, X., Lei, Y., Chen, J., et al. (2019). Vitamin A and vitamin D deficiencies exacerbate symptoms in children with autism spectrum disorders. *Nutr. Neurosci.* 22, 637–647. doi: 10.1080/1028415x.2017.1423268

Halicka, H. D., Zhao, H., Li, J., Traganos, F., Studzinski, G. P., and Darzynkiewicz, Z. (2012). Attenuation of constitutive DNA damage signaling by 1,25-dihydroxyvitamin D3. *Aging* 4, 270–278. doi: 10.18632/aging.100450

Harris, J. (2018). Leo Kanner and autism: a 75-year perspective. *Int. Rev. Psychiatry* 30, 3–17. doi: 10.1080/09540261.2018.1455646

Hazlett, H. C., Poe, M. D., Gerig, G., Styner, M., Chappell, C., Smith, R. G., et al. (2011). Early brain overgrowth in autism associated with an increase in cortical surface area before age 2 years. *Arch. Gen. Psychiatry* 68, 467–476. doi: 10.1001/archgenpsychiatry.2011.39

James, S. J., Melnyk, S., Jernigan, S., Cleves, M. A., Halsted, C. H., Wong, D. H., et al. (2006). Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* 141B, 947–956. doi: 10.1002/ajmg.b.30366

Jia, F., Shan, L., Wang, B., Li, H., Miao, C., Xu, Z., et al. (2018). Bench to bedside review: possible role of vitamin D in autism spectrum disorder. *Psychiatry Res.* 260, 360–365. doi: 10.1016/j.psychres.2017.12.005

Jia, F., Wang, B., Shan, L., Xu, Z., Staal, W. G., and Du, L. (2015). Core symptoms of autism improved after vitamin D supplementation. *Pediatrics* 135, e196–e198. doi: 10.1542/peds.2014-2121

Jiang, H. Y., Xu, L. L., Shao, L., Xia, R. M., Yu, Z. H., Ling, Z. X., et al. (2016). Maternal infection during pregnancy and risk of autism spectrum disorders: a systematic review and meta-analysis. *Brain Behav. Immun.* 58, 165–172. doi: 10.1016/j.bbi.2016.06.005

Kaplan, Y. C., Keskin-Arslan, E., Acar, S., and Sozmen, K. (2016). Prenatal selective serotonin reuptake inhibitor use and the risk of autism spectrum disorder in children: a systematic review and meta-analysis. *Reprod. Toxicol.* 66, 31–43. doi: 10.1016/j.reprotox.2016.09.013

Kardas, F., Bayram, A. K., Demirci, E., Akin, L., Ozmen, S., Kendirci, M., et al. (2016). Increased serum phthalates (MEHP, DEHP) and bisphenol A concentrations in children with autism spectrum disorder: the role of endocrine disruptors in autism etiopathogenesis. *J. Child Neurol.* 31, 629–635. doi: 10.1177/0883073815609150

Karras, S. N., Wagner, C. L., and Castracane, V. D. (2018). Understanding vitamin D metabolism in pregnancy: from physiology to pathophysiology and clinical outcomes. *Metabolism* 86, 112–123. doi: 10.1016/j.metabol.2017.10.001

Kerley, C. P., Power, C., Gallagher, L., and Coghlan, D. (2017). Lack of effect of vitamin D(3) supplementation in autism: a 20-week, placebo-controlled RCT. *Arch. Dis. Child.* 102, 1030–1036. doi: 10.1136/archdischild-2017-312783

Kim, K. C., Kim, P., Go, H. S., Choi, C. S., Park, J. H., Kim, H. J., et al. (2013). Male-specific alteration in excitatory post-synaptic development and social interaction in pre-natal valproic acid exposure model of autism spectrum disorder. *J. Neurochem.* 124, 832–843. doi: 10.1111/jnc.12147

Kim, S., Kim, H., Yim, Y. S., Ha, S., Atarashi, K., Tan, T. G., et al. (2017). Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* 549, 528–532. doi: 10.1038/nature23910

Kirsten, T. B., and Bernardi, M. M. (2017). Prenatal lipopolysaccharide induces hypothalamic dopaminergic hypoactivity and autistic-like behaviors: repetitive self-grooming and stereotypies. *Behav. Brain Res.* 331, 25–29. doi: 10.1016/j.bbr.2017.05.013

Krakowiak, P., Goines, P. E., Tancredi, D. J., Ashwood, P., Hansen, R. L., Hertz-Picciotto, I., et al. (2017). Neonatal cytokine profiles associated with autism spectrum disorder. *Biol. Psychiatry* 81, 442–451. doi: 10.1016/j.biopsych.2015.08.007

Lai, M.-C., Kassee, C., Besney, R., Bonato, S., Hull, L., Mandy, W., et al. (2019). Prevalence of co-occurring mental health diagnoses in the autism population: a systematic review and meta-analysis. *Lancet Psychiatry* 6, 819–829. doi: 10.1016/s2215-0366(19)30289-5

Lai, M.-C., Lombardo, M. V., and Baron-Cohen, S. (2014). Autism. *Lancet* 383, 896–910. doi: 10.1016/s0140-6736(13)61539-1

Landel, V., Stephan, D., Cui, X., Eyles, D., and Feron, F. (2018). Differential expression of vitamin D-associated enzymes and receptors in brain cell subtypes. *J. Steroid Biochem. Mol. Biol.* 177, 129–134. doi: 10.1016/j.jsbmb.2017.09.008

López-Vicente, M., Sunyer, J., Lertxundi, N., González, L., Rodríguez-Dehli, C., Espada Sáenz-Torre, M., et al. (2019). Maternal circulating vitamin D(3) levels during pregnancy and behaviour across childhood. *Sci. Rep.* 9:14792. doi: 10.1038/s41598-019-51325-3

Lord, C., Brugha, T. S., Charman, T., Cusack, J., Dumas, G., Frazier, T., et al. (2020). Autism spectrum disorder. *Nat. Rev. Dis. Primers* 6:5. doi: 10.1038/s41572-019-0138-4

Lord, C., Elsabbagh, M., Baird, G., and Veenstra-Vanderweele, J. (2018). Autism spectrum disorder. *Lancet* 392, 508–520. doi: 10.1016/s0140-6736(18)31129-2

Maenner, M. J., Shaw, K. A., Baio, J., Washington, A., Patrick, M., DiRienzo, M., et al. (2020). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2016. *MMWR Surveill. Summ.* 69, 1–12. doi: 10.15585/mmwr.ss6904a1

Mak, A. (2018). The impact of vitamin D on the immunopathophysiology, disease activity, and extra-musculoskeletal manifestations of systemic lupus erythematosus. *Int. J. Mol. Sci.* 19:2355. doi: 10.3390/ijms19082355

Marini, F., Bartoccini, E., Cascianelli, G., Voccoli, V., Baviglia, M. G., Magni, M. V., et al. (2010). Effect of 1alpha,25-dihydroxyvitamin D3 in embryonic hippocampal cells. *Hippocampus* 20, 696–705. doi: 10.1002/hipo.20670

Martínez-Cerdeño, V., Camacho, J., Fox, E., Miller, E., Ariza, J., Kienzle, D., et al. (2016). Prenatal exposure to autism-specific maternal autoantibodies alters proliferation of cortical neural precursor cells, enlarges brain, and increases neuronal size in adult animals. *Cereb. Cortex* 26, 374–383. doi: 10.1093/cercor/bhu291

Mazahery, H., Camargo, C. A. Jr., Conlon, C., Beck, K. L., Kruger, M. C., and von Hurst, P. R. (2016). Vitamin D and autism spectrum disorder: a literature review. *Nutrients* 8:236. doi: 10.3390/nu8040236

Meltzer, A., and Van de Water, J. (2017). The role of the immune system in autism spectrum disorder. *Neuropsychopharmacology* 42, 284–298. doi: 10.1038/npp.2016.158

Modabbernia, A., Velthorst, E., and Reichenberg, A. (2017). Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. *Mol. Autism* 8:13. doi: 10.1186/s13229-017-0121-4

Mostafa, G. A., and Al-Ayadhi, L. Y. (2012). Reduced serum concentrations of 25-hydroxy vitamin D in children with autism: relation to autoimmunity. *J. Neuroinflammation* 9:201. doi: 10.1186/1742-2094-9-201

Naaijen, J., Zwiers, M. P., Forde, N. J., Williams, S. C., Durston, S., Brandeis, D., et al. (2018). Striatal structure and its association with N-acetylaspartate and glutamate in autism spectrum disorder and obsessive compulsive disorder. *Eur. Neuropsychopharmacol.* 28, 118–129. doi: 10.1016/j.euroneuro.2017.11.010

Nakai, N., Takumi, T., Nakai, J., and Sato, M. (2018). Common defects of spine dynamics and circuit function in neurodevelopmental disorders: a systematic review of findings from in vivo optical imaging of mouse models. *Front. Neurosci.* 12:412. doi: 10.3389/fnins.2018.00412

Nakamura, K., Sekine, Y., Ouchi, Y., Tsujii, M., Yoshikawa, E., Futatsubashi, M., et al. (2010). Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. *Arch. Gen. Psychiatry* 67, 59–68. doi: 10.1001/archgenpsychiatry.2009.137

Napolioni, V., Ober-Reynolds, B., Szelinger, S., Corneveaux, J. J., Pawlowski, T., Ober-Reynolds, S., et al. (2013). Plasma cytokine profiling in sibling pairs discordant for autism spectrum disorder. *J. Neuroinflammation* 10:38. doi: 10.1186/1742-2094-10-38

Pagnozzi, A. M., Conti, E., Calderoni, S., Fripp, J., and Rose, S. E. (2018). A systematic review of structural MRI biomarkers in autism spectrum disorder:

a machine learning perspective. *Int. J. Dev. Neurosci.* 71, 68–82. doi: 10.1016/j.ijdevneu.2018.08.010

Patrick, R. P., and Ames, B. N. (2014). Vitamin D hormone regulates serotonin synthesis. Part 1: relevance for autism. *FASEB J.* 28, 2398–2413. doi: 10.1096/fj.13-246546

Pertile, R. A. N., Cui, X., Hammond, L., and Eyles, D. W. (2018). Vitamin D regulation of GDNF/Ret signaling in dopaminergic neurons. *FASEB J.* 32, 819–828. doi: 10.1096/fj.201700713R

Principi, N., and Esposito, S. (2019). Vitamin D deficiency during pregnancy and autism spectrum disorders development. *Front. Psychiatry* 10:987. doi: 10.3389/fpsyt.2019.00987

Ritterhouse, L. L., Crowe, S. R., Niewold, T. B., Kamen, D. L., Macwana, S. R., Roberts, V. C., et al. (2011). Vitamin D deficiency is associated with an increased autoimmune response in healthy individuals and in patients with systemic lupus erythematosus. *Ann. Rheum. Dis.* 70, 1569–1574. doi: 10.1136/ard.2010.148494

Rossignol, D. A., and Frye, R. E. (2014). Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. *Front. Physiol.* 5:150. doi: 10.3389/fphys.2014.00150

Saad, K., Abdel-Rahman, A., Elserogy, Y., Al-Atram, A., El-Houfey, A., Othman, H., et al. (2019). Retraction: randomized controlled trial of vitamin D supplementation in children with autism spectrum disorder. *J. Child Psychol. Psychiatry Allied Disciplines* 60:711. doi: 10.1111/jcpp.13076

Saad, K., Abdel-Rahman, A. A., Elserogy, Y. M., Al-Atram, A. A., El-Houfey, A. A., Othman, H. A., et al. (2018). Randomized controlled trial of vitamin D supplementation in children with autism spectrum disorder. *J. Child Psychol. Psychiatry Allied Disciplines* 59, 20–29. doi: 10.1111/jcpp.12652

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Hultman, C., Larsson, H., and Reichenberg, A. (2017). The heritability of autism spectrum disorder. *JAMA* 318, 1182–1184. doi: 10.1001/jama.2017.12141

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Larsson, H., Hultman, C. M., and Reichenberg, A. (2014). The familial risk of autism. *JAMA* 311, 1770–1777. doi: 10.1001/jama.2014.4144

Schmidt, R. J., Hansen, R. L., Hartiala, J., Allayee, H., Sconberg, J. L., Schmidt, L. C., et al. (2015). Selected vitamin D metabolic gene variants and risk for autism spectrum disorder in the CHARGE Study. *Early Hum. Dev.* 91, 483–489. doi: 10.1016/j.earlhumdev.2015.05.008

Selim, M. E., and Al-Ayadhi, L. Y. (2013). Possible ameliorative effect of breastfeeding and the uptake of human colostrum against coeliac disease in autistic rats. *World J. Gastroenterol.* 19, 3281–3290. doi: 10.3748/wjg.v19.i21.3281

Shan, L., Hu, X. L., Wang, B., and Jia, F. Y. (2016). [Research advances in the role of vitamin D in autism spectrum disorders]. *Zhongguo Dang Dai Er Ke Za Zhi* 18, 183–188. doi: 10.7499/j.issn.1008-8830.2016.02.016

Siracusano, M., Riccioni, A., Abate, R., Benvenuto, A., Curatolo, P., and Mazzone, L. (2020). Vitamin D deficiency and autism spectrum disorder. *Curr. Pharm. Des.* 26, 2460–2474. doi: 10.2174/1381612826666200415174311

Skalny, A. V., Skalnaya, M. G., Bjørklund, G., Nikonorov, A. A., and Tinkov, A. A. (2016). Mercury as a possible link between maternal obesity and autism spectrum disorder. *Med. Hypotheses* 91, 90–94. doi: 10.1016/j.mehy.2016.04.021

Staal, W. G., de Krom, M., and de Jonge, M. V. (2012). Brief report: the dopamine-3-receptor gene (DRD3) is associated with specific repetitive behavior in autism spectrum disorder (ASD). *J. Autism Dev. Disord.* 42, 885–888. doi: 10.1007/s10803-011-1312-z

Steinman, G. (2020). The putative etiology and prevention of autism. *Prog. Mol. Biol. Transl. Sci.* 173, 1–34. doi: 10.1016/bs.pmbts.2020.04.013

Stubbs, G., Henley, K., and Green, J. (2016). Autism: will vitamin D supplementation during pregnancy and early childhood reduce the recurrence rate of autism in newborn siblings? *Med. Hypotheses* 88, 74–78. doi: 10.1016/j.mehy.2016.01.015

Südhof, T. C. (2017). Synaptic neurexin complexes: a molecular code for the logic of neural circuits. *Cell* 171, 745–769. doi: 10.1016/j.cell.2017.10.024

Sun, X., Allison, C., Wei, L., Matthews, F. E., Auyeung, B., Wu, Y. Y., et al. (2019). Autism prevalence in China is comparable to Western prevalence. *Mol. Autism* 10:7. doi: 10.1186/s13229-018-0246-0

Trifonova, E. A., Klimenko, A. I., Mustafin, Z. S., Lashin, S. A., and Kochetov, A. V. (2019). The mTOR signaling pathway activity and vitamin D availability control the expression of most autism predisposition genes. *Int. J. Mol. Sci.* 20:6332. doi: 10.3390/ijms20246332

Uçar, N., Grant, W. B., Peraita-Costa, I., and Morales Suárez-Varela, M. (2020). How 25(OH)D levels during pregnancy affect prevalence of autism in children: systematic review. *Nutrients* 12:2311. doi: 10.3390/nu12082311

Vinkhuyzen, A. A. E., Eyles, D. W., Burne, T. H. J., Blanken, L. M. E., Kruithof, C. J., Verhulst, F., et al. (2018). Gestational vitamin D deficiency and autism-related traits: the generation R study. *Mol. Psychiatry* 23, 240–246. doi: 10.1038/mp.2016.213

Wai, K. M., Yu, P. K., and Lam, K. S. (2015). Reduction of solar UV radiation due to urban high-rise buildings–a coupled modelling study. *PLoS One* 10:e0135562. doi: 10.1371/journal.pone.0135562

Wang, T., Shan, L., Du, L., Feng, J., Xu, Z., Staal, W. G., et al. (2016). Serum concentration of 25-hydroxyvitamin D in autism spectrum disorder: a systematic review and meta-analysis. *Eur. Child Adolesc. Psychiatry* 25, 341–350. doi: 10.1007/s00787-015-0786-1

Wang, Z., Ding, R., and Wang, J. (2020). The association between vitamin D status and autism spectrum disorder (ASD): a systematic review and meta-analysis. *Nutrients* 13:86. doi: 10.3390/nu13010086

Wong, H., and Hoeffer, C. (2018). Maternal IL-17A in autism. *Exp. Neurol.* 299(Pt A), 228–240. doi: 10.1016/j.expneurol.2017.04.010

Wood, A. G., Nadebaum, C., Anderson, V., Reutens, D., Barton, S., O'Brien, T. J., et al. (2015). Prospective assessment of autism traits in children exposed to antiepileptic drugs during pregnancy. *Epilepsia* 56, 1047–1055. doi: 10.1111/epi.13007

Zablotsky, B., Black, L. I., Maenner, M. J., Schieve, L. A., and Blumberg, S. J. (2015). Estimated prevalence of autism and other developmental disabilities following questionnaire changes in the 2014 national health interview survey. *Natl. Health Stat. Rep.* 87, 1–20.

Zhou, S. H., Wang, X., Fan, M. Y., Li, H. L., Bian, F., Huang, T., et al. (2018). Influence of vitamin D deficiency on T cell subsets and related indices during spinal tuberculosis. *Exp. Ther. Med.* 16, 718–722. doi: 10.3892/etm.2018.6203

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Behavioral Neuroscience

REVIEW
published: 25 May 2022
doi: 10.3389/fnbeh.2022.893955

Transcranial Direct Current Stimulation of the Dorsolateral Prefrontal Cortex for Treatment of Neuropsychiatric Disorders

Qing Li[1,2], Yu Fu[1], Chang Liu[3,4,5] and Zhiqiang Meng[2,4,5]**

[1] Medical School, Kunming University of Science and Technology, Kunming, China, [2] Shenzhen Key Laboratory of Drug Addiction, Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China, [3] Shenzhen Key Laboratory of Viral Vectors for Biomedicine, Brain Cognition and Brain Disease Institute, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China, [4] Shenzhen-Hong Kong Institute of Brain Science, Shenzhen Fundamental Research Institutions, Shenzhen, China, [5] CAS Key Laboratory of Brain Connectome and Manipulation, Shenzhen Institute of Advanced Technology, Chinese Academy of Sciences, Shenzhen, China*

Background: The dorsolateral prefrontal cortex (DLPFC) is a key node of the frontal cognitive circuit. It is involved in executive control and many cognitive processes. Abnormal activities of DLPFC are likely associated with many psychiatric diseases. Modulation of DLPFC may have potential beneficial effects in many neural and psychiatric diseases. One of the widely used non-invasive neuromodulation technique is called transcranial direct current stimulation (or tDCS), which is a portable and affordable brain stimulation approach that uses direct electrical currents to modulate brain functions.

Objective: This review aims to discuss the results from the past two decades which have shown that tDCS can relieve clinical symptoms in various neurological and psychiatric diseases.

Methods: Here, we performed searches on PubMed to collect clinical and preclinical studies that using tDCS as neuromodulation technique, DLPFC as the stimulation target in treating neuropsychiatric disorders. We summarized the stimulation sites, stimulation parameters, and the overall effects in these studies.

Results: Overall, tDCS stimulation of DLPFC could alleviate the clinical symptoms of schizophrenia, depression, drug addiction, attention deficit hyperactivity disorder and other mental disorders.

Conclusion: The stimulation parameters used in these studies were different from each other. The lasting effect of stimulation was also not consistent. Nevertheless, DLPFC is a promising target for non-invasive stimulation in many psychiatric disorders. TDCS is a safe and affordable neuromodulation approach that has potential clinical uses. Larger clinical studies will be needed to determine the optimal stimulation parameters in each condition.

Keywords: non-invasive neuromodulation, dorsolateral prefrontal cortex (DLPFC), schizophrenia, addiction, depression, psychiatric disease

OPEN ACCESS

Edited by:
Dongdong Qin,
Yunnan University of Traditional Chinese Medicine, China

Reviewed by:
Yunpeng Zhang,
Brandeis University, United States
Ti-Fei Yuan,
Shanghai Jiao Tong University, China

****Correspondence:***
Chang Liu
chang.liu3@siat.ac.cn
Zhiqiang Meng
zhiqiang-meng@163.com

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *11 March 2022*
Accepted: *04 April 2022*
Published: *25 May 2022*

Citation:
Li Q, Fu Y, Liu C and Meng Z (2022) Transcranial Direct Current Stimulation of the Dorsolateral Prefrontal Cortex for Treatment of Neuropsychiatric Disorders. Front. Behav. Neurosci. 16:893955. doi: 10.3389/fnbeh.2022.893955

INTRODUCTION

Neuropsychiatric disorders are combinations of psychiatric and neurologic malfunction that deal with mental disorders, including degenerative diseases, addictions, mood disorders, neurotic disorders, etc. Current treatments of neuropsychiatric diseases mainly include drug therapy, physical therapy and psychotherapy. Common physical therapies included electroconvulsive treatment (ECT), deep brain stimulation (DBS), transcranial magnetic stimulation (TMS), transcranial direct current stimulation (tDCS), etc. Among these techniques, tDCS becomes an increasingly employed clinically due to its economical, convenient, non-invasive and mild side effects. However, current dilemma in using tDCS as a option of clinical treatment is that there is no common standard, and the therapeutic effects vary from case to case.

In this review, we discussed: (1) the mechanism of tDCS and the application of tDCS technique in clinical research, focusing on five types of psychiatric disorders; (2) and the potential therapeutic brain target DLPFC.

AN OVERVIEW OF TRANSCRANIAL DIRECT CURRENT STIMULATION TECHNIQUE

Accumulating knowledge has supported that transcranial direct current stimulation (tDCS) can relieve symptoms of various diseases, including pain (Wrigley et al., 2013), depression (Sharafi et al., 2019), schizophrenia (Brunelin et al., 2012a), attention deficit disorder (Cosmo et al., 2015), drug addiction (da Silva et al., 2013), and anxiety disorder (Heeren et al., 2017). In recent years, tDCS has been widely used in clinical research due to the advantages mentioned above. tDCS is a non-invasive brain stimulation technique that uses low-intensity direct current (1–2 mA) to modulate cortical activity (Woods et al., 2016). A common tDCS stimulator consists of a controller to generate a constant current, and at least one pair of stimulation electrodes to attach to the surface of the scalp. Although there is no uniform standard for stimulation parameters in clinical studies, electrodes of 20–35 cm^2, with application of 1–2 mA currents, 20- or 30-min stimulation duration for one session with one or multiple sessions through a certain period have been employed in a large body of studies.

The activity of the brain is based on the electrical activity of neurons. It is believed that tDCS may modulate the brain activity at different scales. First, from a macro perspective, tDCS likely modulate the brain activity via changing the cortical excitability directly. In general, anodal stimulation depolarizes neurons, whereas cathodal stimulation hyperpolarizes neurons (Purpura and McMurtry, 1965; Bikson et al., 2004). In addition, tDCS may regulate the activity of neural networks by influencing other brain regions associated with the target brain region. It has been suggested that neuronal networks were more sensitive than single neuron in the weak electric field (Francis et al., 2003). By using resting-state functional magnetic resonance imaging (fMRI) technique, it has been found that anode tDCS intensified the functional connection among the thalamus, the temporal lobe and the left caudate nucleus (Dalong et al., 2020). At the neuronal levels, tDCS has been shown to modulate the neural oscillations. McDermott et al. (2019) reported that anode tDCS increased spontaneous activity in the theta (4–7 Hz) and alpha (9–14 Hz) bands in prefrontal and occipital cortices in a flanker task. Finally, from the molecular perspective, tDCS may modulate neurotransmitter release to regulate synaptic plasticity. For example, long-term potentiation (LTP) which was observed after anodal tDCS coupling with synaptic activation (Fritsch et al., 2010). Another study found that the effects of tDCS may be related to the polarity-specific changes in neurotransmitter concentrations. Anodal tDCS caused locally reduced GABA concentrations while cathodal stimulation caused reduced glutamatergic neuronal activity with a highly correlated increase in GABA concentration (Stagg et al., 2009). Liebetanz et al. (2002) showed that, dextromethorphan, an antagonist of N-Methyl-D-Aspartic Acid receptors (NMDAR, receptors that are involved in synaptic plasticity regulation), suppressed the post-stimulation effects of both anode and cathode stimulation.

In order to recommend this convenient technique as a powerful therapeutic strategy, a remarkable effort is still needed to further understand how tDCS modulate the brain activity.

DORSOLATERAL PREFRONTAL CORTEX IS A TARGET FOR NON-INVASIVE STIMULATION IN NEUROPSYCHIATRIC DISEASES

One of the most common cortical targets for tDCS is the dorsolateral prefrontal cortex (DLPFC; **Figure 1**). DLPFC is a structurally and functionally heterogeneous region (Glasser et al., 2016), and is closely related with cognitive functions [attention (Vossel et al., 2014; Bidet-Caulet et al., 2015), decision-making (Philiastides et al., 2011; Rahnev et al., 2016), working memory (Barbey et al., 2013), and emotion regulation (Shahani and Russell, 1969; Buhle et al., 2014; Frank et al., 2014)]. The DLPFC is located in the middle frontal gyrus, and it is a part of the prefrontal cortex (PFC) which regulates the marginal reward area, and involves in higher executive function and impulsive behaviors (Fitzpatrick et al., 2013; Xu et al., 2017). The left DLPFC connects to the primary motor area, primary sensory area, etc. It mainly participates in pain perception and emotional cognitive processing through a top-down neural network (Koenigs and Grafman, 2009; Vaseghi et al., 2015). The right DLPFC is selectively involved in processing pessimistic, negative emotions and mediates vigilance and arousal (Hecht, 2010). DLPFC has become an important target in the treatment for mental disorders.

A large number of studies have shown that tDCS targeting at DLPFC can alleviate a variety of neuronal and psychiatric diseases symptoms. For example, anode tDCS (2 mA) can reduce the pain caused by multiple sclerosis (Ayache et al., 2016). Anode stimulation of the right DLPFC, and cathode at the left DLPFC improved the risk preference of the subjects

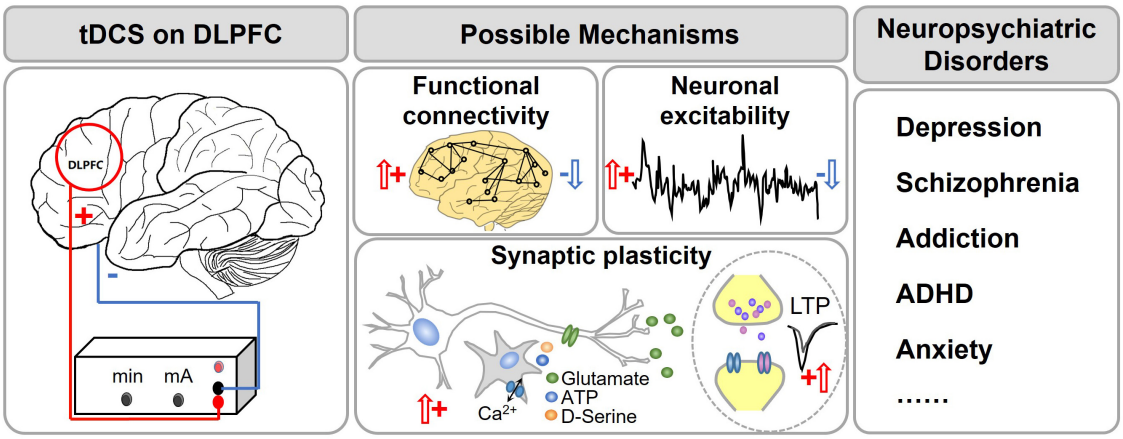


FIGURE 1 | tDCS of the dorsal lateral prefrontal cortex (DLPFC) for treatment of neuropsychiatric disorders. The red circle shows the DLPFC. It is the center for higher brain functions such as working memory, executive function, attention, etc. Dysfunction of DLPFC was found in many psychiatric disorders such as schizophrenia, depression, ADHD, etc. tDCS of DLPFC has become a popular treatment option for these disorders. It has been proposed that tDCS changes the functional connectivity, neuronal excitability and synaptic plasticity of the related brain regions.

(Yang et al., 2017). Studies have shown that anodal tDCS stimulation of left DLPFC could decrease negative emotions and improve cognitive control (Pena-Gomez et al., 2011). Here, we summarize and discuss perspectives of the parameters and effects of tDCS targeting DLPFC in the treatment of different types of neuropsychiatric disorders.

Depression

Depression (also known as depressive disorder) is a mental disease that causes a persistent feeling of sadness and loss of interests, with high recurrence rate, disability rate and suicide rate. In general, it can be classified into major depression, bipolar disorder or treatment-resistant depression. Bipolar disorder, causing extreme mood swings that include emotional highs (mania or hypomania) and lows (depression). Treatment-resistant depression refers to no response to at least two different antidepressant treatments.

Twenty studies collected from PubMed were shown in **Table 1**. Majority of these studies have shown that tDCS targeting at DLPFC (mostly the left DLPFC) can significantly improve depression symptoms for a month or longer. All studies placed the anode electrodes on the left DLPFC and the cathode electrodes on the opposite side (right DLPFC or orbitofrontal region). 17 out of 20 studies reported improvement of depressive symptoms. Besides, tDCS also improved working memory and attention (Loo et al., 2012). Importantly, tDCS in combination with other treatments, such as an antidepressant drug (Brunoni et al., 2013b) or with computerized cognitive behavioral therapy (Welch et al., 2019), can reduce depressive symptoms even better than tDCS alone (Brunoni et al., 2013a). It is important to note that tDCS on DLPFC may have some side effects, such as mania, although this is not common (Loo et al., 2012). For the stimulation parameters, most studies have used a current of 2 mA, electrode sizes of 25–35 cm^2, and a total of more than five sessions (see details in **Table 1**). Though various parameters have shown different effects on depression symptoms, most stimulation protocols with longer stimulation duration for one session and repeated sessions were shown to have therapeutic effects.

Schizophrenia

Schizophrenia is a chronic mental disorder. The most typical symptoms of schizophrenia include hallucinations and delusions, which are often referred to as positive symptoms. Schizophrenia may also experience negative symptoms, such as social withdrawal, anhedonia, hyperboulia, affective blunting and alogia (Carpenter et al., 2016). In recent years, clinical studies have shown that tDCS may be effective in reducing auditory hallucination symptoms in patients with schizophrenia. For example, a study reported that anode tDCS showed a significant increase in short- interval intracortical inhibition in the left motor cortex, but no change in intra-cortical facilitation (ICF) compared to sham stimulation (Gordon et al., 2019). Yoon et al. (2019) found that decreased functional network connectivity was negatively correlated with the increase of hallucinogenic behavior at baseline and was significantly enhanced after anode 2 mA tDCS. This may suggest that fronto-temporal tDCS may regulate abnormal hallucination-related functional network connectivity in patients with schizophrenia. Decline in insight is also one of the main symptoms of schizophrenia. Patients with insight deficits often fail to recognize that they are ill and may refuse treatment. Bose et al. (2014) found that 2 mA anode tDCS stimulation over left DLPFC and cathode over the left temporo-parietal junction, could improve the insight and decrease auditory hallucination symptoms in patients. However, no such effect was observed after 1 mA stimulation, which indicates that the current intensity of tDCS is a key factor (Hill et al., 2016). A combination of medication, physical therapy, and psychotherapy usually have a synergic effect. Non-invasive brain stimulation combined with physical therapy has been shown to improve motor performance and language function

TABLE 1 | Effects of DLPFC tDCS on depression.

References	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
	Anode (+)	Cathode (–)						
Brunoni et al., 2017	DLPFC (F3)	DLPFC (F4)	/	2	30	1/day, 3 weeks + 1/week × 7 weeks	22	Have a significant effect, but it was inferior to escitalopram
Aparicio et al., 2019	DLPFC (F3)	DLPFC (F4)	25	2	30	1/day, 3 weeks, +1/week, 7 weeks	22	Reduced recurrence rate significantly
Moreno et al., 2020	DLPFC (F3)	DLPFC (F4)	/	2	30	1/day, 3 weeks, +1/week, 7 weeks	22	Reduced practice effects in processing speed, but no change in cognitive deficits
Palm et al., 2012	DLPFC (F3)	Orbitofrontal region	35	1/2	20	1/day, 4 weeks	20	No significant effect
Martin et al., 2013	DLPFC (F3)	Arm/opposite Side of track (F8) (two forms of tDCS)	35	2	20	1/week × 3 months + 1/2 weeks × 3 months	18	Reduced the recurrence rate for relapse significantly
Sampaio-Junior et al., 2018	DLPFC (F3)	DLPFC (F4)	25	2	30	1/day, 2 weeks + 2/other week, 6 weeks	16	Have a significant improvement
Brunoni et al., 2013b	DLPFC (F3)	DLPFC (F4)	25	2	30	1/day × 2 weeks + 1/2 weeks × 2	12	Improved mood significantly [tDCS + sertraline (50 mg/d)]
Welch et al., 2019	DLPFC (F3)	DLPFC (F4)	25	2	30	3/week × 4 weeks	12	Reduced depressive symptoms significantly (tDCS + computerized cognitive behavioral therapy)
Brunoni et al., 2014	DLPFC (F3)	DLPFC (F4)	25	2	30	1/day, 2 weeks	10	Reduced depressive symptoms significantly
Blumberger et al., 2012	DLPFC (F3)	DLPFC (F4)	35	2	20	1/day, 3 weeks	15	No significant effect
Loo et al., 2012	DLPFC (F3)	Orbitofrontal region (F8)	35	2	20	1/day, 3 weeks	15	Improved mood significantly
Loo et al., 2010	DLPFC (F3)	Orbitofrontal region	35	1	20	5 active + 5 active sessions	10	Improved overall depression significantly over 10 tDCS treatments, no between-group difference in the five-session, sham-controlled phase
					20	5 sham session + 5 active sessions	10	
Dell'Osso et al., 2012	DLPFC (F3)	Contralateral cortex	32	2	20	2/day × 5 days	10	Have a significant improvement
Sharafi et al., 2019	DLPFC (F3)	DLPFC (F4)	20	2	20	1/day, 2 weeks	10	Have a significant effect (lasted for 1 month after treatment)
Lin et al., 2021	DLPFC (F3)	DLPFC (F4)	35	2	20	2/day × 5 days	10	Improved unipolar and bipolar depression rapidly
Brunoni et al., 2011	DLPFC (F3)	DLPFC (F4)	35	2	20	2/day × 5 days	10	Improved depression for 1 week in MDD group and 1 month in BDD group
Rigonatti et al., 2008	DLPFC (F3)	Contralateral Superior orbital region	35	2	20	1/day × 10 days	10	Have a significant effect (similar to fluoxetine 20 mg/day for 6 weeks)
Boggio et al., 2008a	DLPFC (F3)	Contralateral Supraorbital area	35	2	20	1/day, 2 weeks	10	Reduced depression scores significantly (lasted for 1 month after treatment) after DLPFC tDCS compared to occipital and sham tDCS
Bennabi et al., 2015	DLPFC (F3)	Contralateral superior Orbital region	35	2	20	2/day × 5 days	10	No significant effect
Kumar et al., 2020	left DLPFC (F3) + right DLPFC (F4)	Iz	25	1	30	1/day, 2 weeks	10	No significant effect

MDD, major depressive disorder; BDD, bipolar depressive disorder.

in stroke patients (Barros Galvao et al., 2014; Rubi-Fessen et al., 2015). Orlov et al. (2017) found that anode tDCS stimulation combined with cognitive behavioral training showed significant improvement in working memory and learning. However, Shiozawa et al. (2016) found that tDCS combined with cognitive training failed to produce a synergic effect in schizophrenia patients. This may due to the small sample size and the use of antipsychotics in patients (Orlov et al., 2017).

We summarized 28 studies using tDCS as a treatment strategy for schizophrenia in **Table 2**. Overall, tDCS improved both positive syndromes and negative syndromes in patients with schizophrenia. Only two studies showed no significant improvement after tDCS. For the electrodes positions, in 26 out of 28 studies placed the anode in the left DLPFC (F3) or a point midway between F3 and FP1 and the cathode in the right hemisphere (left temporoparietal junction, FP2, or right contralateral superior orbital region). 20 out of 28 studies used 25–35 cm^2 electrodes. For stimulating current intensity, 26 studies used 2 mA current, only 1 study used 1 mA current, and 1 study used both 1 mA and 2 mA current. For stimulation duration, 26 studies used 20 min/session, 1 study used 30 min/session, and 1 study used 15 min/session. All studies adopted multiple stimulation sessions (from 5 to 20 sessions), only two studies used one single session of tDCS. Most multiple sessions of tDCS brought a better curative effect, pointing to a repeated application of tDCS as therapeutic strategy. In studies with one single session of tDCS, 2 mA but not 1 mA was shown to induce a positive effect. Taken together, 2 mA multi-session anodal tDCS of the left DLPFC or left temporoparietal junction area has the most potential to improve symptoms in patients with schizophrenia.

Addiction

Addiction is a chronic brain disease characterized by compulsive use of drugs, with loss of self-control and a high relapse rate (Berke and Hyman, 2000; Preller et al., 2013). Patients may experience negative emotions during withdrawal, such as sadness, restlessness, subdued pleasure. The relapse tendency indicates that a solid memory of drugs, a pathological memory, also called drug memory formed in addiction patients (Boning, 2009; Nestler, 2013). Drug memory is signaled by dynamic neuronal activity patterns in the brain areas such as prefrontal cortex, hippocampus and the ventral tegmental area (VTA; Berke and Hyman, 2000). Drugs increase the activity of VTA dopaminergic neurons as well as the concentration of dopamine in the projection area (Hyman and Malenka, 2001; Pierce and Kumaresan, 2006). The downstream targets of VTA dopaminergic neurons mainly includes ventral striatum, which is responsible for processing reward information, and prefrontal cortex, which is responsible for higher brain functions such as decision making, executive function, etc. (Robbins and Everitt, 2002; Hyman et al., 2006). Reward related perception and executive function can be modulated by the release of dopamine in the frontal lobe (Goldstein and Volkow, 2002).

Many studies have shown that tDCS can significantly relieve the symptoms of addictions (such as craving for cocaine, cigarette, alcohol, etc.). Bilateral DLPFC tDCS stimulation reduced cocaine craving with a linear decrease within 4 weeks, and improved anxiety symptoms and overall quality of life in patients (Batista et al., 2015). In addition to cocaine, tDCS stimulation can also reduce cravings for alcohol and cigarettes. Klauss et al. (2018b) showed that bilateral DLPFC tDCS stimulation significantly reduced alcohol cravings and reduced recurrence rates. Fecteau et al. (2014) found that the number of cigarettes consumed decreased significantly after bilateral DLPFC stimulation, and the effect could last for 4 days after the stimulation. Besides, non-substance addiction, such as food addiction, gambling addiction and internet addiction, shows executive function (such as decision-making and risk- taking processes) and working memory deficits similar to those in drug addiction (Fernandez-Serrano et al., 2010; Marazziti et al., 2014; Potenza, 2014). Studies have shown that anode tDCS stimulation of the right DLPFC decreased craving and negative emotions in addicted internet gaming players (Wu et al., 2020). Fregni et al. (2008b) found that the bilateral tDCS stimulation, left anode/right cathode or right anode/left cathode, reduced the food craving as well.

In **Table 3**, we summarized 21 studies evaluated tDCS treatment in substance addiction. Four studies didn't observe any improvement after tDCS treatment. All other studies showed tDCS reduced craving, improved behavioral control and reduced likelihood of relapse. Most studies used 25–35 cm^2 electrodes. For stimulating current intensity, 14 studies used 2 mA current, and 7 studies used a lower current. For stimulation duration, 4 studies used 10∼15 min/session, other studies used 20 min/session. There are 18 studies applied stimulation sessions from 1 to 4, and three of these studies showed no positive effects the rest studies used stimulation sessions from 5 to 20, which induced significant improvement of addiction symptoms except for one study. Roughly half of the studies placed anodal electrode on the right DLPFC, and the other half on the left. A couple of studies tried both montages. Together, tDCS of the DLPFC (left and/or right) has the potential to improve symptoms and reduce craving in substance addiction.

Attention Deficit Hyperactivity Disorder

Attention Deficit Hyperactivity Disorder (ADHD) is a brain disorder that characterized with inattention, impulsivity, hyperactivity and learning disabilities. ADHD mainly occurs in primary and middle schools (6–17 years old), and the prevalence is as high as over 6% (Rowland et al., 2015). The prevalence of ADHD is higher in boys than girls, and the risk for premature infants is also higher (Polanczyk et al., 2015). Neuroimaging studies have shown that the symptoms in ADHD patients may be related to abnormalities in fronto–striato–cerebellar neural circuit, especially the prefrontal lobe (Cubillo et al., 2012; Christakou et al., 2013). Specifically, the activity of bilateral striato-thalamus, left DLPFC and superior parietal cortex was significantly reduced in ADHD patients, and the activity of precuneus was significantly increased (Hart et al., 2013). Adults with childhood ADHD showed reduced activation in bilateral inferior prefrontal cortex, caudate and thalamus compared to controls. Neuro-functional abnormalities in ADHD patients are likely to persist from childhood to

TABLE 2 | Effects of tDCS of DLPFC on schizophrenia.

References	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
	Anode (+)	Cathode (–)						
Weickert et al., 2019	Right DLPFC (F4)	Left Temporoparietal junction	35	2	20	1/day, 4 weeks	20	Improved language-based working memory after 2 weeks, and oral fluency after 2 and 4 weeks significantly
Bose et al., 2015	Right DLPFC (a point midway between F4 and FP2)	Right left temporoparietal junction	35	2	20	2/day × 9 days	18	Right DLPFC tDCS reduced auditory hallucinations, but no change after left DLPFC tDCS
	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction						
Fitzgerald et al., 2014	Left DLPFC (F3)	Left temporo-parietal junction (unilaterally F3/TP3 or bilaterally F3 + F4/TP3 + TP4)	35	2	20	1/day, 3 weeks	15	No significant effect
Brunelin et al., 2012a	Left DLPFC (F3)	Left temporo-parietal cortex	35	2	20	2/day × 5 days	10	Reduced AVH significantly (lasted for 3 months after treatment), improved negative symptoms
Brunelin et al., 2012b	Left DLPFC (F3)	Left temporo-parietal cortex	35	2	20	2/day × 5 days	10	Have a significant effect (lasted for 3 months after treatment)
Shiozawa et al., 2013	Left DLPFC (F3)	Cathode: right DLPFC (F4)	35	2	20	1/day × 10 days	10	Improved catatonic symptoms significantly (remained for 4 weeks after treatment)
Jacks et al., 2014	Left DLPFC (F3)	Left temporo-parietal cortex	/	2	20	2/day × 5 days	10	Improved mood, feelings of hope, and fewer AVH, but no change in PANSS score
Jeon et al., 2018	Left DLPFC (F3)	Right DLPFC (F4)	25	2	30	1/day, 2 weeks	10	Improved working memory over time
Valiengo et al., 2020	Left DLPFC (F3)	Left temporoparietal junction	35	2	20	2/day × 5 days	10	Improved PANSS score significantly
Narayanaswamy et al., 2014	Left DLPFC (F3)	Cathode: left temporo-parietal cortex	/	2	20	2/day × 5 days	10	Improved in negative symptoms and AVH significantly (lasted for 6 months after treatment)
Palm et al., 2016	Left DLPFC (F3)	Right contralateral superior orbital region	35	2	20	1/day, 2 weeks	10	Improved negative and positive symptoms significantly
Palm et al., 2013	Left DLPFC	Right contralateral superior orbital region	/	2	20	1/day × 10 days	10	Improved negative and positive symptoms significantly
Brunelin et al., 2015	Left DLPFC	Left temporoparietal junction	35	2	20	2/day × 5 days	10	Reduced AVH significantly
Bose et al., 2014	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	35	2	20	2/day × 5 days	10	Improved insight and reduced AVH
Mondino et al., 2015	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	35	2	20	2/day × 5 days	10	Reduced AVH significantly
Mondino et al., 2016	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	35	2	20	2/day × 5 days	10	Improved in negative symptoms and AVH significantly

(Continued)

TABLE 2 | (Continued)

References	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
	Anode (+)	Cathode (–)						
Nawani et al., 2014a	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	/	2	20	2/day × 5 days	10	Have a significant reduction in AHRS score
Rakesh et al., 2013	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	/	2	20	2/day × 5 days	10	Reduced AVH significantly
Shenoy et al., 2015	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	/	2	20	2/day × 5 days	10	Reduced AVH significantly (lasted for 1 month after treatment)
Chang et al., 2019	Left DLPFC (a point midway between F3 and FP1)	Left temporo-parietal junction	35	2	20	2/day × 5 days	10	Improved overall symptoms
Chang et al., 2020	Left DLPFC (a point midway between F3 and FP1) + right DLPFC (a point midway between F4 and Fp2)	Forearms	35	2	20	2/day × 5 days	10	Reduced AVH significantly (lasted for 3 months after treatment)
Homan et al., 2011	Left temporo-parietal cortex	Right supraorbital area	35	1	15	1/day, 2 weeks	10	Reduced AVH significantly (lasted for 6 weeks after treatment)
Praharaj et al., 2015	Left DLPFC (F3)	Midway between T3 and P3	25	2	20	1/day × 5 days	5	Reduced AVH temporarily
Nawani et al., 2014b	Left prefrontal	Left temporoparietal	/	2	20	1/day × 5 days	5	Reduced AVH significantly
Smith et al., 2015	Left DLPFC (F3)	Right contralateral superior orbital region	5.08	2	20	1/day × 5 days	5	Improved memory, attention, and cognitive function significantly
Frohlich et al., 2016	Left DLPFC (a point midway between F3 and FP1)	Left temporoparietal junction	35	2	20	1/day × 5 days	5	Reduced AVH, but overall symptoms did not change significantly
Schilling et al., 2021	Left DLPFC (F3)	FP2	25	2	20	1/day	1	No enhancement in executive functions
Hoy et al., 2014	Left DLPFC (F3)	Right contralateral superior orbital region	35	1/2	20	1/day	1	Improved cognitive performance only after 2 mA tDCS

adulthood (Cubillo et al., 2010). fMRI studies also showed that striatum activation was abnormal in ADHD children (Durston et al., 2003).

In recent years, tDCS has been considered to have an ameliorative effect on ADHD symptoms. Studies have shown that 1 mA anode tDCS of the left DLPFC improved the executive function in adolescent ADHD patients. After tDCS, they showed better inhibitory control, interference control, working memory and cognitive flexibility (Nejati et al., 2020). Blair's research showed that inhibitory control is the main executive problem for adolescents with ADHD, and the problems with inhibitory control will lead to dysfunctions in memory, emotion regulation and other executive functions (Blair and Razza, 2007). tDCS improves the symptoms not only in adolescent patients, but also in adult ADHD patients. Left DLPFC tDCS in adult ADHD patients improved the impulsiveness symptoms (Allenby et al., 2018), and bilateral tDCS (anode over right DLPFC, cathode over left DLPFC) improved the inattention symptoms (Cachoeira et al., 2017). Only several studies were collected here which were shown in **Table 4**. All these studies targeted left DLPFC with

TABLE 3 | Effects of DLPFC tDCS on addiction behaviors.

References	Substance	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
		Anode (+)	Cathode (–)						
Ghorbani Behnam et al., 2019	Smoking	Left DLPFC (F3)	Right DLPFC (F4)	35/100	2	20	1/day, 4 weeks	20	Reduced smoking addiction only in active group (20 sessions, 12 weeks). The effect was similar to 300 g bupropion
							1/day, 2 weeks + 1/week, 10 weeks	20	
Mondino et al., 2018	Smoking	Right DLPFC (F4)	Left occipital region	35/100	2	20	2/day × 5 days	10	Reduced smoking cue related craving significantly and increased brain reactivity in the right posterior cingulate cortex
Klauss et al., 2018a	Cocaine	Right DLPFC (F4)	Left DLPFC (F3)	35	2	20	1/every other day	10	No significant effect
Klauss et al., 2018b	Alcohol	Right DLPFC (F4)	Left DLPFC (F3)	35	2	20	1/every other day	10	Reduced alcohol cravings and recurrence rates significantly
da Silva et al., 2013	Alcohol	Left DLPFC (F3)	Contralateral (right) supradeltoid area	35	2	20	1/day × 5 days	5	Improved depressive symptoms and reduced alcohol craving
Holla et al., 2020	Alcohol	Right DLPFC (F4)	Left DLPFC (F3)	35	2	20	1/day × 5 days	5	Increase the global efficiency of brain networks significantly with a concurrent significant reduction in global clustering
Batista et al., 2015	Cocaine	Left DLPFC (F3)	Right DLPFC (F4)	35	2	20	1/every other day	5	Decreased craving for crack-cocaine use, anxiety, and improved quality of life
Vitor de Souza Brangioni et al., 2018	Smoking	Left DLPFC (F3)	Right supra-orbital area	35	1	20	1/day × 5 days	5	Reduced cigarette consumption up to 4-weeks post-intervention coupled with high motivation to quite
Boggio et al., 2009	Smoking	Left DLPFC (F3)	Right DLPFC (F4)	35/100	2	20	1/day × 5 days	5	A significant cumulative effect on modifying smoking cue-provoked craving, with significant decrease in the number of cigarettes
Fecteau et al., 2014	Smoking	Right DLPFC (F4)	Left DLPFC (F3)	35	2	30	1/day × 4 days	4	Decreased the amount of smoking significantly (lasted for 4 days after stimulation)
den Uyl et al., 2017	Alcohol	Left DLPFC (F3)	Right DLPFC (F4)	35/100	2	20	1/day × 4 days	4	No significant effect
den Uyl et al., 2016	Alcohol	Contralateral supraorbital region	Left DLPFC (F3)	35	1	15	1/day × 3 days	3	Decreased cue-induced craving (but not overall craving) on post assessment, but no effects on cognitive bias modification (CBM)
Alghamdi et al., 2019	Smoking	Left DLPFC (F3)	Right DLPFC (F4)	25	1.5	20	1/day × 3 days	3	No significant effect

(Continued)

TABLE 3 | (Continued)

References	Substance	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
		Anode (+)	Cathode (–)						
Boggio et al., 2008b	Alcohol	Right DLPFC (F4)	Left DLPFC (F3)	35	2	20	1/day	1	Reduced alcohol craving significantly in two active stimulation groups, and alcohol craving did not increase further after treatment
		left DLPFC (F3)	Right DLPFC (F4)						
den Uyl et al., 2015	Alcohol	Left DLPFC (F3)	Contralateral supraorbital region	35	1	10	1/day	1	Anodal tDCS over the DLPFC reduced alcohol craving significantly, stimulation of the IFG did not decrease craving
		Right inferior frontal gyrus (IFG)	Contralateral supraorbital region						
Wietschorke et al., 2016	Alcohol	Right DLPFC (F4)	Left DLPFC (F3)	35	1	20	1/day	1	Reduced alcohol craving
		Left DLPFC (F3)	Right DLPFC (F4)						
Fregni et al., 2008a	Smoking	Left DLPFC (F3)	Contralateral hemisphere	35/100	2	20	1/day	1	Both anodal and cathodal tDCS to left DLPFC significantly reduced craving
		Right DLPFC (F4)	Contralateral hemisphere						
Xu et al., 2013	Smoking	Left DLPFC (F3)	Right supra-orbital area	35	2	20	1/day	1	Reduced negative emotions, but no reduction in cigarette craving
Kroczek et al., 2016	Smoking	Left DLPFC (F3)	Contralateral right supradeltoid area	35	2	15	1/day	1	No significant effect
Falcone et al., 2016	Smoking	Left DLPFC (F3)	Right supra-orbital area	25	1	20	1/day	1	Increased latency to smoke and decreased the total number of cigarettes smoked significantly
Gorini et al., 2014	Cocaine	Left DLPFC (F3)	Right DLPFC (F4)	32	1.5	20	1/day	1	Increased safe behavior after right DLPFC anodal stimulation, increased risk-taking behavior after left DLPFC anodal stimulation
		Right DLPFC (F4)	Left DLPFC (F3)						

anodal stimulation. One out of six studies (used a single session protocol) showed negative results, and all the rest found tDCS improved ADHD related symptoms. The stimulation current was 1 mA or 2 mA, 1 session to 5 sessions in total. While the potential of tDCS of the DLPFC to treat ADHD is promising, the published studies are relatively fewer compared to other diseases.

Anxiety

Anxiety disorders are the most common form of emotional disorder characterized by nervousness, worry and fear. There are several types of anxiety disorders, including generalized anxiety disorder (GAD), Social anxiety disorder (SAD), post-traumatic stress disorder (PTSD), panic disorder (PD), obsessive compulsive disorder (OCD), agoraphobe and specific phobia. Studies have shown that OCD symptoms are related to the cortico-striato-thalamocortical circuitry, including DLPFC, orbital frontal lobe (OFC), medial prefrontal lobe (MPF), and anterior cingulate cortex (ACC; Del Casale et al., 2011; Fineberg et al., 2011). Striatal dysfunction may lead to hypothalamic gating problems and hyperactivity in the

TABLE 4 | Effects of DLPFC tDCS on ADHD.

References	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
	Anode (+)	Cathode (–)						
Soff et al., 2017	DLPFC (F3)	Vertex	3.14/12.5	1	20	1/day × 5 days	5	Improved inattention and impulsivity, and the effect lasted for 7 days
Cachoeira et al., 2017	DLPFC (F3)	DLPFC (F4)	35	2	20	1/day × 5 days	5	Improved inattention
Allenby et al., 2018	DLPFC (F3)	Supra-orbital area	25	2	20	3/week	3	Improved impulsivity symptoms acutely (conners continuous performance task) but not the stop signal task
Dubreuil-Vall et al., 2021	DLPFC (F3)	Contralateral Supraorbital region (Fp1 or Fp2)	3.14	2	30	1/day	1	Modulated reaction time and P300 amplitude in the Eriksen flanker task, but not in the stop signal task
	DLPFC (F4)							
Cosmo et al., 2015	DLPFC (F3)	Right DLPFC (F4)	35	1	20	1/day	1	No significant differences in behavioral performance
Gogler et al., 2017	DLPFC (F3)	Right contralateral Superior orbital region	25	2	20	1/day	1	Improved inattention

TABLE 5 | Effects of tDCS on OCD and anxiety.

References	Disease	Electrode montage		Electrode size (cm^2)	Current intensity (mA)	Stimulation duration (min)	Stimulation sessions	Total sessions	Key findings
		Anode (+)	Cathode (–)						
Narayanaswamy et al., 2015	OCD	Fz2	Right supra-orbital area	35	2	20	2/day × 10 days	20	Clinical improvement, enhanced pre-SMA/SMA activation
D'Urso et al., 2016a	OCD	Presupplementary motor area (pre-SMA)	Right deltoid	35	2	20	1/day × 20 days	20	Improved OCD symptoms
Shiozawa et al., 2014	OCD	Contralateral deltoid	Right DLPFC	25	2	20	1/day, 3 weeks	15	Improved anxiety symptoms
Volpato et al., 2013	OCD	Posterior neck-base	Left DLPFC (F3)	35	2	20	1/day, 10 days	10	Improved depression and anxiety, reduced interhemispheric imbalance
Ahmadizadeh et al., 2019	PTSD	DLPFC (F3)	Right DLPFC	35	2	20	1/day, 2 weeks	10	Reduced PTSD symptoms, hyper-arousal and negative alterations in cognition and mood sub-symptoms as well as depressive and anxiety symptoms
Jafari et al., 2021	SAD	DLPFC (F3)	Medial PFC (Fpz)	35	1/2	20	2/day × 5 days	10	Reduced fear/avoidance symptoms, worries and improved emotion regulation
de Lima et al., 2019	GAD	DLPFC (F3)	Contralateral supraorbital area (Fp2)	35	2	20	1/day, week	5	Improved in physical symptoms significantly, but no improvements in anxiety, mood symptoms of stress, affectivity, or depression
Heeren et al., 2017	SAD	DLPFC (F3)	Vertically at the ipsilateral arm	35	2	25	1/day	1	Decreased attentional bias

orbitofrontal cortex and anterior cingulate cortex in OCD patients (Milad and Rauch, 2012). Sakai et al. (2011) found that functional connections of the orbitofrontal cortex, medial prefrontal cortex, DLPFC and ventral striatum were significantly increased in patients with OCD, but there was no significant correlation between symptom severity and connection strength. D'Urso et al. (2016b) reported that patients received cathode stimulation over the left DLPFC showed significant improvement in OCD symptoms.

Generalized anxiety disorder is characterized by persistent unspecified nervousness, excessive anxiety and worry about everyday life events (Locke et al., 2015; Stein et al., 2017). Previous studies have shown that brain regions related to rumination and introspection in GAD patients were overactivated (Locke et al., 2015). Patients also showed autonomic nervous dysfunction, vagus-mediated decreased heart rate variability, and neurostructural abnormalities in the rostral ACC, left medial orbitofrontal cortex, and right isthmic cingulate gyrus (Etkin and Wager, 2007; Carnevali et al., 2019). Neuroplasticity in prefrontal and limbic regions is also altered in patients with a variety of subtypes of anxiety disorders (Ironside et al., 2019). Vicario et al. (2019) reviewed the using of non-invasive brain stimulation techniques for the treatment of anxiety previously. A study showed that stimulation of the left DLPFC with 2 mA tDCS significantly improved physical stress symptoms in patients, however, there was no significant improvement in major psychological symptoms, such as anxiety, tension, emotion, or depression (de Lima et al., 2019). In another case report, a total of 15 sessions of 2 mA cathode tDCS stimulation improved anxiety symptoms in patients with GAD (Shiozawa et al., 2014).

Social anxiety disorder is an anxiety disorder characterized by extreme fear in getting involved in social interactions. Studies have shown that patients with SAD have attentional bias brought by social threats, and the attentional bias will increase the anxiety of patients with SAD (Klosowska et al., 2015). Anode tDCS of the left DLPFC significantly reduced attentional bias compared to the sham stimulation (Heeren et al., 2017). In addition, a single dose of 1 mA of tDCS reduced pain anxiety caused by burns (Hosseini Amiri et al., 2016), and improved anxiety symptoms caused by major depression (Nishida et al., 2019). Although there are only a few studies on the tDCS treatment of anxiety, these findings indicate that this technique can be an effective therapeutic option. We have summarized some of the published studies in **Table 5**.

SUMMARY AND OUTLOOK

In recent years, tDCS is increasingly being studied for the therapeutic potential in neurological and psychiatric disorders. DLPFC is involved in many higher brain functions such as working memory, decision making, impulsivity, attention, etc. DLPFC also plays an important role in cognition and emotion. These brain functions were often disrupted in neurological and psychiatric diseases. Thus, modulation of the activity of DLPFC is a major strategy in treatment of these diseases. Although the neural mechanisms of tDCS is still not quite clear. It is believed that anodal stimulation increases brain activity while cathodal stimulation inhibits brain activity. One of the major problems of tDCS treatment of neuropsychiatric diseases is that each study used slightly different stimulation parameters. For instance, the current intensities were from 1 to 2 mA, tDCS sessions were from one session to more than 20 sessions. The tDCS frequency varies from twice daily to once every other day. Thus, it's not appropriate to compare the current results directly side by side. Future studies will need to investigate the effects of tDCS using the different parameters in the same study or the same parameters in different studies. Nevertheless, this review demonstrates clearly that tDCS of DLPFC has a great potential to treat neuropsychiatric disorders.

AUTHOR CONTRIBUTIONS

CL and ZM discussed and initiated the review topic and edited the manuscript substantially. QL drafted the manuscript. All authors interpreted the results together, revised the manuscript critically, and contributed to the article and approved the submitted version.

FUNDING

This work was supported by the Science and Technology Innovation 2030-Major Project (2021ZD0202103), National Natural Science Foundation of China (U20A2017, U20A6005, and 32071009), Science, Technology and Innovation Commission of Shenzhen Municipality (ZDSYS20190902093601675, NYKFKT20190020, and ZDSYS20200811142401005), Key Laboratory of Brain Connectome and Manipulation, Chinese Academy of Sciences (2019DP173024), and Natural Science Foundation of Guangdong Province (2020A1515011055).

REFERENCES

Ahmadizadeh, M. J., Rezaei, M., and Fitzgerald, P. B. (2019). Transcranial direct current stimulation (tDCS) for post-traumatic stress disorder (PTSD): a randomized, double-blinded, controlled trial. *Brain Res. Bull.* 153, 273–278. doi: 10.1016/j.brainresbull.2019.09.011

Alghamdi, F., Alhussien, A., Alohali, M., Alatawi, A., Almusned, T., Fecteau, S., et al. (2019). Effect of transcranial direct current stimulation on the number of smoked cigarettes in tobacco smokers. *PLoS One* 14:e0212312. doi: 10.1371/journal.pone.0212312

Allenby, C., Falcone, M., Bernardo, L., Wileyto, E. P., Rostain, A., Ramsay, J. R., et al. (2018). Transcranial direct current brain stimulation decreases impulsivity in ADHD. *Brain Stimul.* 11, 974–981. doi: 10.1016/j.brs.2018.04.016

Aparicio, L. V. M., Rosa, V., Razza, L. M., Sampaio-Junior, B., Borrione, L., Valiengo, L., et al. (2019). Transcranial direct current stimulation (tDCS) for preventing major depressive disorder relapse: results of a 6-month follow-up. *Depress. Anxiety* 36, 262–268. doi: 10.1002/da.22878

Ayache, S. S., Palm, U., Chalah, M. A., Al-Ani, T., Brignol, A., Abdellaoui, M., et al. (2016). Prefrontal tDCS decreases pain in patients with multiple sclerosis. *Front. Neurosci.* 10:147. doi: 10.3389/fnins.2016.00147

Barbey, A. K., Koenigs, M., and Grafman, J. (2013). Dorsolateral prefrontal contributions to human working memory. *Cortex* 49, 1195–1205. doi: 10.1016/j.cortex.2012.05.022

Barros Galvao, S. C., Borba Costa dos Santos, R., Borba dos Santos, P., Cabral, M. E., and Monte-Silva, K. (2014). Efficacy of coupling repetitive transcranial magnetic stimulation and physical therapy to reduce upper-limb spasticity in patients with stroke: a randomized controlled trial. *Arch. Phys. Med. Rehabil.* 95, 222–229. doi: 10.1016/j.apmr.2013.10.023

Batista, E. K., Klauss, J., Fregni, F., Nitsche, M. A., and Nakamura-Palacios, E. M. (2015). A randomized placebo-controlled trial of targeted prefrontal cortex modulation with bilateral tDCS in patients with crack-cocaine dependence. *Int. J. Neuropsychopharmacol.* 18:pyv066. doi: 10.1093/ijnp/pyv066

Bennabi, D., Nicolier, M., Monnin, J., Tio, G., Pazart, L., Vandel, P., et al. (2015). Pilot study of feasibility of the effect of treatment with tDCS in patients suffering from treatment-resistant depression treated with escitalopram. *Clin. Neurophysiol.* 126, 1185–1189. doi: 10.1016/j.clinph.2014.09.026

Berke, J. D., and Hyman, S. E. (2000). Addiction, dopamine, and the molecular mechanisms of memory. *Neuron* 25, 515–532. doi: 10.1016/s0896-6273(00)81056-9

Bidet-Caulet, A., Buchanan, K. G., Viswanath, H., Black, J., Scabini, D., Bonnet-Brilhault, F., et al. (2015). Impaired facilitatory mechanisms of auditory attention after damage of the lateral prefrontal cortex. *Cereb. Cortex* 25, 4126–4134. doi: 10.1093/cercor/bhu131

Bikson, M., Inoue, M., Akiyama, H., Deans, J. K., Fox, J. E., Miyakawa, H., et al. (2004). Effects of uniform extracellular DC electric fields on excitability in rat hippocampal slices in vitro. *J. Physiol.* 557(Pt 1), 175–190. doi: 10.1113/jphysiol.2003.055772

Blair, C., and Razza, R. P. (2007). Relating effortful control, executive function, and false belief understanding to emerging math and literacy ability in kindergarten. *Child. Dev.* 78, 647–663. doi: 10.1111/j.1467-8624.2007.01019.x

Blumberger, D. M., Tran, L. C., Fitzgerald, P. B., Hoy, K. E., and Daskalakis, Z. J. (2012). A randomized double-blind sham-controlled study of transcranial direct current stimulation for treatment-resistant major depression. *Front. Psychiatry* 3:74. doi: 10.3389/fpsyt.2012.00074

Boggio, P. S., Liguori, P., Sultani, N., Rezende, L., Fecteau, S., and Fregni, F. (2009). Cumulative priming effects of cortical stimulation on smoking cue-induced craving. *Neurosci. Lett.* 463, 82–86. doi: 10.1016/j.neulet.2009.07.041

Boggio, P. S., Rigonatti, S. P., Ribeiro, R. B., Myczkowski, M. L., Nitsche, M. A., Pascual-Leone, A., et al. (2008a). A randomized, double-blind clinical trial on the efficacy of cortical direct current stimulation for the treatment of major depression. *Int. J. Neuropsychopharmacol.* 11, 249–254. doi: 10.1017/S1461145707007833

Boggio, P. S., Sultani, N., Fecteau, S., Merabet, L., Mecca, T., Pascual-Leone, A., et al. (2008b). Prefrontal cortex modulation using transcranial DC stimulation reduces alcohol craving: a double-blind, sham-controlled study. *Drug Alcohol Depend.* 92, 55–60. doi: 10.1016/j.drugalcdep.2007.06.011

Boning, J. (2009). Addiction memory as a specific, individually learned memory imprint. *Pharmacopsychiatry* 42(Suppl. 1), S66–S68. doi: 10.1055/s-0029-1216357

Bose, A., Shivakumar, V., Narayanaswamy, J. C., Nawani, H., Subramaniam, A., Agarwal, S. M., et al. (2014). Insight facilitation with add-on tDCS in schizophrenia. *Schizophr. Res.* 156, 63–65. doi: 10.1016/j.schres.2014.03.029

Bose, A., Sowmya, S., Shenoy, S., Agarwal, S. M., Chhabra, H., Narayanaswamy, J. C., et al. (2015). Clinical utility of attentional salience in treatment of auditory verbal hallucinations in schizophrenia using transcranial direct current stimulation (tDCS). *Schizophr. Res.* 164, 279–280. doi: 10.1016/j.schres.2015.01.040

Brunelin, J., Hasan, A., Haesebaert, F., Nitsche, M. A., and Poulet, E. (2015). Nicotine smoking prevents the effects of frontotemporal transcranial direct current stimulation (tDCS) in hallucinating patients with schizophrenia. *Brain Stimul.* 8, 1225–1227. doi: 10.1016/j.brs.2015.08.002

Brunelin, J., Mondino, M., Gassab, L., Haesebaert, F., Gaha, L., Suaud-Chagny, M. F., et al. (2012a). Examining transcranial direct-current stimulation (tDCS) as a treatment for hallucinations in schizophrenia. *Am. J. Psychiatry* 169, 719–724. doi: 10.1176/appi.ajp.2012.11071091

Brunelin, J., Mondino, M., Haesebaert, F., Saoud, M., Suaud-Chagny, M. F., and Poulet, E. (2012b). Efficacy and safety of bifocal tDCS as an interventional treatment for refractory schizophrenia. *Brain Stimul.* 5, 431–432. doi: 10.1016/j.brs.2011.03.010

Brunoni, A. R., Boggio, P. S., De Raedt, R., Bensenor, I. M., Lotufo, P. A., Namur, V., et al. (2014). Cognitive control therapy and transcranial direct current stimulation for depression: a randomized, double-blinded, controlled trial. *J. Affect. Disord.* 162, 43–49. doi: 10.1016/j.jad.2014.03.026

Brunoni, A. R., Ferrucci, R., Bortolomasi, M., Scelzo, E., Boggio, P. S., Fregni, F., et al. (2013a). Interactions between transcranial direct current stimulation (tDCS) and pharmacological interventions in the major depressive episode: findings from a naturalistic study. *Eur. Psychiatry* 28, 356–361. doi: 10.1016/j.eurpsy.2012.09.001

Brunoni, A. R., Valiengo, L., Baccaro, A., Zanao, T. A., de Oliveira, J. F., Goulart, A., et al. (2013b). The sertraline vs. electrical current therapy for treating depression clinical study: results from a factorial, randomized, controlled trial. *JAMA Psychiatry* 70, 383–391. doi: 10.1001/2013.jamapsychiatry.32

Brunoni, A. R., Ferrucci, R., Bortolomasi, M., Vergari, M., Tadini, L., Boggio, P. S., et al. (2011). Transcranial direct current stimulation (tDCS) in unipolar vs. bipolar depressive disorder. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 35, 96–101. doi: 10.1016/j.pnpbp.2010.09.010

Brunoni, A. R., Moffa, A. H., Sampaio-Junior, B., Borrione, L., Moreno, M. L., Fernandes, R. A., et al. (2017). Trial of electrical direct-current therapy versus escitalopram for depression. *N. Engl. J. Med.* 376, 2523–2533. doi: 10.1056/NEJMoa1612999

Buhle, J. T., Silvers, J. A., Wager, T. D., Lopez, R., Onyemekwu, C., Kober, H., et al. (2014). Cognitive reappraisal of emotion: a meta-analysis of human neuroimaging studies. *Cereb. Cortex* 24, 2981–2990. doi: 10.1093/cercor/bht154

Cachoeira, C. T., Leffa, D. T., Mittelstadt, S. D., Mendes, L. S. T., Brunoni, A. R., Pinto, J. V., et al. (2017). Positive effects of transcranial direct current stimulation in adult patients with attention-deficit/hyperactivity disorder – a pilot randomized controlled study. *Psychiatry Res.* 247, 28–32. doi: 10.1016/j.psychres.2016.11.009

Carnevali, L., Mancini, M., Koenig, J., Makovac, E., Watson, D. R., Meeten, F., et al. (2019). Cortical morphometric predictors of autonomic dysfunction in generalized anxiety disorder. *Auton. Neurosci.* 217, 41–48. doi: 10.1016/j.autneu.2019.01.001

Carpenter, W. T., Blanchard, J. J., and Kirkpatrick, B. (2016). New standards for negative symptom assessment. *Schizophr. Bull.* 42, 1–3. doi: 10.1093/schbul/sbv160

Chang, C. C., Kao, Y. C., Chao, C. Y., and Chang, H. A. (2019). Enhancement of cognitive insight and higher-order neurocognitive function by fronto-temporal transcranial direct current stimulation (tDCS) in patients with schizophrenia. *Schizophr. Res.* 208, 430–438. doi: 10.1016/j.schres.2018.12.052

Chang, C. C., Kao, Y. C., Chao, C. Y., Tzeng, N. S., and Chang, H. A. (2020). Examining bi-anodal transcranial direct current stimulation (tDCS) over bilateral dorsolateral prefrontal cortex coupled with bilateral extracephalic references as a treatment for negative symptoms in non-acute schizophrenia patients: a randomized, double-blind, sham-controlled trial. *Prog. Neuropsychopharmacol. Biol. Psychiatry.* 96:109715. doi: 10.1016/j.pnpbp.2019.109715

Christakou, A., Murphy, C. M., Chantiluke, K., Cubillo, A. I., Smith, A. B., Giampietro, V., et al. (2013). Disorder-specific functional abnormalities during sustained attention in youth with attention deficit hyperactivity disorder (ADHD) and with autism. *Mol. Psychiatry* 18, 236–244. doi: 10.1038/mp.2011.185

Cosmo, C., Baptista, A. F., de Araujo, A. N., do Rosario, R. S., Miranda, J. G., Montoya, P., et al. (2015). A randomized, double-blind, sham-controlled trial of transcranial direct current stimulation in attention-deficit/hyperactivity disorder. *PLoS One* 10:e0135371. doi: 10.1371/journal.pone.0135371

Cubillo, A., Halari, R., Ecker, C., Giampietro, V., Taylor, E., and Rubia, K. (2010). Reduced activation and inter-regional functional connectivity of fronto-striatal networks in adults with childhood attention-deficit hyperactivity disorder (ADHD) and persisting symptoms during tasks of motor inhibition and cognitive switching. *J. Psychiatr. Res.* 44, 629–639. doi: 10.1016/j.jpsychires.2009.11.016

Cubillo, A., Halari, R., Smith, A., Taylor, E., and Rubia, K. (2012). A review of fronto-striatal and fronto-cortical brain abnormalities in children and adults with attention deficit hyperactivity disorder (ADHD) and new evidence for

dysfunction in adults with ADHD during motivation and attention. *Cortex* 48, 194–215. doi: 10.1016/j.cortex.2011.04.007

da Silva, M. C., Conti, C. L., Klauss, J., Alves, L. G., do Nascimento Cavalcante, H. M., Fregni, F., et al. (2013). Behavioral effects of transcranial direct current stimulation (tDCS) induced dorsolateral prefrontal cortex plasticity in alcohol dependence. *J. Physiol. Paris* 107, 493–502. doi: 10.1016/j.jphysparis.2013.07.003

Dalong, G., Jiyuan, L., Ying, Z., Lei, Z., Yanhong, H., and Yongcong, S. (2020). Transcranial direct current stimulation reconstructs diminished thalamocortical connectivity during prolonged resting wakefulness: a resting-state fMRI pilot study. *Brain Imaging Behav.* 14, 278–288. doi: 10.1007/s11682-018-9979-9

de Lima, A. L., Braga, F. M. A., da Costa, R. M. M., Gomes, E. P., Brunoni, A. R., and Pegado, R. (2019). Transcranial direct current stimulation for the treatment of generalized anxiety disorder: a randomized clinical trial. *J. Affect. Disord.* 259, 31–37. doi: 10.1016/j.jad.2019.08.020

Del Casale, A., Kotzalidis, G. D., Rapinesi, C., Serata, D., Ambrosi, E., Simonetti, A., et al. (2011). Functional neuroimaging in obsessive-compulsive disorder. *Neuropsychobiology* 64, 61–85. doi: 10.1159/000325223

Dell'Osso, B., Zanoni, S., Ferrucci, R., Vergari, M., Castellano, F., D'Urso, N., et al. (2012). Transcranial direct current stimulation for the outpatient treatment of poor-responder depressed patients. *Eur. Psychiatry* 27, 513–517. doi: 10.1016/j.eurpsy.2011.02.008

den Uyl, T. E., Gladwin, T. E., Rinck, M., Lindenmeyer, J., and Wiers, R. W. (2017). A clinical trial with combined transcranial direct current stimulation and alcohol approach bias retraining. *Addict. Biol.* 22, 1632–1640. doi: 10.1111/adb.12463

den Uyl, T. E., Gladwin, T. E., and Wiers, R. W. (2015). Transcranial direct current stimulation, implicit alcohol associations and craving. *Biol. Psychol.* 105, 37–42. doi: 10.1016/j.biopsycho.2014.12.004

den Uyl, T. E., Gladwin, T. E., and Wiers, R. W. (2016). Electrophysiological and behavioral effects of combined transcranial direct current stimulation and alcohol approach bias retraining in hazardous drinkers. *Alcohol Clin. Exp. Res.* 40, 2124–2133. doi: 10.1111/acer.13171

Dubreuil-Vall, L., Gomez-Bernal, F., Villegas, A. C., Cirillo, P., Surman, C., Ruffini, G., et al. (2021). Transcranial direct current stimulation to the left dorsolateral prefrontal cortex improves cognitive control in patients with attention-deficit/hyperactivity disorder: a randomized behavioral and neurophysiological study. *Biol. Psychiatry Cogn. Neurosci. Neuroimaging* 6, 439–448. doi: 10.1016/j.bpsc.2020.11.006

D'Urso, G., Brunoni, A. R., Anastasia, A., Micillo, M., de Bartolomeis, A., and Mantovani, A. (2016a). Polarity-dependent effects of transcranial direct current stimulation in obsessive-compulsive disorder. *Neurocase* 22, 60–64. doi: 10.1080/13554794.2015.1045522

D'Urso, G., Brunoni, A. R., Mazzaferro, M. P., Anastasia, A., de Bartolomeis, A., and Mantovani, A. (2016b). Transcranial direct current stimulation for obsessive-compulsive disorder: a randomized, controlled, partial crossover trial. *Depress. Anxiety* 33, 1132–1140. doi: 10.1002/da.22578

Durston, S., Tottenham, N. T., Thomas, K. M., Davidson, M. C., Eigsti, I. M., Yang, Y., et al. (2003). Differential patterns of striatal activation in young children with and without ADHD. *Biol. Psychiatry* 53, 871–878. doi: 10.1016/s0006-3223(02)01904-2

Etkin, A., and Wager, T. D. (2007). Functional neuroimaging of anxiety: a meta-analysis of emotional processing in PTSD, social anxiety disorder, and specific phobia. *Am. J. Psychiatry* 164, 1476–1488. doi: 10.1176/appi.ajp.2007.07030504

Falcone, M., Bernardo, L., Ashare, R. L., Hamilton, R., Faseyitan, O., McKee, S. A., et al. (2016). Transcranial direct current brain stimulation increases ability to resist smoking. *Brain Stimul.* 9, 191–196. doi: 10.1016/j.brs.2015.10.004

Fecteau, S., Agosta, S., Hone-Blanchet, A., Fregni, F., Boggio, P., Ciraulo, D., et al. (2014). Modulation of smoking and decision-making behaviors with transcranial direct current stimulation in tobacco smokers: a preliminary study. *Drug Alcohol Depend.* 140, 78–84. doi: 10.1016/j.drugalcdep.2014.03.036

Fernandez-Serrano, M. J., Perez-Garcia, M., Perales, J. C., and Verdejo-Garcia, A. (2010). Prevalence of executive dysfunction in cocaine, heroin and alcohol users enrolled in therapeutic communities. *Eur. J. Pharmacol.* 626, 104–112. doi: 10.1016/j.ejphar.2009.10.019

Fineberg, N. A., Chamberlain, S. R., Hollander, E., Boulougouris, V., and Robbins, T. W. (2011). Translational approaches to obsessive-compulsive disorder: from animal models to clinical treatment. *Br. J. Pharmacol.* 164, 1044–1061. doi: 10.1111/j.1476-5381.2011.01422.x

Fitzgerald, P. B., McQueen, S., Daskalakis, Z. J., and Hoy, K. E. (2014). A negative pilot study of daily bimodal transcranial direct current stimulation in schizophrenia. *Brain Stimul.* 7, 813–816. doi: 10.1016/j.brs.2014.08.002

Fitzpatrick, S., Gilbert, S., and Serpell, L. (2013). Systematic review: are overweight and obese individuals impaired on behavioural tasks of executive functioning? *Neuropsychol. Rev.* 23, 138–156. doi: 10.1007/s11065-013-9224-7

Francis, J. T., Gluckman, B. J., and Schiff, S. J. (2003). Sensitivity of neurons to weak electric fields. *J. Neurosci.* 23, 7255–7261. doi: 10.1523/JNEUROSCI.23-19-07255.2003

Frank, D. W., Dewitt, M., Hudgens-Haney, M., Schaeffer, D. J., Ball, B. H., Schwarz, N. F., et al. (2014). Emotion regulation: quantitative meta-analysis of functional activation and deactivation. *Neurosci. Biobehav. Rev.* 45, 202–211. doi: 10.1016/j.neubiorev.2014.06.010

Fregni, F., Liguori, P., Fecteau, S., Nitsche, M. A., Pascual-Leone, A., and Boggio, P. S. (2008a). Cortical stimulation of the prefrontal cortex with transcranial direct current stimulation reduces cue-provoked smoking craving: a randomized, sham-controlled study. *J. Clin. Psychiatry* 69, 32–40. doi: 10.4088/jcp.v69n0105

Fregni, F., Orsati, F., Pedrosa, W., Fecteau, S., Tome, F. A., Nitsche, M. A., et al. (2008b). Transcranial direct current stimulation of the prefrontal cortex modulates the desire for specific foods. *Appetite* 51, 34–41. doi: 10.1016/j.appet.2007.09.016

Fritsch, B., Reis, J., Martinowich, K., Schambra, H. M., Ji, Y., Cohen, L. G., et al. (2010). Direct current stimulation promotes BDNF-dependent synaptic plasticity: potential implications for motor learning. *Neuron* 66, 198–204. doi: 10.1016/j.neuron.2010.03.035

Frohlich, F., Burrello, T. N., Mellin, J. M., Cordle, A. L., Lustenberger, C. M., Gilmore, J. H., et al. (2016). Exploratory study of once-daily transcranial direct current stimulation (tDCS) as a treatment for auditory hallucinations in schizophrenia. *Eur. Psychiatry* 33, 54–60. doi: 10.1016/j.eurpsy.2015.11.005

Ghorbani Behnam, S., Mousavi, S. A., and Emamian, M. H. (2019). The effects of transcranial direct current stimulation compared to standard bupropion for the treatment of tobacco dependence: a randomized sham-controlled trial. *Eur. Psychiatry* 60, 41–48. doi: 10.1016/j.eurpsy.2019.04.010

Glasser, M. F., Coalson, T. S., Robinson, E. C., Hacker, C. D., Harwell, J., Yacoub, E., et al. (2016). A multi-modal parcellation of human cerebral cortex. *Nature* 536, 171–178. doi: 10.1038/nature18933

Gogler, N., Willacker, L., Funk, J., Strube, W., Langgartner, S., Napiorkowski, N., et al. (2017). Single-session transcranial direct current stimulation induces enduring enhancement of visual processing speed in patients with major depression. *Eur. Arch. Psychiatry Clin. Neurosci.* 267, 671–686. doi: 10.1007/s00406-016-0761-y

Goldstein, R. Z., and Volkow, N. D. (2002). Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. *Am. J. Psychiatry* 159, 1642–1652. doi: 10.1176/appi.ajp.159.10.1642

Gordon, P. C., Valiengo, L., de Paula, V. J. R., Galhardoni, R., Ziemann, U., de Andrade, D. C., et al. (2019). Changes in motor cortical excitability in schizophrenia following transcranial direct current stimulation. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 90, 43–48. doi: 10.1016/j.pnpbp.2018.11.004

Gorini, A., Lucchiari, C., Russell-Edu, W., and Pravettoni, G. (2014). Modulation of risky choices in recently abstinent dependent cocaine users: a transcranial direct-current stimulation study. *Front. Hum. Neurosci.* 8:661. doi: 10.3389/fnhum.2014.00661

Hart, H., Radua, J., Nakao, T., Mataix-Cols, D., and Rubia, K. (2013). Meta-analysis of functional magnetic resonance imaging studies of inhibition and attention in attention-deficit/hyperactivity disorder: exploring task-specific, stimulant medication, and age effects. *JAMA Psychiatry* 70, 185–198. doi: 10.1001/jamapsychiatry.2013.277

Hecht, D. (2010). Depression and the hyperactive right-hemisphere. *Neurosci. Res.* 68, 77–87. doi: 10.1016/j.neures.2010.06.013

Heeren, A., Billieux, J., Philippot, P., De Raedt, R., Baeken, C., de Timary, P., et al. (2017). Impact of transcranial direct current stimulation on attentional bias for threat: a proof-of-concept study among individuals with social anxiety disorder. *Soc. Cogn. Affect. Neurosci.* 12, 251–260. doi: 10.1093/scan/nsw119

Hill, A. T., Fitzgerald, P. B., and Hoy, K. E. (2016). Effects of anodal transcranial direct current stimulation on working memory: a systematic review and meta-analysis of findings from healthy and neuropsychiatric populations. *Brain Stimul.* 9, 197–208. doi: 10.1016/j.brs.2015.10.006

Holla, B., Biswal, J., Ramesh, V., Shivakumar, V., Bharath, R. D., Benegal, V., et al. (2020). Effect of prefrontal tDCS on resting brain fMRI graph measures in alcohol use disorders: a randomized, double-blind, sham-controlled study. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 102:109950. doi: 10.1016/j.pnpbp.2020.109950

Homan, P., Kindler, J., Federspiel, A., Flury, R., Hubl, D., Hauf, M., et al. (2011). Muting the voice: a case of arterial spin labeling-monitored transcranial direct current stimulation treatment of auditory verbal hallucinations. *Am. J. Psychiatry* 168, 853–854. doi: 10.1176/appi.ajp.2011.11030496

Hosseini Amiri, M., Tavousi, S. H., Mazlom, S. R., and Manzari, Z. S. (2016). Effect of transcranial direct current stimulation on pain anxiety during burn wound care. *Burns* 42, 872–876. doi: 10.1016/j.burns.2016.01.006

Hoy, K. E., Arnold, S. L., Emonson, M. R., Daskalakis, Z. J., and Fitzgerald, P. B. (2014). An investigation into the effects of tDCS dose on cognitive performance over time in patients with schizophrenia. *Schizophr. Res.* 155, 96–100. doi: 10.1016/j.schres.2014.03.006

Hyman, S. E., and Malenka, R. C. (2001). Addiction and the brain: the neurobiology of compulsion and its persistence. *Nat. Rev. Neurosci.* 2, 695–703. doi: 10.1038/35094560

Hyman, S. E., Malenka, R. C., and Nestler, E. J. (2006). Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu. Rev. Neurosci.* 29, 565–598. doi: 10.1146/annurev.neuro.29.051605.113009

Ironside, M., Browning, M., Ansari, T. L., Harvey, C. J., Sekyi-Djan, M. N., Bishop, S. J., et al. (2019). Effect of prefrontal cortex stimulation on regulation of amygdala response to threat in individuals with trait anxiety: a randomized clinical trial. *JAMA Psychiatry* 76, 71–78. doi: 10.1001/jamapsychiatry.2018.2172

Jacks, S., Kalivas, B., Mittendorf, A., Kindt, C., and Short, E. B. (2014). Transcranial direct-current stimulation as an adjunct to electroconvulsive therapy and clozapine for refractory psychosis. *Prim. Care Companion CNS Disord.* 16:PCC.14l01635. doi: 10.4088/PCC.14l01635

Jafari, E., Alizadehgoradel, J., Pourmohseni Koluri, F., Nikoozadehkordmirza, E., Refahi, M., Taherifard, M., et al. (2021). Intensified electrical stimulation targeting lateral and medial prefrontal cortices for the treatment of social anxiety disorder: a randomized, double-blind, parallel-group, dose-comparison study. *Brain Stimul.* 14, 974–986. doi: 10.1016/j.brs.2021.06.005

Jeon, D. W., Jung, D. U., Kim, S. J., Shim, J. C., Moon, J. J., Seo, Y. S., et al. (2018). Adjunct transcranial direct current stimulation improves cognitive function in patients with schizophrenia: a double-blind 12-week study. *Schizophr. Res.* 197, 378–385. doi: 10.1016/j.schres.2017.12.009

Klauss, J., Anders, Q. S., Felippe, L. V., Ferreira, L. V. B., Cruz, M. A., Nitsche, M. A., et al. (2018a). Lack of effects of extended sessions of transcranial direct current stimulation (tDCS) over dorsolateral prefrontal cortex on craving and relapses in crack-cocaine users. *Front. Pharmacol.* 9:1198. doi: 10.3389/fphar.2018.01198

Klauss, J., Anders, Q. S., Felippe, L. V., Nitsche, M. A., and Nakamura-Palacios, E. M. (2018b). Multiple sessions of transcranial direct current stimulation (tDCS) reduced craving and relapses for alcohol use: a randomized placebo-controlled trial in alcohol use disorder. *Front. Pharmacol.* 9:716. doi: 10.3389/fphar.2018.00716

Klosowska, J., Blaut, A., and Paulewicz, B. (2015). [Attentional bias training in reducing symptoms of anxiety]. *Psychiatr. Pol.* 49, 57–66. doi: 10.12740/PP/27628

Koenigs, M., and Grafman, J. (2009). The functional neuroanatomy of depression: distinct roles for ventromedial and dorsolateral prefrontal cortex. *Behav. Brain Res.* 201, 239–243. doi: 10.1016/j.bbr.2009.03.004

Kroczek, A. M., Haussinger, F. B., Rohe, T., Schneider, S., Plewnia, C., Batra, A., et al. (2016). Effects of transcranial direct current stimulation on craving, heart-rate variability and prefrontal hemodynamics during smoking cue exposure. *Drug Alcohol Depend.* 168, 123–127. doi: 10.1016/j.drugalcdep.2016.09.006

Kumar, S., Batist, J., Ghazala, Z., Zomorrodi, R. M., Brooks, H., Goodman, M., et al. (2020). Effects of bilateral transcranial direct current stimulation on working memory and global cognition in older patients with remitted major depression: a pilot randomized clinical trial. *Int. J. Geriatr. Psychiatry* 35, 1233–1242. doi: 10.1002/gps.5361

Liebetanz, D., Nitsche, M. A., Tergau, F., and Paulus, W. (2002). Pharmacological approach to the mechanisms of transcranial DC-stimulation-induced after-effects of human motor cortex excitability. *Brain* 125(Pt 10), 2238–2247. doi: 10.1093/brain/awf238

Lin, Y. Y., Chang, C. C., Huang, C. C., Tzeng, N. S., Kao, Y. C., and Chang, H. A. (2021). Efficacy and neurophysiological predictors of treatment response of adjunct bifrontal transcranial direct current stimulation (tDCS) in treating unipolar and bipolar depression. *J. Affect. Disord.* 280(Pt A), 295–304. doi: 10.1016/j.jad.2020.11.030

Locke, A. B., Kirst, N., and Shultz, C. G. (2015). Diagnosis and management of generalized anxiety disorder and panic disorder in adults. *Am. Fam. Phys.* 91, 617–624.

Loo, C. K., Alonzo, A., Martin, D., Mitchell, P. B., Galvez, V., and Sachdev, P. (2012). Transcranial direct current stimulation for depression: 3-week, randomised, sham-controlled trial. *Br. J. Psychiatry* 200, 52–59. doi: 10.1192/bjp.bp.111.097634

Loo, C. K., Sachdev, P., Martin, D., Pigot, M., Alonzo, A., Malhi, G. S., et al. (2010). A double-blind, sham-controlled trial of transcranial direct current stimulation for the treatment of depression. *Int. J. Neuropsychopharmacol.* 13, 61–69. doi: 10.1017/S1461145709990411

Marazziti, D., Presta, S., Baroni, S., Silvestri, S., and Dell'Osso, L. (2014). Behavioral addictions: a novel challenge for psychopharmacology. *CNS Spectr.* 19, 486–495. doi: 10.1017/S1092852913001041

Martin, D. M., Alonzo, A., Ho, K. A., Player, M., Mitchell, P. B., Sachdev, P., et al. (2013). Continuation transcranial direct current stimulation for the prevention of relapse in major depression. *J. Affect. Disord.* 144, 274–278. doi: 10.1016/j.jad.2012.10.012

McDermott, T. J., Wiesman, A. I., Mills, M. S., Spooner, R. K., Coolidge, N. M., Proskovec, A. L., et al. (2019). tDCS modulates behavioral performance and the neural oscillatory dynamics serving visual selective attention. *Hum. Brain Mapp.* 40, 729–740. doi: 10.1002/hbm.24405

Milad, M. R., and Rauch, S. L. (2012). Obsessive-compulsive disorder: beyond segregated cortico-striatal pathways. *Trends Cogn. Sci.* 16, 43–51. doi: 10.1016/j.tics.2011.11.003

Mondino, M., Haesebaert, F., Poulet, E., Suaud-Chagny, M. F., and Brunelin, J. (2015). Fronto-temporal transcranial direct current stimulation (tDCS) reduces source-monitoring deficits and auditory hallucinations in patients with schizophrenia. *Schizophr. Res.* 161, 515–516. doi: 10.1016/j.schres.2014.10.054

Mondino, M., Jardri, R., Suaud-Chagny, M. F., Saoud, M., Poulet, E., and Brunelin, J. (2016). Effects of fronto-temporal transcranial direct current stimulation on auditory verbal hallucinations and resting-state functional connectivity of the left temporo-parietal junction in patients with schizophrenia. *Schizophr. Bull.* 42, 318–326. doi: 10.1093/schbul/sbv114

Mondino, M., Luck, D., Grot, S., Januel, D., Suaud-Chagny, M. F., Poulet, E., et al. (2018). Effects of repeated transcranial direct current stimulation on smoking, craving and brain reactivity to smoking cues. *Sci. Rep.* 8:8724. doi: 10.1038/s41598-018-27057-1

Moreno, M. L., Goerigk, S. A., Bertola, L., Suemoto, C. K., Razza, L. B., Moffa, A. H., et al. (2020). Cognitive changes after tDCS and escitalopram treatment in major depressive disorder: results from the placebo-controlled ELECT-TDCS trial. *J. Affect. Disord.* 263, 344–352. doi: 10.1016/j.jad.2019.12.009

Narayanaswamy, J. C., Jose, D., Chhabra, H., Agarwal, S. M., Shrinivasa, B., Hegde, A., et al. (2015). Successful application of add-on transcranial direct current stimulation (tDCS) for treatment of SSRI resistant OCD. *Brain Stimul.* 8, 655–657. doi: 10.1016/j.brs.2014.12.003

Narayanaswamy, J. C., Shivakumar, V., Bose, A., Agarwal, S. M., Venkatasubramanian, G., and Gangadhar, B. N. (2014). Sustained improvement of negative symptoms in schizophrenia with add-on tDCS: a case report. *Clin. Schizophr. Relat. Psychoses* 8, 135–136. doi: 10.3371/CSRP.JNVS.061314

Nawani, H., Bose, A., Agarwal, S. M., Shivakumar, V., Chhabra, H., Subramaniam, A., et al. (2014a). Modulation of corollary discharge dysfunction in schizophrenia by tDCS: preliminary evidence. *Brain Stimul.* 7, 486–488. doi: 10.1016/j.brs.2014.01.003

Nawani, H., Kalmady, S. V., Bose, A., Shivakumar, V., Rakesh, G., Subramaniam, A., et al. (2014b). Neural basis of tDCS effects on auditory verbal hallucinations in schizophrenia: a case report evidence for cortical neuroplasticity modulation. *J. ECT* 30, e2–e4. doi: 10.1097/YCT.0b013e3182a35492

Nejati, V., Salehinejad, M. A., Nitsche, M. A., Najian, A., and Javadi, A. H. (2020). Transcranial direct current stimulation improves executive dysfunctions in ADHD: implications for inhibitory control, interference control, working memory, and cognitive flexibility. *J. Atten. Disord.* 24, 1928–1943. doi: 10.1177/1087054717730611

Nestler, E. J. (2013). Cellular basis of memory for addiction. *Dialog. Clin. Neurosci.* 15, 431–443. doi: 10.31887/DCNS.2013.15.4/enestler

Nishida, K., Koshikawa, Y., Morishima, Y., Yoshimura, M., Katsura, K., Ueda, S., et al. (2019). Pre-stimulus brain activity is associated with state-anxiety changes during single-session transcranial direct current stimulation. *Front. Hum. Neurosci.* 13:266. doi: 10.3389/fnhum.2019.00266

Orlov, N. D., Tracy, D. K., Joyce, D., Patel, S., Rodzinka-Pasko, J., Dolan, H., et al. (2017). Stimulating cognition in schizophrenia: a controlled pilot study of the effects of prefrontal transcranial direct current stimulation upon memory and learning. *Brain Stimul.* 10, 560–566. doi: 10.1016/j.brs.2016.12.013

Palm, U., Keeser, D., Blautzik, J., Pogarell, O., Ertl-Wagner, B., Kupka, M. J., et al. (2013). Prefrontal transcranial direct current stimulation (tDCS) changes negative symptoms and functional connectivity MRI (fcMRI) in a single case of treatment-resistant schizophrenia. *Schizophr. Res.* 150, 583–585. doi: 10.1016/j.schres.2013.08.043

Palm, U., Keeser, D., Hasan, A., Kupka, M. J., Blautzik, J., Sarubin, N., et al. (2016). Prefrontal transcranial direct current stimulation for treatment of schizophrenia with predominant negative symptoms: a double-blind, sham-controlled proof-of-concept study. *Schizophr. Bull.* 42, 1253–1261. doi: 10.1093/schbul/sbw041

Palm, U., Schiller, C., Fintescu, Z., Obermeier, M., Keeser, D., Reisinger, E., et al. (2012). Transcranial direct current stimulation in treatment resistant depression: a randomized double-blind, placebo-controlled study. *Brain Stimul.* 5, 242–251. doi: 10.1016/j.brs.2011.08.005

Pena-Gomez, C., Vidal-Pineiro, D., Clemente, I. C., Pascual-Leone, A., and Bartres-Faz, D. (2011). Down-regulation of negative emotional processing by transcranial direct current stimulation: effects of personality characteristics. *PLoS One* 6:e22812. doi: 10.1371/journal.pone.0022812

Philiastides, M. G., Auksztulewicz, R., Heekeren, H. R., and Blankenburg, F. (2011). Causal role of dorsolateral prefrontal cortex in human perceptual decision making. *Curr. Biol.* 21, 980–983. doi: 10.1016/j.cub.2011.04.034

Pierce, R. C., and Kumaresan, V. (2006). The mesolimbic dopamine system: the final common pathway for the reinforcing effect of drugs of abuse? *Neurosci. Biobehav. Rev.* 30, 215–238. doi: 10.1016/j.neubiorev.2005.04.016

Polanczyk, G. V., Salum, G. A., Sugaya, L. S., Caye, A., and Rohde, L. A. (2015). Annual research review: a meta-analysis of the worldwide prevalence of mental disorders in children and adolescents. *J. Child Psychol. Psychiatry* 56, 345–365. doi: 10.1111/jcpp.12381

Potenza, M. N. (2014). Non-substance addictive behaviors in the context of DSM-5. *Addict. Behav.* 39, 1–2. doi: 10.1016/j.addbeh.2013.09.004

Praharaj, S. K., Behere, R. V., and Sharma, P. S. (2015). Cathodal transcranial direct current stimulation over left temporoparietal area for treatment-refractory delusions and auditory hallucinations in schizophrenia: a case study. *J. ECT* 31, 277–278. doi: 10.1097/YCT.0000000000000237

Preller, K. H., Wagner, M., Sulzbach, C., Hoenig, K., Neubauer, J., Franke, P. E., et al. (2013). Sustained incentive value of heroin-related cues in short- and long-term abstinent heroin users. *Eur. Neuropsychopharmacol.* 23, 1270–1279. doi: 10.1016/j.euroneuro.2012.11.007

Purpura, D. P., and McMurtry, J. G. (1965). Intracellular activities and evoked potential changes during polarization of motor cortex. *J. Neurophysiol.* 28, 166–185. doi: 10.1152/jn.1965.28.1.166

Rahnev, D., Nee, D. E., Riddle, J., Larson, A. S., and D'Esposito, M. (2016). Causal evidence for frontal cortex organization for perceptual decision making. *Proc. Natl. Acad. Sci. U.S.A.* 113, 6059–6064. doi: 10.1073/pnas.1522551113

Rakesh, G., Shivakumar, V., Subramaniam, A., Nawani, H., Amaresha, A. C., Narayanaswamy, J. C., et al. (2013). Monotherapy with tDCS for schizophrenia: a case report. *Brain Stimul.* 6, 708–709. doi: 10.1016/j.brs.2013.01.007

Rigonatti, S. P., Boggio, P. S., Myczkowski, M. L., Otta, E., Fiquer, J. T., Ribeiro, R. B., et al. (2008). Transcranial direct stimulation and fluoxetine for the treatment of depression. *Eur. Psychiatry.* 23, 74–76. doi: 10.1016/j.eurpsy.2007.09.006

Robbins, T. W., and Everitt, B. J. (2002). Limbic-striatal memory systems and drug addiction. *Neurobiol. Learn. Mem.* 78, 625–636. doi: 10.1006/nlme.2002.4103

Rowland, A. S., Skipper, B. J., Umbach, D. M., Rabiner, D. L., Campbell, R. A., Naftel, A. J., et al. (2015). The prevalence of ADHD in a population-based sample. *J. Atten. Disord.* 19, 741–754. doi: 10.1177/1087054713513799

Rubi-Fessen, I., Hartmann, A., Huber, W., Fimm, B., Rommel, T., Thiel, A., et al. (2015). Add-on effects of repetitive transcranial magnetic stimulation on subacute aphasia therapy: enhanced improvement of functional communication and basic linguistic skills. A randomized controlled study. *Arch. Phys. Med. Rehabil.* 96, 1935–1944.e1932. doi: 10.1016/j.apmr.2015.06.017

Sakai, Y., Narumoto, J., Nishida, S., Nakamae, T., Yamada, K., Nishimura, T., et al. (2011). Corticostriatal functional connectivity in non-medicated patients with obsessive-compulsive disorder. *Eur. Psychiatry* 26, 463–469. doi: 10.1016/j.eurpsy.2010.09.005

Sampaio-Junior, B., Tortella, G., Borrione, L., Moffa, A. H., Machado-Vieira, R., Cretaz, E., et al. (2018). Efficacy and safety of transcranial direct current stimulation as an add-on treatment for bipolar depression: a randomized clinical trial. *JAMA Psychiatry* 75, 158–166. doi: 10.1001/jamapsychiatry.2017.4040

Schilling, T. M., Bossert, M., Konig, M., Wirtz, G., Weisbrod, M., and Aschenbrenner, S. (2021). Acute effects of a single dose of 2 mA of anodal transcranial direct current stimulation over the left dorsolateral prefrontal cortex on executive functions in patients with schizophrenia-a randomized controlled trial. *PLoS One* 16:e0254695. doi: 10.1371/journal.pone.0254695

Shahani, B., and Russell, W. R. (1969). Motor neurone disease. An abnormality of nerve metabolism. *J. Neurol. Neurosurg. Psychiatry* 32, 1–5. doi: 10.1136/jnnp.32.1.1

Sharafi, E., Taghva, A., Arbabi, M., Dadarkhah, A., and Ghaderi, J. (2019). Transcranial direct current stimulation for treatment-resistant major depression: a double-blind randomized sham-controlled trial. *Clin. EEG Neurosci.* 50, 375–382. doi: 10.1177/1550059419863209

Shenoy, S., Bose, A., Chhabra, H., Dinakaran, D., Agarwal, S. M., Shivakumar, V., et al. (2015). Transcranial direct current stimulation (tDCS) for auditory verbal hallucinations in schizophrenia during pregnancy: a case report. *Brain Stimul.* 8, 163–164. doi: 10.1016/j.brs.2014.10.013

Shiozawa, P., da Silva, M. E., Cordeiro, Q., Fregni, F., and Brunoni, A. R. (2013). Transcranial direct current stimulation (tDCS) for catatonic schizophrenia: a case study. *Schizophr. Res.* 146, 374–375. doi: 10.1016/j.schres.2013.01.030

Shiozawa, P., Gomes, J. S., Ducos, D. V., Akiba, H. T., Dias, A. M., Trevizol, A. P., et al. (2016). Effect of transcranial direct current stimulation (tDCS) over the prefrontal cortex combined with cognitive training for treating schizophrenia: a sham-controlled randomized clinical trial. *Trends Psychiatry Psychother.* 38, 175–177. doi: 10.1590/2237-6089-2015-0043

Shiozawa, P., Leiva, A. P., Castro, C. D., da Silva, M. E., Cordeiro, Q., Fregni, F., et al. (2014). Transcranial direct current stimulation for generalized anxiety disorder: a case study. *Biol. Psychiatry* 75, e17–e18. doi: 10.1016/j.biopsych.2013.07.014

Smith, R. C., Boules, S., Mattiuz, S., Youssef, M., Tobe, R. H., Sershen, H., et al. (2015). Effects of transcranial direct current stimulation (tDCS) on cognition, symptoms, and smoking in schizophrenia: a randomized controlled study. *Schizophr. Res.* 168, 260–266. doi: 10.1016/j.schres.2015.06.011

Soff, C., Sotnikova, A., Christiansen, H., Becker, K., and Siniatchkin, M. (2017). Transcranial direct current stimulation improves clinical symptoms in adolescents with attention deficit hyperactivity disorder. *J. Neural. Transm (Vienna).* 124, 133–144. doi: 10.1007/s00702-016-1646-y

Stagg, C. J., Best, J. G., Stephenson, M. C., O'Shea, J., Wylezinska, M., Kincses, Z. T., et al. (2009). Polarity-sensitive modulation of cortical neurotransmitters by transcranial stimulation. *J. Neurosci.* 29, 5202–5206. doi: 10.1523/JNEUROSCI.4432-08.2009

Stein, D. J., Scott, K. M., de Jonge, P., and Kessler, R. C. (2017). Epidemiology of anxiety disorders: from surveys to nosology and back. *Dialog. Clin. Neurosci.* 19, 127–136. doi: 10.31887/DCNS.2017.19.2/dstein

Valiengo, L., Goerigk, S., Gordon, P. C., Padberg, F., Serpa, M. H., Koebe, S., et al. (2020). Efficacy and safety of transcranial direct current stimulation for treating negative symptoms in schizophrenia: a randomized clinical trial. *JAMA Psychiatry* 77, 121–129. doi: 10.1001/jamapsychiatry.2019.3199

Vaseghi, B., Zoghi, M., and Jaberzadeh, S. (2015). A meta-analysis of site-specific effects of cathodal transcranial direct current stimulation on sensory perception and pain. *PLoS One* 10:e0123873. doi: 10.1371/journal.pone.0123873

Vicario, C. M., Salehinejad, M. A., Felmingham, K., Martino, G., and Nitsche, M. A. (2019). A systematic review on the therapeutic effectiveness of non-invasive brain stimulation for the treatment of anxiety disorders. *Neurosci. Biobehav. Rev.* 96, 219–231. doi: 10.1016/j.neubiorev.2018.12.012

Vitor de Souza Brangioni, M. C., Pereira, D. A., Thibaut, A., Fregni, F., Brasil-Neto, J. P., and Boechat-Barros, R. (2018). Effects of prefrontal transcranial direct current stimulation and motivation to quit in tobacco smokers: a randomized, sham controlled, double-blind trial. *Front. Pharmacol.* 9:14. doi: 10.3389/fphar.2018.00014

Volpato, C., Piccione, F., Cavinato, M., Duzzi, D., Schiff, S., Foscolo, L., et al. (2013). Modulation of affective symptoms and resting state activity by brain stimulation in a treatment-resistant case of obsessive-compulsive disorder. *Neurocase* 19, 360–370. doi: 10.1080/13554794.2012.667131

Vossel, S., Geng, J. J., and Fink, G. R. (2014). Dorsal and ventral attention systems: distinct neural circuits but collaborative roles. *Neuroscientist* 20, 150–159. doi: 10.1177/1073858413494269

Weickert, T. W., Salimuddin, H., Lenroot, R. K., Bruggemann, J., Loo, C., Vercammen, A., et al. (2019). Preliminary findings of four-week, task-based anodal prefrontal cortex transcranial direct current stimulation transferring to other cognitive improvements in schizophrenia. *Psychiatry Res.* 280:112487. doi: 10.1016/j.psychres.2019.112487

Welch, E. S., Weigand, A., Hooker, J. E., Philip, N. S., Tyrka, A. R., Press, D. Z., et al. (2019). Feasibility of computerized cognitive-behavioral therapy combined with bifrontal transcranial direct current stimulation for treatment of major depression. *Neuromodulation* 22, 898–903. doi: 10.1111/ner.12807

Wietschorke, K., Lippold, J., Jacob, C., Polak, T., and Herrmann, M. J. (2016). Transcranial direct current stimulation of the prefrontal cortex reduces cue-reactivity in alcohol-dependent patients. *J. Neural. Transm (Vienna).* 123, 1173–1178. doi: 10.1007/s00702-016-1541-6

Woods, A. J., Antal, A., Bikson, M., Boggio, P. S., Brunoni, A. R., Celnik, P., et al. (2016). A technical guide to tDCS, and related non-invasive brain stimulation tools. *Clin. Neurophysiol.* 127, 1031–1048. doi: 10.1016/j.clinph.2015.11.012

Wrigley, P. J., Gustin, S. M., McIndoe, L. N., Chakiath, R. J., Henderson, L. A., and Siddall, P. J. (2013). Longstanding neuropathic pain after spinal cord injury is refractory to transcranial direct current stimulation: a randomized controlled trial. *Pain* 154, 2178–2184. doi: 10.1016/j.pain.2013.06.045

Wu, L. L., Potenza, M. N., Zhou, N., Kober, H., Shi, X. H., Yip, S. W., et al. (2020). A role for the right dorsolateral prefrontal cortex in enhancing regulation of both craving and negative emotions in internet gaming disorder: a randomized trial. *Eur. Neuropsychopharmacol.* 36, 29–37. doi: 10.1016/j.euroneuro.2020.04.003

Xu, J., Fregni, F., Brody, A. L., and Rahman, A. S. (2013). Transcranial direct current stimulation reduces negative affect but not cigarette craving in overnight abstinent smokers. *Front. Psychiatry* 4:112. doi: 10.3389/fpsyt.2013.00112

Xu, X., Deng, Z. Y., Huang, Q., Zhang, W. X., Qi, C. Z., and Huang, J. A. (2017). Prefrontal cortex-mediated executive function as assessed by stroop task performance associates with weight loss among overweight and obese adolescents and young adults. *Behav. Brain Res.* 321, 240–248. doi: 10.1016/j.bbr.2016.12.040

Yang, X., Gao, M., Shi, J., Ye, H., and Chen, S. (2017). Modulating the activity of the DLPFC and OFC has distinct effects on risk and ambiguity decision-making: a tDCS study. *Front. Psychol.* 8:1417. doi: 10.3389/fpsyg.2017.01417

Yoon, Y. B., Kim, M., Lee, J., Cho, K. I. K., Kwak, S., Lee, T. Y., et al. (2019). Effect of tDCS on aberrant functional network connectivity in refractory hallucinatory schizophrenia: a pilot study. *Psychiatry Investig.* 16, 244–248. doi: 10.30773/pi.2018.11.18

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 30 June 2022
doi: 10.3389/fnbeh.2022.919485

Brain Somatic Variant in Ras-Like Small GTPase *RALA* Causes Focal Cortical Dysplasia Type II

Han Xu[1], Kai Gao[1], Qingzhu Liu[2], Tianshuang Wang[1], Zhongbin Zhang[1], Lixin Cai[2], Ye Wu[1,2] and Yuwu Jiang[1,2,3,4,5]**

[1] Department of Pediatrics, Peking University First Hospital, Beijing, China, [2] Children Epilepsy Center, Peking University First Hospital, Beijing, China, [3] Beijing Key Laboratory of Molecular Diagnosis and Study on Pediatric Genetic Diseases, Beijing, China, [4] Key Laboratory for Neuroscience, Ministry of Education/National Health and Family Planning Commission, Peking University, Beijing, China, [5] Institute for Brain Disorders, Beijing, China

OPEN ACCESS

Edited by:
Dongdong Qin,
Yunnan University of Chinese Medicine, China

Reviewed by:
Jianmin Liang,
First Affiliated Hospital of Jilin University, China
Hoon-Chul Kang,
Yonsei University, South Korea

***Correspondence:**
Ye Wu
dryewu@263.net
Yuwu Jiang
jiangyuwu@bjmu.edu.cn;
jiangyw@263.net

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: 13 April 2022
Accepted: 03 June 2022
Published: 30 June 2022

Citation:
Xu H, Gao K, Liu Q, Wang T, Zhang Z, Cai L, Wu Y and Jiang Y (2022) Brain Somatic Variant in Ras-Like Small GTPase RALA Causes Focal Cortical Dysplasia Type II. Front. Behav. Neurosci. 16:919485. doi: 10.3389/fnbeh.2022.919485

Purpose: In our group's previous study, we performed deep whole-exome sequencing and targeted amplicon sequencing in the postoperative brain tissue of epilepsy patients with focal cortical dysplasia type II (FCD II). We identified the first somatic variant of *RALA* in the brain tissue of a child with FCD type IIb. *RALA* encodes a small GTPase of the Ras superfamily. To date, the role of *RALA* in brain development is not yet known. In this study, we reported that the *RALA* somatic variant led to FCD type II through activation of the mammalian target of rapamycin (mTOR) pathways.

Materials and Methods: HEK293T cells were transfected *in vitro* to analyze the expression of the RalA protein, as well as phosphorylated S6 (P-S6), one of the major markers of mTOR pathway activation, RalA GTPase activity, and the interaction between RalA and its downstream binding effectors. *In vivo*, wild-type, and mutant *RALA* plasmids were transfected into the local cortex of mice using *in utero* electroporation to evaluate the effect of *RALA* c.G482A on neuronal migration.

Results: The *RALA* c.G482A mutation increased RalA protein expression, the abnormal activation of the mTOR pathways, RalA GTPase activity, and binding to downstream effectors. *RALA* c.G482A local transfection in the embryonic brain *in utero* induced abnormal cortical neuron migration in mice.

Conclusion: This study demonstrated for the first time that the somatic gain-of-function variant of *RALA* activates the mTOR pathway and leads to neuronal migration disorders in the brain, facilitating the development of FCD II. Therefore, *RALA* brain somatic mutation may be one of the pathogenic mechanisms leading to FCD II, which is always related to drug-resistant epilepsy in children. However, more somatic variations of this gene are required to be confirmed in more FCD II patient brain samples.

Keywords: pediatric drug-resistant epilepsy, FCD II, somatic mutation, RalA, mTOR pathway

INTRODUCTION

Malformation of cortical development (MCD) is a common cause of drug-refractory epilepsy in children. Focal cortical dysplasia (FCD), a type of MCD, is characterized by the impaired migration and proliferation of localized cortical neurons during embryonic development, and this is accompanied by abnormal cell proliferation (Mariasavina et al., 2020). In 2011, the International League Against Epilepsy (ILAE) proposed a classification consensus of FCD that divided FCD into three categories (Ingmar et al., 2011), among which FCD II has received widespread attention due to its unique pathological features. FCD II is associated with specific cytological abnormalities, such as dysmorphic neurons (DNs) and balloon cells (BCs), in addition to disrupted cortical lamination. FCD II was further divided into two subtypes, FCD IIa with dysmorphic neurons but without balloon cells and FCD IIb with both dysmorphic neurons and balloon cells. Evidence from research statistics shows that FCD accounts for approximately 75% of the surgically resected MCD brain tissue samples from children undergoing epilepsy surgery, with FCD II being the most common pathological type, accounting for approximately 45.3% (Blumcke et al., 2017).

The underlying molecular genetic etiology of FCD II remains unclarified, but recent genetic studies have shown that somatic mutations in the mammalian target of rapamycin (mTOR) pathway gene have a pivotal role in the pathogenesis of FCD II. Through deep sequencing of surgically resected FCD II brain tissue samples and using matched peripheral blood cell sequencing results of patients as a control, somatic variants were detected in 10–63% of the brain tissues, with mutated genes including *MTOR*, *PIK3CA*, *AKT3*, *RHEB*, *DEPDC5*, *NPRL2*, *NPRL3*, *TSC2*, and *PTEN* primarily located in the mTOR pathways (Lim et al., 2015; Nakashima et al., 2015; Moller et al., 2016; Baulac et al., 2019; Oegema et al., 2020; Blumcke et al., 2021). In addition, the variant allele frequency (VAF) in the tissues was only 0.93–12.63% (Marsan and Baulac, 2018; Baulac et al., 2019; Zhang et al., 2020). Among them, somatic activating mutations in the *MTOR* gene accounted for approximately 15.6–46% of the FCD samples, which is the most common somatic genetic cause of FCD II at present (Lim et al., 2015). mTOR is an evolutionarily conserved serine/threonine-protein kinase, and the PI3K-AKT-mTOR signaling pathway is one of the vital intracellular signaling pathways that regulate cellular transcription, translation, metabolism, proliferation, and migration. When external stimuli act on cells, PI3K can be phosphorylated, and this in turn phosphorylates AKT. Activated AKT inhibits the TSC1/TSC2 complex, and this deactivates the downstream Rheb, leading to the activation of the mTOR complex 1 (mTORC1). Then, the downstream substrates, namely, P70-S6K and S6, are phosphorylated, exerting regulatory effects on protein synthesis, proliferation, and autophagy. In the central nervous system (CNS), the mTOR pathway is involved in regulating neural development, neuronal morphology, neural circuits, and synaptic plasticity (Saxton and Sabatini, 2017). Nevertheless, pathogenic mutations have not been detected in more than half of FCD II brain tissues, and it is unclear whether the occurrence of FCD II is associated with somatic mutations in other pathway-related genes or new mTOR pathway-regulated genes.

The somatic variant of the Ras-Like Proto-Oncogene A gene (*RALA*, c.G482A, p.Arg161Gln) with a VAF of 5.50% was detected for the first time in surgically resected FCD IIb brain tissue by our group earlier, and the activation of the mTOR pathway was also confirmed in the lesions (Zhang et al., 2020). *RALA* encodes a small GTPase of the Ras superfamily, RalA, that is implicated in a range of biological functions including metabolic and transcriptional regulation. In the CNS, RalA is involved in neuronal development, plasticity, polarization, migration, and renewal of synaptic vesicles as well as NMDA, AMPA, and dopamine receptor regulation (Teodoro et al., 2013; Zheng et al., 2016). Previous studies have focused on the role of *RALA* variants in tumor cell proliferation and metastasis, but *RALA* has never been reported to be associated with FCD. In this study, we performed functional studies on the newly discovered somatic variant of *RALA* to explore the effect of this variant on the PI3K-AKT-mTOR signaling pathway and thus on neuronal proliferation and migration. Using in-depth studies of the *RALA* variant, we expected to provide significant clues for further interpretation of the pathogenesis of FCD and the mechanism of epileptogenesis.

MATERIALS AND METHODS

Clinical Data Collection

This study was approved by the Ethics Committee of Peking University First Hospital, and informed consent was signed by the guardians of the participant. Clinical data of the child were collected, including gender, age, epilepsy seizure symptoms, video-electroencephalogram (V-EEG), MRI imaging, and postoperative histopathology.

Plasmid Constructs for Generating the Wild-Type and the Mutant *RALA*

GV141 Flag-tagged wild-type *RALA* (NM_005402) and GV141 Flag-tagged mutant *RALA* constructs were purchased from the GeneChem Company in Shanghai. After generating the mutant constructs, we checked the full sequence of the coding region for each construct and found no secondary missense or truncated mutation.

Cell Culture, Transfection, and Western Blotting

Human embryonic kidney 293T (HEK293T) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 100 units/ml of penicillin, 100 mg/ml of streptomycin, and 10% of fetal bovine serum at 37°C and 5% CO_2. The cells were transfected with flag-tagged wild-type and mutant *RALA* plasmids while growing to a 70% density. For the immunoblotting, the cells were lysed to extract the total proteins. The membranes were incubated with primary antibodies including anti-phospho-S6 ribosomal protein, anti-S6 ribosomal protein, anti-β-actin, and anti-RalA in TBST overnight

at 4°C after electrophoresis. Then, the secondary antibody was incubated and developed for imaging.

Quantitative Real-Time PCR Analysis

The total RNA was isolated and transcribed into cDNA. Quantitative real-time PCR (qPCR) was performed using the qPCR Kit (Promega) with specific primers designed for the target gene and housekeeping gene. GAPDH was used as the internal control.

Protein Purification and the RalA GTPase Assays

Flag-tagged proteins were purified using Pierce DYKDDDDK magnetic agarose (Thermo Fisher Scientific) according to the manufacturer's protocol. Protein purity was assessed using standard SDS-PAGE blotting. The protein concentration was quantified and normalized among samples in an elution buffer prior to use in the assays. The GTPase activity of 5 μg of purified, recombinant proteins was assessed using the GTPase-Glo Assay. The luminescence was quantified using a microplate reader.

RalA Effector Binding Assays

The binding of the purified, recombinant proteins to a proprietary Ral effector protein was assessed using the RalA G-LISA Activation Assay Kit following the protocols. In brief, purified RalA protein was incubated in the presence or absence of 15 μM of GTP for 1.5 h at 25°C, and then 50 ng of the purified RalA/GTP mixture was applied to the Ral-BP binding plate.

Immunofluorescence of the Focal Cortical Dysplasia Brain Tissues

The FCD brain specimens were collected after surgery. Surgical tissues were fixed in 4% paraformaldehyde (PFA) overnight, cryoprotected overnight in 20% buffered sucrose, and made into OCT-embedded tissue blocks. Then, cryostat-cut sections (20 μm thick) were performed. Then, cryostat-cut sections were immune-stained with anti-NeuN/SMI-311/Vimentin (V9) antibodies, the mouse antibody to RalA, and then stained with the Alexa Fluor 488-conjugated and Alexa Fluor 568-conjugated secondary antibodies separately. Then, the images were scanned and analyzed using a laser scanning confocal microscope.

Tandem Mass Tag-Labeled Quantitative Proteome Assay

The transfected cell samples were prepared according to the requirements described in the literature (Wiśniewski et al., 2009; Carolyn et al., 2015; Gillette et al., 2020), and three biological replicates were set for each sample group. Quantitative proteomics analysis was performed by Novogene Company.

In utero Electroporation and Image Analysis

Pregnant ICR mice at embryonic day 14.5 (E14.5) were used in this experiment, and the detailed procedures are described in the previous literature (Wang and Mei, 2013; Baumgart and Baumgart, 2016). The mouse brains were harvested at E18.5, and 20 μm thick Cryostat brain sections were immune-stained with the anti-GFP antibody and fluorescence-conjugated secondary antibody. Images were collected using a confocal microscope. GFP-positive cells in the different cortical layers were analyzed using ImageJ. All animal experiments were approved by the Animal Ethics Committee of Peking University First Hospital.

Statistical Analysis

All experimental data were processed using SPSS 25.0 for Mac and GraphPad Prism statistical software. The normal distribution data of the continuous variables were represented by the mean ± standard deviation (SD). A student's *t*-test was used for comparison between two groups, and a one-way ANOVA was used for comparison between three groups and more than three groups. An LSD analysis was used for comparisons between the two groups. A $P < 0.05$ was considered statistically significant.

RESULTS

Clinical Characteristics of a Patient With the Brain *RALA* c.G482A Somatic Variant

Male focal seizures began at 8 months of age, and these were characterized by squinting of the eyes to the left, clenching of the right hand, flexion, and stiffness of the right limb lasting for several minutes, and relieving by dozens to hundreds of attacks per day. The patient was treated with valproic acid, clonazepam, levetiracetam, and oxcarbazepine successively, which were poorly controlled and accompanied by severe developmental retardation. The video electroencephalogram (EEG) monitored multiple left frontopolar onset seizures during the waking hours. The cranial magnetic resonance imaging (MRI; **Figure 1A**) suggested dysplasia of the left frontal cortex. At the age of 1 year and 11 months, the child underwent resection of the left frontal epileptogenic focus, and the postoperative pathology suggested FCD Type IIb (**Figure 1B**). There was no recurrence of epilepsy at the 3-year postoperative follow-up, and there existed an Engel grade of I. Development was also significantly improved compared with the preoperative period.

RALA Variation Site and the RalA Protein Structure

We identified the first somatic variant of *RALA* (c.G482A, p.Arg161Gln) in the brain tissue of FCD II in our previous study, which was not retrieved as a minor allele frequency in the dbSNP, ExAC, and GnomAD databases and was predicted to be pathogenic by various mutation hazard analysis software such as SIFT and PolyPhen-2. The VAF was 5.50% in the lesions and was not detected in the perilesional brain tissues or peripheral blood. RalA is a small GTPase encoded by the *RALA* gene, a member of the Ral subfamily of the Ras superfamily of proto-oncogenes. The RalA structural domain primarily consists of three components that include three GTP/GDP binding domains, one effector binding domain, and a posttranslational modification site (containing the phosphorylation site of serine/threonine kinase)

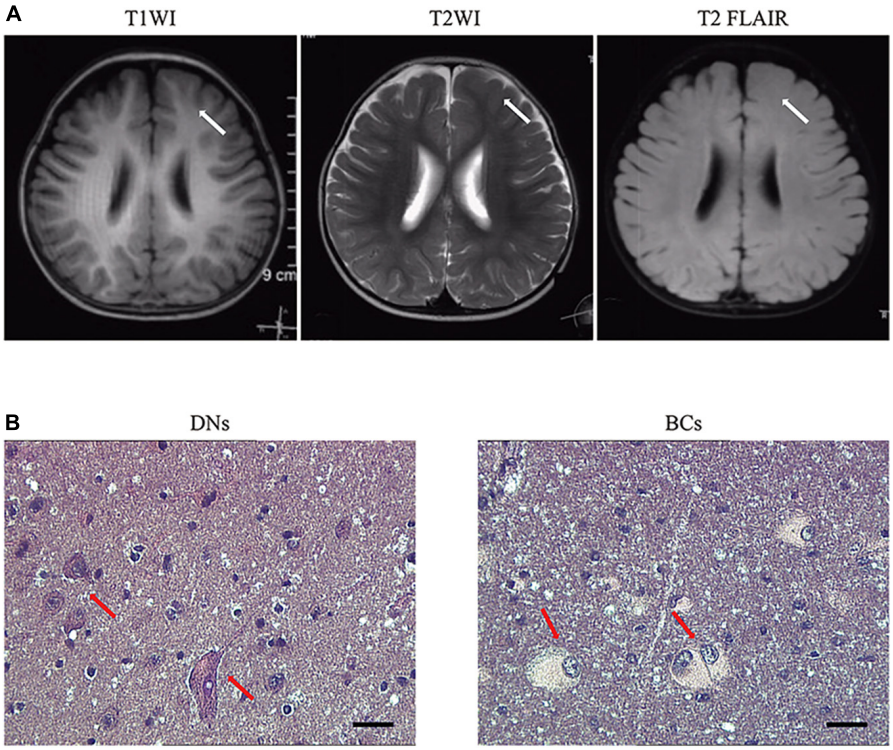


FIGURE 1 | Representative MRI and pathology of patients with *RALA* c.G482A somatic variant. **(A)** MRI axial representations of the *RALA* somatic mutation patient. T1WI, T1-weighed image; T2WI, T2-weighed image; T2 FLAIR, T2 fluid-attenuated inversion recovery. The location of the lesion is indicated by a white arrow. **(B)** Histopathological results of the patient. Dysmorphic neurons and balloon cells are shown in the red arrow. HE, 10 × 40; DNs, dysmorphic neurons; BCs, balloon cells.

located at the COOH end (**Figure 2A**). Analysis of the RalA secondary structural domain showed that the p.Arg161Gln variant was located near the GTP/GDP binding domain. The homology alignment of multispecies sequences revealed that the variation loci were relatively conservative among species (**Figure 2B**). In addition, a predicted comparison of the tertiary protein structure before and after the mutation showed that the *RALA* p.Arg161Gln variant changed the arginine to glutamine at position 161 of the RalA protein, and this may lead to a reduction in the binding of hydrogen bonds and loosening of the protein structure. This results in the exposure and activation of the GTP/GDP binding domain and promotes the binding and hydrolysis of GTP while activating the corresponding downstream effector molecules (**Figure 2C**).

RALA c.G482A Increased the RalA Expression

After transfection of the HEK293T cells *in vitro*, we found that transfection of the *RALA* c.G482A mutant significantly increased the expression of the RalA protein. Compared with the vector group (0.64 ± 0.06 vs. 0.09 ± 0.04, $P = 0.000$, $n = 6$) and the wild-type group (0.64 ± 0.06 vs. 0.38 ± 0.08, $P = 0.000$, $n = 6$), the RalA protein expression was significantly increased in the *RALA* mutant group (**Figure 3A**). In addition, the immunofluorescence staining also exhibited consistent results (**Figure 3B**). The mRNA expression was also significantly upregulated in the *RALA* c.G482A group compared with the vector group (1.46 ± 0.23 vs. 0.32 ± 0.06, $P = 0.000$, $n = 4$) and the wild-type group (1.46 ± 0.23 vs. 0.83 ± 0.12, $P = 0.024$, $n = 4$) (**Figure 3C**).

RALA c.G482A Led to Increased RalA GTPase Activity and the Activation of Downstream Effectors

To illustrate the effect of this variant on RalA GTPase activity and downstream effectors, subsequent experiments were conducted. According to the characteristic tags carried by the transfected plasmids, Flag-tagged protein purification was first performed on the extracted total cell proteins using specific DYKDDDDK magnetic agar to obtain different groups of RalA proteins. Following that, the RalA GTPase activity was assayed using the Ral GTPase kit. The results demonstrated that the RalA GTPase activity was enhanced in the *RALA* c.G482A group compared with the vector group (35.68 ± 0.53 vs. 0.37 ± 0.20, $P = 0.000$, $n = 3$) and the wild-type group (35.68 ± 0.53 vs. 18.54 ± 0.39, $P = 0.000$, $n = 3$) (**Figure 3D**).

Then, RalA downstream effector binding assays were implemented based on the purified proteins harvested above. The results showed that the downstream effector binding was also significantly increased (**Figure 3E**) compared with the

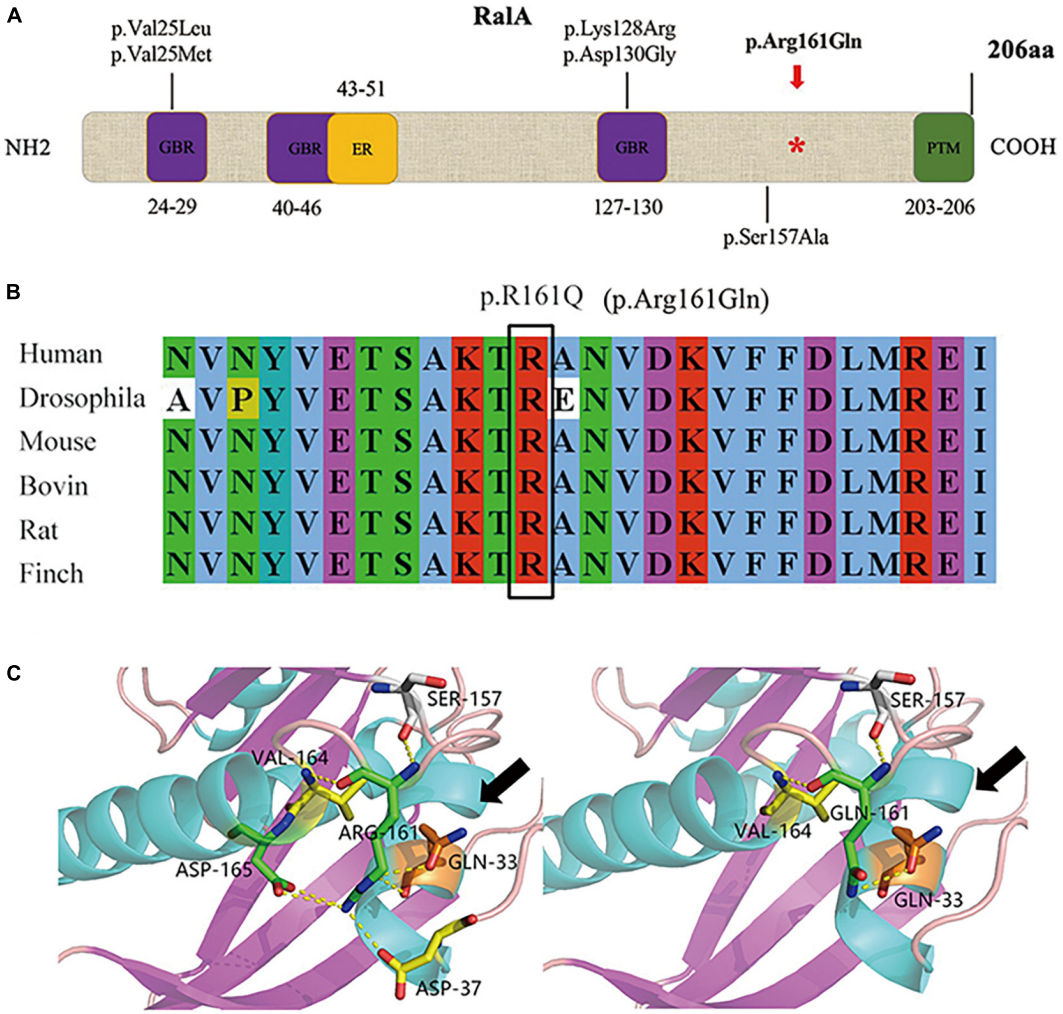


FIGURE 2 | Domain diagram of human RalA and *RALA* variants. **(A)** Domains of RalA. GBR, GTP/GDP binding regions; ER, effector region; PTM, posttranslational modification. A red asterisk *: mutation site of *RALA* we identified in FCD type II tissue. The other variants associated with neurological diseases are also shown. **(B)** Homology comparison between species of *RALA* variant. **(C)** Protein structure before and after mutation of *RALA* c.G482A (p.Arg161Gln), as indicated by the black arrow.

vector group (3.2384 ± 0.0436 vs. 0.0017 ± 0.0010, $P = 0.000$, $n = 3$) and the wild-type transfected group (3.3284 ± 0.0436 vs. 1.7440 ± 0.0719, $P = 0.000$, $n = 3$).

The above results indicated that *RALA* c.G482A brought about a significant increase in RalA GTPase activity as well as activation of downstream effectors. This implied that the *RALA* c.G482A mutation resulted in a gain of function.

RALA c.G482A Caused Aberrant Activation of the Mammalian Target of Rapamycin Pathway

To verify whether the *RALA* mutation leads to mTORC1 overactivation, we examined the level of ribosomal S6 protein phosphorylation in HEK293T cells after transfection, a typical marker of mTOR pathway activation. We observed that cells expressing the *RALA* c.G482A mutant had significantly higher levels of S6 phosphorylation compared with wild-type or vector-transfected group cells (**Figures 3F,G**). These results suggest that the *RALA* c.G482A mutation leads to abnormal activation of the mTOR pathway.

Immunofluorescence staining was performed on the brain tissue sections of patients carrying the somatic variant of *RALA* c.G482A labeled with NeuN, SMI-311, and Vimentin (V9) for neurons, DNs, and BCs, respectively. The phosphorylation level of the S6 protein was significantly higher at the FCD lesion than in the perilesional tissues and colocated with DNs and BCs (**Figure 3H**), indicating the overactivation of the mTOR pathway in the patient's brain tissue, which was consistent with the results of the *in vitro* cellular experiments. Then, we detected the expression and localization of the RalA protein in the lesioned brain tissue, the results proved that the expression of the RalA protein was significantly colocalized with BCs and DNs, and the

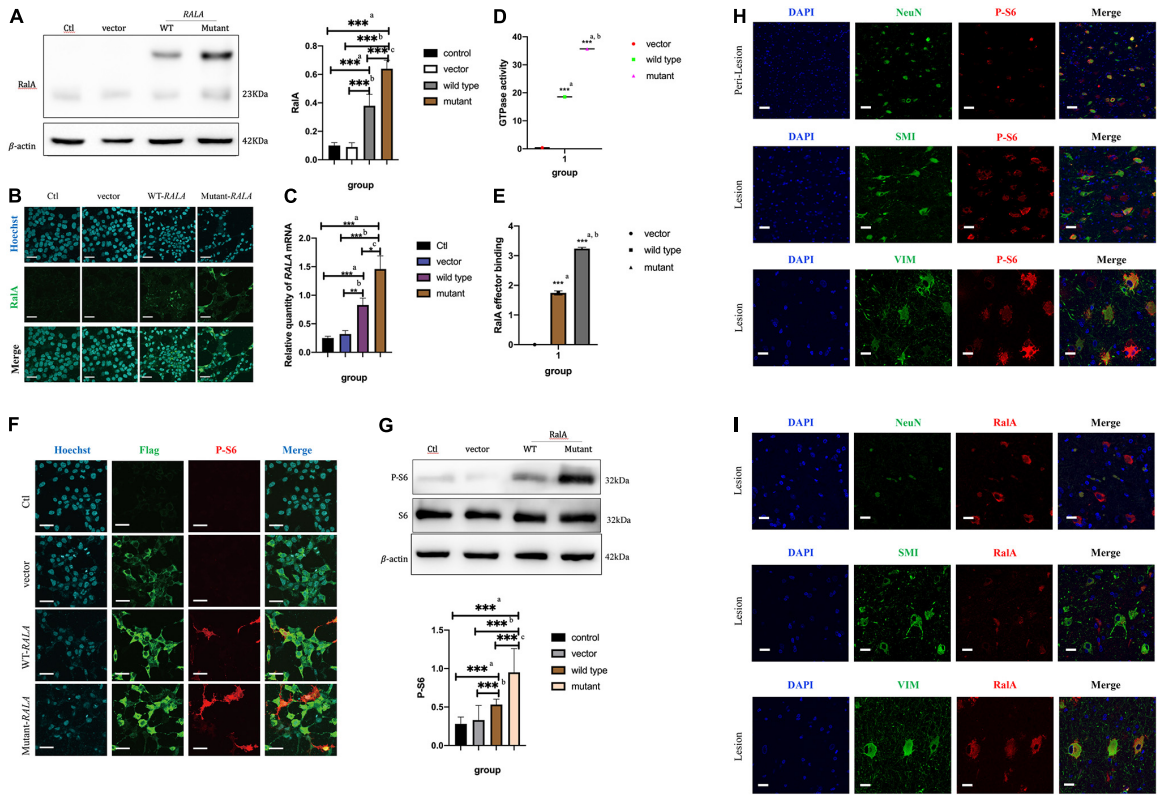


FIGURE 3 | *RALA* c.G482A increases the expression of RalA and promotes mTOR pathway overactivation. **(A)** Expression of RalA protein between different groups after *RALA* c.G482A transfection. Ctl, control; WT, wild type of *RALA*; a, compared with Ctl; b, compared with vector; c, compared with WT. $n = 6$; $^{***}P < 0.001$. **(B)** Immunofluorescence of RalA protein in different groups after *RALA* mutant transfection. **(C)** Changes of *RALA* mRNA expression in different groups. $n = 4$; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. **(D)** Detection of GTPase activity of RalA. a, compared with vector group; b, compared with wild type. $n = 3$; $^{***}P < 0.001$. **(E)** Downstream effector activation assay after *RALA* mutation. a, compared with vector group; b, compared with wild type. $n = 3$; $^{***}P < 0.001$. **(F,G)** Immunofluorescence staining and Western blotting showed the activation of the mTOR pathway. P-S6, phospho-S6 Ribosomal protein (Ser240/244), one of the typical markers of mTOR pathway activation. Bar = 100 μm; magnification: 10 × 60. $n = 6$; $^{***}P < 0.001$. **(H)** Activation of mTOR pathway in brain tissue. NeuN, marker of neurons; SMI-311, marker of dysmorphic neurons; Vimentin (V9), marker of balloon cells. **(I)** Expression of RalA protein in brain tissue. Bar = 50 μm; magnification: 10 × 40.

RalA expression was more enriched in the BCs than the DNs (**Figure 3I**).

RALA c.G482A Might Mediate Activation of Downstream Mammalian Target of Rapamycin Pathways via Downstream Effectors Phospholipase D1 or EXOC2

Furthermore, to inquire about the pathway through which RalA activates the mTOR pathway, we further conducted quantitative proteomic analysis on the transfected cells of *RALA* wild-type and *RALA* mutant. A total of 10 FC > 1.2-fold differentially expressed proteins (DEPs) were identified in the mutant and wild-type groups by proteomic mass spectrometry, with a total of 7,208 proteins detected in each sample. Gene ontology (GO) enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment results of DEPs are shown in **Figures 4A–F**, which are mainly involved in regulating cytoskeleton assembly, microtubule movement, phospholipase activity, and docking of exocytosis vesicles. Finally, we used the String DB protein interaction database to map the top 10 protein molecules that directly interact with RalA (**Figure 4G** and **Table 1**) and hypothesized that *RALA* c.G482A may be involved in the regulation of cell polarity and cell migration through the downstream pathway of phospholipase D1 (PLD1)-mediated activation of mTOR, or through EXOC8/EXOC2 (**Figure 4H**) mediated exocyst complex to recruit downstream kinases, which in turn leads to aberrant activation of the mTOR pathway (Zaman et al., 2021). Whether the *RALA* c.G482A variant mediates downstream mTOR pathway activation *via* PLD1/EXOC2 is currently only a hypothesis and requires further experimental validation.

In utero Electroporation of the *RALA* c.G482A Somatic Mutation-Induced Migration Disorders of Mice Cortex Neurons

We electrotransfected wild-type or *RALA* c.G482A IRES-GFP plasmids into E14.5 mice embryos (**Figure 5A**) and sacrificed the

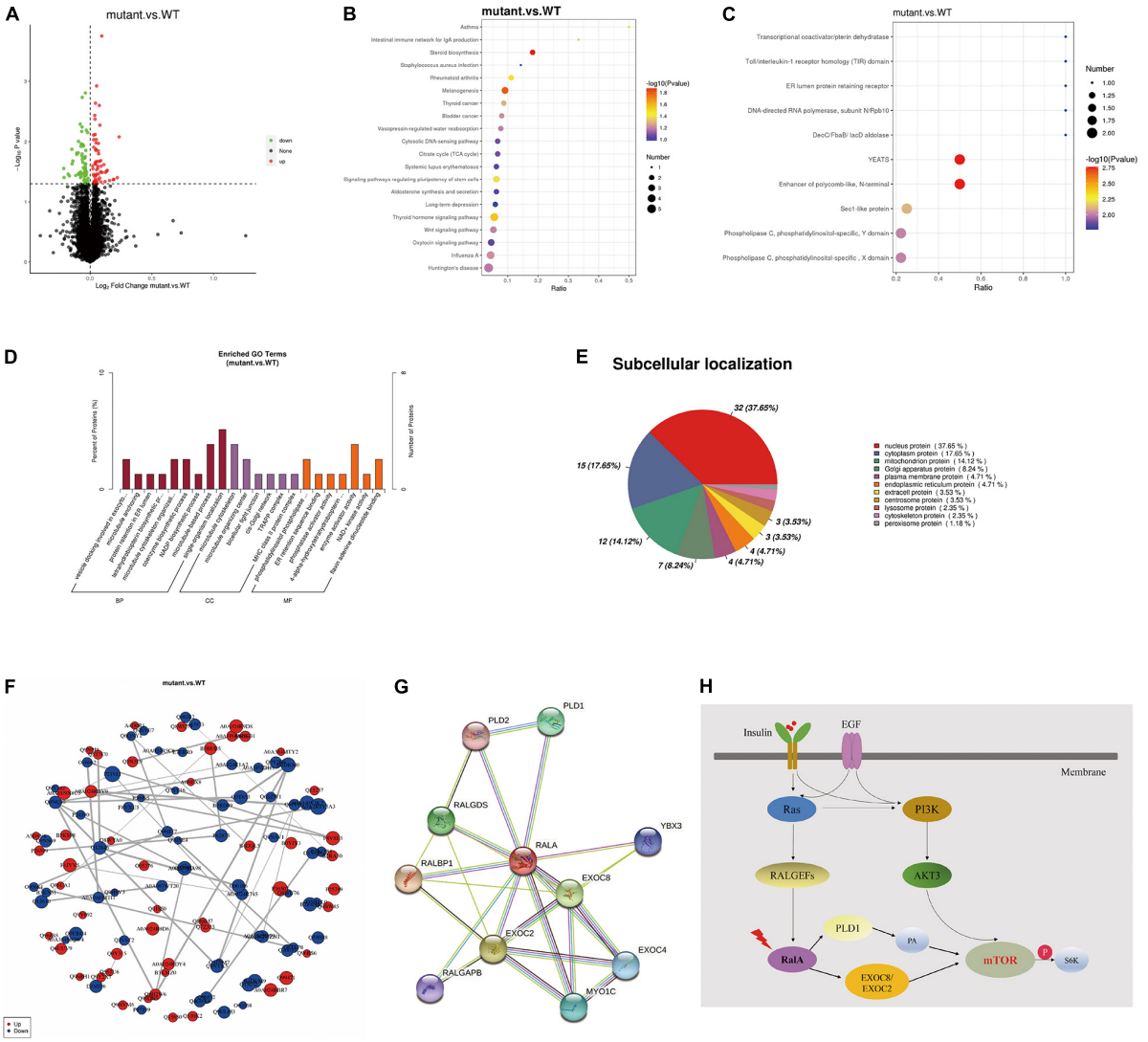


FIGURE 4 | Tandem mass tag (TMT) quantitative proteomics assay. **(A)** Differential proteins volcano map. The horizontal axis represents the multiple of difference (Log2 value) of differential proteins, the vertical axis represents *P*-value (–log10 value), the black represents the protein with no significant difference, the red represents the upregulated protein, and the green represents the downregulated protein. **(B)** Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment bubble chart. The abscissa in the figure is the ratio of the number of differential proteins in the corresponding pathway to the total number of proteins identified in the pathway. The size of the point represents the number of differential proteins in the corresponding pathway. The larger the point, the more differential proteins in the pathway. **(C)** Interproscan software enrichment scatterplot. The abscissa is the ratio of the number of differential proteins in the corresponding domain to the total number of proteins identified in the domain. The size of the point represents the number of differential proteins in the corresponding domain. The larger the point, the more differential proteins in the domain. **(D)** Gene ontology enrichment analysis histogram. BP, GO_Class, biological process; CC, GO_Class, cellular component; MF, GO_Class, molecular function. *x*, the number of differential proteins associated with the gene ontology (GO); *n*, number of differential proteins annotated by GO. The diagram shows the enrichment of the three categories as a result, each show at most 20 (*P*-value $\leq$ 0.05), and the percentage of the ordinate represents the *x*/*n* in the table. **(E)** Subcellular localization analysis of differential proteins. **(F)** Differential protein-protein interaction analysis. Each node in the interaction network represents a protein, and the node size represents the number of proteins interacting with it. The larger the node, the more the proteins interacting with it. The color of the node indicates the expression level of this protein in the comparison pair. The red color represents the upregulated proteins, and the blue color represents the significantly downregulated proteins. **(G)** Top 10 proteins that interact with RalA, identified using the String DB protein interaction database (http://string-db.org/). **(H)** RALA somatic variant might activate mTOR pathway through downstream effectors PLD1 or EXOC8/EXOC2.

mice at E18.5 for brain slices to measure the radial migration of GFP-positive cells in the cortex and the distribution of neurons in different cortical areas. The immunofluorescence results showed that neurons in the *RALA* c.G482A group migrated less from the paraventricular to the cortical plate (CP) and were more concentrated in the intermediate zone (IZ) compared with the wild-type group (**Figure 5B**). Neurons were counted in different parts of the mouse cerebral cortex (Fata et al., 2014), and the results displayed that the number of neurons migrating to the CP was significantly decreased in the

TABLE 1 | A list of the top 10 proteins interacting with RalA.

Predicted partners	Functional annotations
RalA-binding protein 1 (RalBP1)	As a downstream effector of RalA and RalB. As a GTPase-activating protein/GAP inactivated CDC42 and RAC1. As an effector of RalA controlling mitochondrial fission in mitosis. Regulating ligand-dependent EGF and insulin receptors-mediated endocytosis.
Exocyst complex component 2 (EXOC2)	Component of the exocyst complex involved in the docking of exocytic vesicles. Belongs to the SEC5 family.
Exocyst complex component 8 (EXOC8)	Component of the exocyst complex involved in the docking of exocytic vesicles. Belongs to the EXO84 family.
Ral guanine nucleotide dissociation stimulator (RALGDS)	Stimulating GTP binding and activation of the RalA and RalB GTPases. Interacts and acts as an effector molecule for R-Ras, H-Ras, K-Ras.
Phospholipase D1 (PLD1)	Implicated in signal transduction, membrane trafficking, and the regulation of mitosis.
Unconventional myosin-Ic (MYO1C)	Actin-based motor molecules with ATPase activity served in intracellular movements. Involved in glucose transporter recycling by regulating movement of intracellular GLUT4-containing vesicles to the plasma membrane. Acts as a mediator of adaptation of mechanoelectrical transduction.
Exocyst complex component 4 (EXOC4)	Component of the exocyst complex involved in the docking of exocytic vesicles with fusion sites on the plasma membrane.
Y-box-binding protein 3 (YBX3)	Binds to the GM-CSF promoter as a repressor. Binds to full-length mRNA and to short RNA sequences containing the consensus site 5′-UCCAUCA-3′ acted as translation repression.
Ral GTPase-activating protein subunit beta (RALGAPB)	Non-catalytic subunit of the heterodimeric RalGAP1 and RalGAP2 complexes. GTPase activators for the Ras-like small GTPases RALA and RALB.
Phospholipase D2 (PLD2)	Involved in signal-induced cytoskeletal regulation and/or endocytosis.

RALA c.G482A group mice compared with the wild-type group (77.33 ± 16.21 vs. 129.00 ± 16.46, $P = 0.000$, $n = 6$), while the number of neurons in the IZ region was significantly increased (137.33 ± 15.96 vs. 77.17 ± 11.41, $P = 0.000$, $n = 6$). The number of neurons in the subventricular area (SVZ) in the two groups was 72.83 ± 12.14 vs. 75.50 ± 10.33 ($P = 0.747$, $n = 6$), and the difference was not statistically significant (**Figure 5C**). Mice expressing *RALA* c.G482A had reduced numbers of GFP-positive cells in the CP areas and increased numbers of GFP-positive cells in the ventricular and paraventricular areas, suggesting that the expression of *RALA* c.G482A can lead to neuronal migration disorders.

To further confirm the effect of *RALA* variation on neuronal migration, we labeled the superficial layer II–IV neurons (CUX-1) and the deep layer V/VI neurons (TBR1) separately, and the staining results revealed that more cortical neurons in the mutant group resided in the layer V/VI cortex and fewer neurons migrated to the layer II–IV compared with the wild-type mice (**Figure 5D**).

DISCUSSION

RALA encodes a small GTPase of the Ras superfamily, RalA, that is widely expressed in various tissues throughout the body and is involved in a series of biological functions, such as gene expression, cell migration, cell proliferation, and membrane transport by interacting with different downstream effectors (Yan and Theodorescu, 2018). In the CNS, RalA is engaged in neuronal development, plasticity, polarization, migration, synaptic vesicle fusion, NMDA, AMPA, and dopamine receptor regulation (Teodoro et al., 2013; Zheng et al., 2016).

Our group first detected the *RALA* somatic variant in FCD II brain tissue using whole-exome sequencing (WES), and we verified this using amplicon sequencing with a VAF of 5.50%. In this study, we discovered that the *in vitro* transfection of the *RALA* c.G482A variant resulted in increased RalA protein expression and RalA GTPase activity and the activation of its downstream effectors. In addition, we observed an obvious increase in S6 phosphorylation and overactivation of the mTOR pathway after transfection. Immunofluorescence staining of the brain tissue specimens from this patient also confirmed the overactivation of the mTOR pathway in brain tissue, and that the *RALA* c.G482A variant was more enriched in the BCs and DNs. Then, by constructing an *in vivo* electrotransfected mice model, we found that the *RALA* c.G482A variant apparently affected cortical neuronal migration, and this resulted in more cortical neurons residing in the paraventricular region and the layer V/VI cortex and less migration in the superficial II-IV cortex. These results suggest that in FCD type II brain tissue lesions, the somatic variant of the *RALA* gene is primarily distributed in BCs and DNs, and this variant leads to the functional gain of RalA, activating its downstream effector molecules, further activating the PI3K-AKT-mTOR pathway, thus affecting neuronal migration, and ultimately leading to the development of FCD.

Somatic variants of *RALA* have been previously reported in cancers, and a total of 492 *RALA* somatic variants have been found in various tissues and organs, suggesting that pathogenic *RALA* somatic variants are associated with a wide range of disease phenotypes. Among them, the *RALA* c.G482A somatic variant we identified has been reported to be associated with adult T-cell lymphoma leukemia (Kataoka, 2015) and malignant melanoma (Hayward et al., 2017; Wilmott et al.,

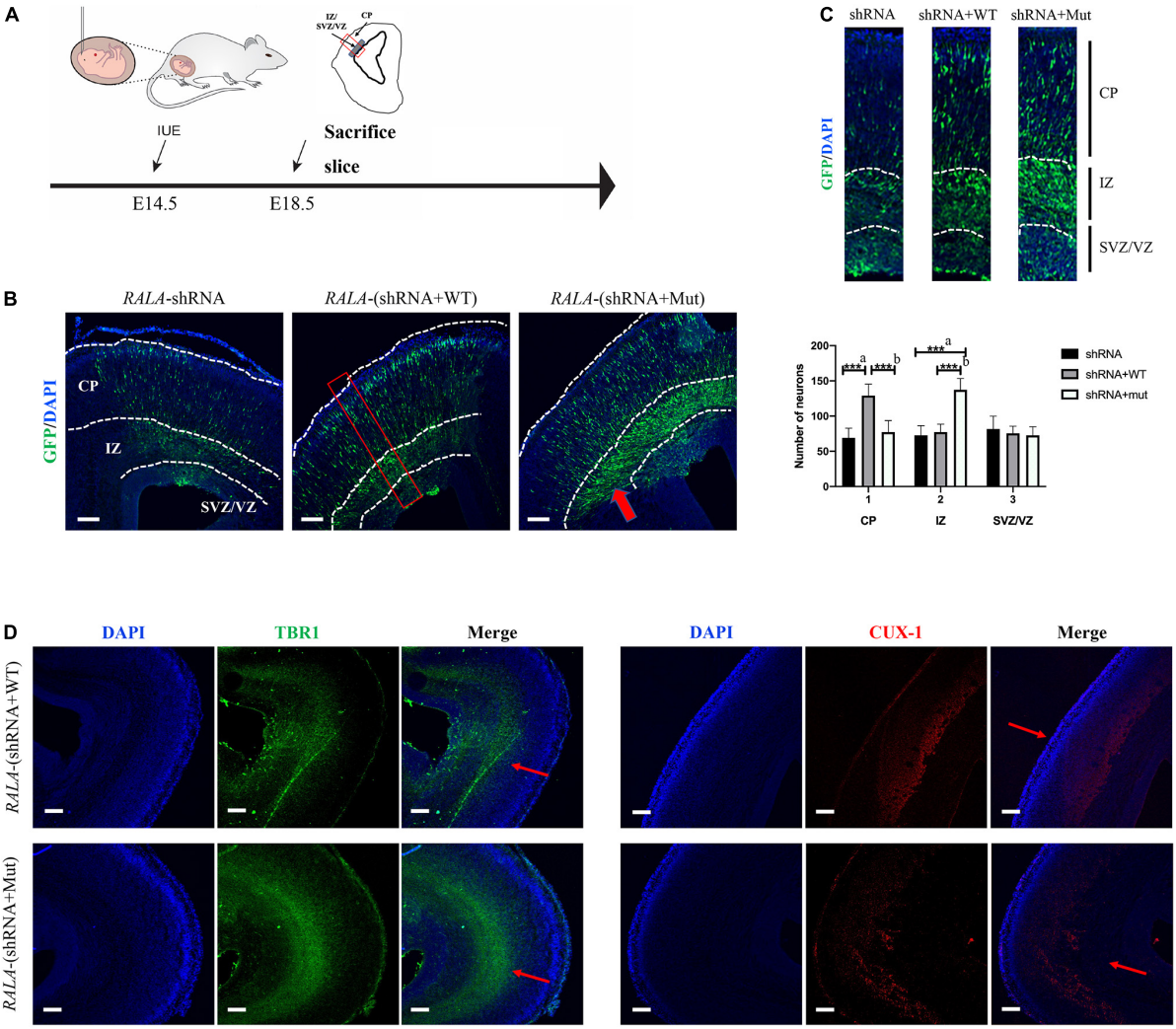


FIGURE 5 | Simulation of cortical dysplasia in mouse models *via in utero* electroporation. **(A)** Schematic diagram of animal experiment. **(B)** *RALA* c.G482A causes disorders of cortical neuron migration, as indicated by the thick red arrow. **(C)** Count and statistics of neurons in different areas of mouse cortex. Local amplification figure from the red square area. **(D)** Immunofluorescence labeled layer II/III/IV and layer V/VI neurons, thin red arrows. IUE, *in utero* electroporation; E14.5, embryo 14.5 days; VZ, ventricular zone; SVZ, subventricular zone; IZ, intermediate zone; CP, cortical plate; EEG, Electroencephalogram; shRNA, short hairpin RNA; TBR1, T-box brain transcription factor 1; CUX-1, Cut Like Homeobox 1. a, compared with *RALA*-shRNA group; b, compared with *RALA*-(shRNA + WT) group. $n = 6$, $^{***}P < 0.001$. Bar = 10 μm; magnification: 10 × 10.

2019), and no studies have been performed to associate them with neurological diseases. Moreover, Hiatt et al. (2018) reported cases of *de novo RALA* germline variants causing Hiatt-Neu-Cooper neurodevelopmental syndromes (HINCONS) with the variation that included six missense variants of c.G73A, c.G73T, c.A383G, c.A389G, c.T469G, and c.C526T and one chromosomal microdeletion of c.472_474delGCT. HINCONS is an autosomal dominant neurodevelopmental disorder characterized by generalized developmental delay, intellectual disability, language deficits, and facial dysmorphism. It may also be accompanied by epilepsy, autism, and structural brain abnormalities, such as corpus callosum dysplasia and polymicrogyria. The phenotypic heterogeneity between germline variants and somatic variants of the same gene has also been appeared in other variants associated with neurological disorders. Bonduelle et al. (2021) investigated 20 cases of mild malformations of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE) children using brain tissue sequencing. *SLC35A2* somatic mutations were detected in 9/20 (45%) patient brains, while the germline variants of the *SLC35A2* gene led to developmental and epileptic encephalopathies. Their findings highlight the importance of brain somatic mutations in the etiology of focal epilepsy associated with MCD.

Currently, the genes located in the mTOR pathway identified in the FCD II brain tissues are classified into three types: (Mariasavina et al., 2020) gain-of-function variation in the mTOR upstream regulatory genes, such as *PIK3CA*, *AKT3,* and *RHEB*; (Ingmar et al., 2011) loss-of-function variation of the

mTOR pathway suppressor genes, such as *DEPDC5*, *NPRL2*, *NPRL3,* and *PTEN*; and (Blumcke et al., 2017) somatic gain-of-function mutations in the *MTOR* gene. Several of these genes also have somatic second-hit mutations, including *AKT3*, *DEPDC5*, *MTOR*, *PIK3CA*, *RHEB*, *TSC1*, and *TSC2*. Jansen et al. (2015) identified *AKT3* and *PIK3CA* somatic mutations in the brain tissue of FCD II patients using targeted sequencing of 10 genes of the mTOR pathway. Mirzaa et al. (2016) performed WES of lesioned brain tissues and peripheral blood samples to find *MTOR* somatic mutations, and these somatic pathogenic variants were enriched in the DNs and BCs, suggesting that DNs and BCs may be the few abnormally differentiated somatic cells that carry pathogenic variants. Our group (Zhang et al., 2020) previously screened seven potential FCD II-related somatic variants using WES and amplicon sequencing in 6/17 (35%) brain tissue specimens from FCD II patients. Except for *MTOR* and *TSC2*, which are the reported genes, the remaining five genes, *IRS1*, *ZNF337*, *HTR6*, *RALA*, and *RAB6B*, were not yet associated with FCD. Nevertheless, no clear pathogenic somatic mutations have been detected in the brain tissue of more than half of the patients. Therefore, it remains to be determined whether other genetic variants in the upstream pathway related to mTOR and abnormal regulation of other signaling pathways are also implicated in the pathogenesis of FCD. Our findings suggest that there are other genetic variants in FCD II brain tissue. However, our findings show that *RALA* c.G482A can also activate the mTOR pathway, and we hypothesized that the *RALA* variant can lead to the aberrant activation of the mTOR pathway through PLD1-mediated downstream signaling pathways. Several previous studies have also reported that activation of PLD1, a downstream effector of RalA, can lead to an increased intracellular phosphatidic acid concentration that in turn activates the mTOR pathway (Xu et al., 2011; Bernfeld et al., 2018). Therefore, mTOR pathway activation may also be a common pathway for FCD II pathogenesis.

However, there are still some limitations in this study: (Mariasavina et al., 2020) all of the FCD II-related somatic variant genes discovered and reported so far are in the mTOR pathway and its regulatory pathways, including the *RALA* gene that we newly identified in this study, and no variant of genes in other pathways outside this pathway has been found (Ingmar et al., 2011). Due to the limitation of the sequencing depth and cost of WES, the somatic variants were only detected in a small number of FCD II brain tissue samples, among which the *RALA* somatic variant was detected in the brain tissue of only one FCD IIb patient, and more than half of the samples were still negative for genetic testing, pending further sequencing (Blumcke et al., 2017). Although our study found that the *RALA* c.G482A variant activated the mTOR pathway and caused cortical neuronal migration disorders, considering that children carrying this variant have epilepsy, further studies are required to better correlate genotype with phenotype as to how *RALA* c.G482A causes FCD and subsequently affects the electrophysiological properties of neurons, ultimately leading to epilepsy.

In summary, we investigated the role of *RALA* gene dysfunction in the formation of FCD II using functional studies of the newly identified brain *RALA* somatic variant at the cellular and animal levels in terms of their effects on the PI3K-AKT-mTOR signaling pathway and on cortical neuronal migration, thus confirming that *RALA* is a new pathogenic gene related to FCD II. The results of the study help to expand the understanding of the pathogenesis of FCD II, enrich the gene spectrum of somatic variants associated with MCD, and provide a basis for clarifying the mechanism of FCD II to explore new therapeutic targets. However, the brain *RALA* somatic variant was found in only one patient with FCD II, and this result needs to be confirmed in more patient samples.

DATA AVAILABILITY STATEMENT

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of Peking University First Hospital. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin. The animal study was reviewed and approved by the Animal Ethics Committee of Peking University First Hospital. Written informed consent was obtained from the individual(s), and minor(s)' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

HX was the executor of this study, who completed experiment procedures, and manuscript writing. KG, QL, TW, ZZ, and LC participated in clinical data collection and result analysis. YW and YJ were the corresponding authors in charge of the study and directed the experimental design, data analysis, manuscript writing, and revision. All authors agreed to be accountable for the content of this work.

FUNDING

This work was supported by the National Key Research and Development Program of China (Grant No. 2020YFA0804000), the National Natural Science Foundation of China (Grant Nos. 82171435, 81971211, and 81601131), the Key Project of Clinical Medicine Research of National Clinical Research Center for Child Health and Disorders, Children's Hospital of Chongqing Medical University (Grant No. NCRCCHD-2021-KP-02), Beijing Natural Science Foundation (Grant No. 7212109), the Beijing Key Laboratory of Molecular Diagnosis and Study on Pediatric Genetic Diseases (Grant No. BZ0317), and the Fundamental Research Funds for the Central Universities (Grant Nos. BMU2017JI002, BMU2018XY006, and PKU2017LCX06).

REFERENCES

Baulac, S., Baldassari, S., Ribierre, T., Marsan, E., Georg, D., Mathilde, C., et al. (2019). Dissecting the genetic basis of focal cortical dysplasia: a large cohort study. *Acta Neuropathol.* 138, 885–900. doi: 10.1007/s00401-019-02061-5

Baumgart, J., and Baumgart, N. (2016). Cortex-, Hippocampus-, Thalamus-, Hypothalamus-, Lateral Septal Nucleus–and Striatum–specific In Utero Electroporation in the C57BL/6 Mouse. *J. Vis. Exp.* 107:e53303. doi: 10.3791/53303

Bernfeld, E., Menon, D., Vaghela, V., Zerin, I., Frias, M. A., Foster, D. A., et al. (2018). Phospholipase D-dependent mTOR complex 1 (mTORC1) activation by glutamine. *J. Biol. Chem.* 293, 16390–16401. doi: 10.1074/jbc.RA118.004972

Blumcke, I., Coras, R., Busch, R. M., Marcia, M. S., Lal, D., Najm, I., et al. (2021). Toward a better definition of focal cortical dysplasia: an iterative histopathological and genetic agreement trial. *Epilepsia* 62, 1416–1428. doi: 10.1111/epi.16899

Blumcke, I., Spreafico, R., Haaker, G., Coras, R., Kobow, K., Bien, C. G., et al. (2017). Histopathological Findings in Brain Tissue Obtained during Epilepsy Surgery. *N. Engl. J. Med.* 377, 1648–1656. doi: 10.1056/NEJMoa1703784

Bonduelle, T., Hartlieb, T., Baldassari, S., Sim, N. S., Kim, S. H., Baulac, S., et al. (2021). Frequent SLC35A2 brain mosaicism in mild malformation of cortical development with oligodendroglial hyperplasia in epilepsy (MOGHE). *Acta Neuropathol. Commun.* 9, 3–15. doi: 10.1186/s40478-020-01085-3

Carolyn, K., Kegan, S., and Alan, D. (2015). Comparison of sodium dodecyl sulfate depletion techniques for proteome analysis by mass spectrometry. *J. Chromatogr. A* 1418, 158–166. doi: 10.1016/j.chroma.2015.09.042

Fata, G. L., Carlos, G., Meredith, R. M., Achsel, T., Dotti, C. G., Bagni, C., et al. (2014). FMRP regulates multipolar to bipolar transition affecting neuronal migration and cortical circuitry. *Nat. Neurosci.* 17, 1693–1700. doi: 10.1038/nn.3870

Gillette, M. A., Satpathy, S., Mannan, R., Song, C., Dhanasekaran, S. M., Vasaikar, S. V., et al. (2020). Proteogenomic Characterization Reveals Therapeutic Vulnerabilities in Lung Adenocarcinoma. *Cell* 182, 200–225. doi: 10.1016/j.cell.2020.06.013

Hayward, N. K., Wilmott, J. S., Nicholas, K., James, S., Waddell, N., Mann, G. L., et al. (2017). Whole-genome landscapes of major melanoma subtypes. *Nature* 545, 175–192. doi: 10.1038/nature22071

Hiatt, S. M., Neu, M. B., Ramaker, R. C., Hardigan, A. A., Sedlacek, Z., Cooper, G. M., et al. (2018). De novo mutations in the GTP/GDP-binding region of RALA, a RAS-like small GTPase, cause intellectual disability and developmental delay. *PLoS Genet.* 14:e1007671. doi: 10.1371/journal.pgen.1007671

Ingmar, B., Maria, T., Eleonora, A., Dawna, D., Harry, V., Andre, P., et al. (2011). The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* 52, 158–174. doi: 10.1111/j.1528-1167.2010.02777.x

Jansen, L. A., Mirzaa, G. M., Ishak, G. E., Hiatt, J. B., Hevner, R. F., Dobyns, W. B., et al. (2015). PI3K/AKT pathway mutations cause a spectrum of brain malformations from megalencephaly to focal cortical dysplasia. *Brain* 138, 1613–1628. doi: 10.1093/brain/awv045

Kataoka, K. (2015). Nagata1 Y, Kitanaka A, Shiraishi Y, Shimamura T, Ogawa S et al. Integrated molecular analysis of adult T cell leukemia/lymphoma. *Nat. Genet.* 47, 1304–1315. doi: 10.1038/ng.3415

Lim, J. S., Kim, W. I., Kang, H. C., Kim, S. H., Park, A. H., Park, E. K., et al. (2015). Brain somatic mutations in MTOR cause focal cortical dysplasia type II leading to intractable epilepsy. *Nat. Med.* 21, 395–400. doi: 10.1038/nm.3824

Mariasavina, S., Ana, F. G., Norbert, U., Domenico, T., Ivana, P., Andrea, R., et al. (2020). Definitions and classification of malformations of cortical development: practical guidelines. *Brain* 143, 2874–2894. doi: 10.1093/brain/awaa174

Marsan, E., and Baulac, S. (2018). Review: mechanistic target of rapamycin (mTOR) pathway, focal cortical dysplasia and epilepsy. *Neuropathol. Appl. Neurobiol.* 44, 6–17. doi: 10.1111/nan.12463

Mirzaa, G. M., Campbell, C. D., Solovieff, N., Goold, C., Winckler, W., Dobyns, W. B., et al. (2016). Association of MTOR Mutations With Developmental Brain Disorders, Including Megalencephaly, Focal Cortical Dysplasia, and Pigmentary Mosaicism. *JAMA Neurol.* 73, 836–845. doi: 10.1001/jamaneurol.2016.0363

Moller, R. S., Weckhuysen, S., Chipaux, M., Marsan, E., Taly, V., Bebin, E. M., et al. (2016). Germline and somatic mutations in the MTOR gene in focal cortical dysplasia and epilepsy. *Neurol. Genet.* 2:e118. doi: 10.1212/NXG.0000000000000118

Nakashima, M., Saitsu, H., Takei, N., Tohyama, J., Kato, M., Kitaura, H., et al. (2015). Somatic Mutations in the MTOR gene cause focal cortical dysplasia type IIb. *Ann. Neurol.* 78, 375–386. doi: 10.1002/ana.24444

Oegema, R., Barakat, T. S., Wilke, M., Stouffs, K., Amrom, D., Aronica, E., et al. (2020). International consensus recommendations on the diagnostic work-up for malformations of cortical development. *Nat. Rev. Neurol.* 16, 618–635. doi: 10.1038/s41582-020-0395-6

Saxton, R. A., and Sabatini, D. M. (2017). mTOR Signaling in Growth, Metabolism, and Disease. *Cell* 168, 960–976. doi: 10.1016/j.cell.2017.02.004

Teodoro, R. O., Pekkurnaz, G., Nasser, A., Maria, B., Camonis, J., Schwarz, T. L., et al. (2013). Ral mediates activity-dependent growth of postsynaptic membranes via recruitment of the exocyst. *EMBO J.* 32, 2039–2055. doi: 10.1038/emboj.2013.147

Wang, C., and Mei, L. (2013). "Utero Electroporation in Mice," in *Neural Development*, eds R. Zhou and L. Mei (Berlin: Springer Science Press), 151–163.

Wilmott, J. S., Johansson, P. A., Newell, F., Waddell, N., Hayward, N. K., Scolyer, R. A., et al. (2019). Whole genome sequencing of melanomas in adolescent and young adults reveals distinct mutation landscapes and the potential role of germline variants in disease susceptibility. *Int. J. Cancer* 144, 1049–1060. doi: 10.1002/ijc.31791

Wiśniewski, J. R., Zougman, A., Nagaraj, N., and Mann, M. (2009). Universal sample preparation method for proteome analysis. *Nat. Methods* 6, 359–362. doi: 10.1038/nmeth.1322

Xu, L., Salloum, D., Medlin, P. S., Saqcena, M., Yellen, P., Foster, D. A., et al. (2011). Phospholipase D mediates nutrient input to mammalian target of rapamycin complex 1 (mTORC1). *J. Biol. Chem.* 286, 25477–25486. doi: 10.1074/jbc.M111.249631

Yan, C., and Theodorescu, D. (2018). RAL GTPases: Biology and Potential as Therapeutic Targets in Cancer. *Pharmacol. Rev.* 70, 1–11. doi: 10.1124/pr.117.014415

Zaman, A., Wu, X., Trever, G., Yadavalli, S., Lee, J., Wang, C., et al. (2021). Exocyst protein subnetworks integrate Hippo and mTOR signaling to promote virus detection and cancer. *Cell Rep.* 36:109491. doi: 10.1016/j.celrep.2021.109491

Zhang, Z., Gao, K., Liu, Q., Jiang, Y., Cai, L., Wu, Y., et al. (2020). Somatic variants in new candidate genes identified in focal cortical dysplasia type II. *Epilepsia* 61, 667–678. doi: 10.1111/epi.16481

Zheng, M., Zhang, X., Sun, N., Min, C., Zhang, X., Kim, K. M., et al. (2016). RalA employs GRK2 and beta-arrestins for the filamin A-mediated regulation of trafficking and signaling of dopamine D2 and D3 receptor. *Biochim. Biophys. Acta* 1863, 2072–2083. doi: 10.1016/j.bbamcr.2016.05.010

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers | Frontiers in Behavioral Neuroscience

MINI REVIEW
published: 01 July 2022
doi: 10.3389/fnbeh.2022.924319

Application of Cognitive Bias Testing in Neuropsychiatric Disorders: A Mini-Review Based on Animal Studies

Yu-Han Zhang[1,2], Ning Wang[1,2], Xiao-Xiao Lin[1,2], Jin-Yan Wang[1,2] and Fei Luo[1,2]*

[1] CAS Key Laboratory of Mental Health, Institute of Psychology, Chinese Academy of Sciences, Beijing, China, [2] Department of Psychology, University of Chinese Academy of Sciences, Beijing, China

Cognitive biases can arise from cognitive processing under affective states and reflect the impact of emotion on cognition. In animal studies, the existing methods for detecting animal emotional state are still relatively limited, and cognitive bias test has gradually become an important supplement. In recent years, its effectiveness in animal research related to neuropsychiatric disorders has been widely verified. Some studies have found that cognitive bias test is more sensitive than traditional test methods such as forced swimming test and sucrose preference test in detecting emotional state. Therefore, it has great potential to become an important tool to measure the influence of neuropsychiatric disorder-associated emotions on cognitive processing. Moreover, it also can be used in early drug screening to effectively assess the potential effects or side effects of drugs on affective state prior to clinical trials. In this mini-review, we summarize the application of cognitive bias tests in animal models of neuropsychiatric disorders such as depression, anxiety, bipolar disorder, and pain. We also discussed its critical value in the identification of neuropsychiatric disorders and the validation of therapeutic approaches.

Keywords: cognitive bias test, animal research, affective state, application, memory bias, interpretation bias

OPEN ACCESS

Edited by:
Jiaojian Wang,
University of Electronic Science and Technology of China, China

Reviewed by:
Rainer Schwarting,
University of Marburg, Germany

***Correspondence:**
Ning Wang
wangn@psych.ac.cn

Specialty section:
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

Received: *20 April 2022*
Accepted: *13 June 2022*
Published: *01 July 2022*

Citation:
Zhang Y-H, Wang N, Lin X-X, Wang J-Y and Luo F (2022) Application of Cognitive Bias Testing in Neuropsychiatric Disorders: A Mini-Review Based on Animal Studies. Front. Behav. Neurosci. 16:924319. doi: 10.3389/fnbeh.2022.924319

INTRODUCTION

Emotions can cause the brain to distort the truth, leading to a discrepancy between what we believe is true and reality. Cognitive bias is the tendency of the brain to process information in favor of certain emotional valence (Lovibond and Lovibond, 1995). Positive emotions lead to positive cognitive biases, while negative emotions cause negative biases, affecting multiple cognitive processes such as attention, memory, and decision-making (Everaert et al., 2012). The phenomenon of cognitive bias is widespread, especially in neuropsychiatric disorders. The concept of "cognitive bias" was first proposed by Beck in the study of patients with depression (Beck, 1967). Based on Beck's theory, early adverse experiences can trigger negative cognitive schemas leading to negative views of the self, the world, and the future, which in turn lead to biases in cognitive processing (Segal, 1988). According to Bower's theory of mood congruity (Bower, 1981), during cognitive processing, individuals tend to focus, process, and recall information that is consistent with their emotional state, resulting in cognitive biases.

Cognitive biases can be divided into three types: attentional bias, interpretation bias, and memory bias. Attentional bias indicates that individuals are more likely to allocate attention to stimuli consistent with their current emotional state (Mennen et al., 2019). In animal research, attentional bias can be investigated by analyzing the behavioral response to threatening stimuli (Lee et al., 2016; Luo et al., 2019). Interpretation bias affects decision-making processes. Individuals are more likely to interpret ambiguous cues to be consistent with their current affective state (Everaert, 2021). Interpretation bias in animal research is often measured using the judgment bias test (JBT) (Nguyen et al., 2020), which relies on certain behaviors (like bar-pressing) and these results are then interpreted with respect to certain human constructs, one of them being "attitude" (see more details in **Table 1**). For example, animals in a more positive affective state tend to interpret ambiguous cues in a more positive way. Memory bias is most often measured through the affective bias test (ABT) and the modified affective bias test (mABT) in animals (Mitte, 2008). The ABT is based on the assumption that emotional state during the memory coding stage affects the perception of reward value (Stuart et al., 2013), while the mABT examines the ability of an animal to form memory bias based on reward value (Stuart et al., 2015).

Animal experiments are an important complement to human research, especially in the study of neurological and psychological phenomena. Animal research has unique advantages to investigate the underlying mechanisms of these phenomena. For ethical considerations, pharmacological, genetic, and invasive human research is greatly limited, while neurophysiological methods that simulate abnormal states and pharmacological experiments in animals can be conducted to explore specific brain regions, neurons, and even molecules, to better understand the mechanisms behind phenomena, leading to targeted interventions. Harding et al. (2004) were the first to use cognitive bias testing in animals. The presented mini-review briefly summarizes the application of cognitive bias tests in animal research to further explore cognitive bias alterations in neuropsychiatric disorders and the neuropsychological mechanism of cognitive bias, which can ultimately lead to the early identification and treatment of these disorders.

APPLICATION OF ANIMAL COGNITIVE BIAS TESTING

Cognitive Bias in Neuropsychiatric Disorders

Many neuropsychiatric disorders are accompanied by emotional alterations which in turn can lead to cognitive biases. One application of cognitive bias testing is to reflect the affective state under different disorders. Currently, cognitive bias tests have been applied in animal models of depression, anxiety, bipolar disorder, and pain (see more details in **Table 2**). The next section briefly discusses the application of cognitive bias tests in some disorders.

Depression

Depression is a mood disorder accompanied by low self-esteem, impaired cognitive function, and decreased pleasure (Monroe and Anderson, 2015). In human studies of cognitive bias, it was found that depressed subjects are more inclined to focus on negative stimuli (Armstrong and Olatunji, 2012), choose more negative words as self-descriptive (Dainer-Best et al., 2018), and recall more negative items and less positive items on memory tests (Bianchi et al., 2020). Harding et al. were the first to apply the judgment bias paradigm to investigate the cognitive bias of rats (Harding et al., 2004), demonstrating that the JBT can be used to detect negative emotions in animals.

Animal models of depression include chronic stress, learned helplessness, deficits in the serotonin system, and adverse experiences in early life (Czéh et al., 2016). Rats exposed to chronic physical stress or chronic psychosocial stress negatively interpret ambiguous cues, approach rewards more slowly, and experience a series of long-term cognitive and behavioral changes (Salmeto et al., 2011; Hymel and Sufka, 2012; Chaby et al., 2013; Papciak et al., 2013). Compared with congenitally non-helpless rats, congenitally helpless rats showed decreased positive responses and increased negative responses to ambiguous cues (Enkel et al., 2010; Richter et al., 2012). A study found that inhibiting serotonin synthesis through para-chlorophenylalanine (pCPA) dosing in pigs leads to a shift to more pessimistic judgments of ambiguous stimuli (Stracke et al., 2017). Results from early adverse experience models have shown lowered expectation of reward in response to ambiguous information (Bateson et al., 2015). Of particular interest, Stuart et al. (2019) found that rats experiencing maternal separation were more prone to corticosterone-induced negative bias and showed a deficit in reward-associated positive bias in mABT, whereas no significant difference was found in the sucrose preference test. This finding indicates that cognitive bias testing is a sensitive and important tool in depression-like state assessment.

Forced swimming test, sucrose preference test, and open-field test are widely used in animal studies to detect depression-like behaviors such as behavioral despair, anhedonia, and exploratory behaviors (Hu et al., 2017). These tests do not require training, while cognitive biased tasks require long-term and complex conditional training, as shown in **Table 1**. Although the cognitive bias test needs more experimental efforts, the affective bias measured by it could not be replaced by other tests (Robinson, 2018). Therefore, cognitive bias test can be used as a good supplement to the commonly used depression-like behavior test and plays a unique role in mechanism research (Stuart et al., 2015) and drug screening (Stuart et al., 2017).

Anxiety

Negative cognitive biases induced by anxiety can help an organism attend to threatening stimuli quickly, leading to an avoidance of potential danger. In a human study, it was found that anxious subjects exhibit an exaggerated attentional bias toward threats and overestimate detrimental consequences of events (Aue and Okon-Singer, 2015). In a JBT study of chicks under anxiety-like state, more pessimistic-like approach

TABLE 1 | Some methodological details of representative cognitive bias paradigms.

Paradigm	Stimuli/Cues	Reward	Punishment	Training duration	Testing duration	References
Auditory judgment bias test	Tones	Sweetened condensed milk	Electric shock	3 phases, 21–27 days, 1 session/day, 30 min or 20 trials/session	6 days, 1 session/day, 23 trials/session	Enkel et al., 2010
		One food pellet	Air-puff	3 phases, 15–40 days, 1 session/day	40 min or when 66 trials were completed	Jones et al., 2018
				3 phases, 16–22 days, 1 session/day	40 min or when 60 trials were completed	
		Four reward pellets (high reward); one reward pellet (low reward)	\	4 phases, 23–29 days, 1 session/day, maximum 100 trials or 60 min/session	2 sessions of 100 trials, 2 sessions of 120 trials, 1 session/day	Hales et al., 2020
Spatial judgment bias test	Positions	Overhead light off and 20 mg chocolate flavored pellet paired with one arm	Overhead light on paired with another arm	6 days, 10 min/day	10 min	Novak et al., 2015
		One food pellet (high reward); one quinine-soaked pellet (low reward)	\	2 days, 12 trials/day	3 days, 13 trials/day	Burman et al., 2009
Tactile judgment bias test	Sandpapers	Chocolate (high reward); cheerio (low reward)	\	Minimum 10 days, 4 trials/day	5 days, 4 trials/day	Brydges et al., 2012
Visual judgment bias test	Bars	Sweet condensed milk	Houselight on	9 phases, 89–111 days, maximum 50 trials/day	5 days, 50 trials/day and no more than 30 min/day	Krakenberg et al., 2019
Olfactory judgment bias test	Scents	Dried, sweetened banana chips (high reward), regular rodent chow (low reward)	\	3 phases, 17–19 days, 2–4 trials/day	3 trials, 2 min/trial	Resasco et al., 2021
Affective bias test	Substrates	One sugar tablet	\	5 days	5 days, including 4 days for reward pairing, and 1 day for preference testing (1 session, 30 trials)	Stuart et al., 2013, 2015
Modified affective bias test	Substrates	Two sugar tablets (high reward); one sugar tablet (low reward)	\	*5 days	5 days, including 4 days for reward pairing, and 1 day for preference testing (1 session, 30 trials)	Stuart et al., 2013; Hinchcliffe et al., 2017

**In the current studies, the modified affective bias test is often carried out after the affective bias test, therefore no additional training is required before testing.*

behaviors were exhibited to ambiguous aversive cues (Salmeto et al., 2011; Hymel and Sufka, 2012). Using pharmacological methods, one study found that sheep injected with the anxiety-stimulating drug 1-methyl-chlorophenylpiperazine (m-CPP) show increased attention toward threats accompanied by increased vigilance (Lee et al., 2016), leading to negative attentional bias. Other studies found that acute injection of anxiogenic drug FG7142 in rats led to negative cognitive bias in both judgment bias tests (Hales et al., 2016) and affective bias tests (Stuart et al., 2013, 2015; Hinchcliffe et al., 2017).

Studies have shown that high-intensity light and white light are aversive to rodents, while dim light and red light are more neutral (Burman et al., 2009; Boleij et al., 2012) and therefore, alterations in lighting can be used to manipulate anxiety level in rodents. There is strong evidence that rats trained in dim lighting conditions but tested in bright lighting conditions have longer approach latencies when exposed to ambiguous cues (Burman et al., 2009; Boleij et al., 2012), indicating that acute increase in anxiety leads to negative judgment bias.

Bipolar Disorder and Mania

Depression and mania are the two core components of bipolar disorder. The cognitive and emotional correlates of depression have been extensively studied, but related research on mania is relatively lacking. Chronic administration of the psychostimulant d-amphetamine has been used to cause manic-like symptoms in animals (Valvassori et al., 2019). Some studies have shown that acute d-amphetamine administration can induce an optimistic bias in rats (Rygula et al., 2014; Hales et al., 2017), while another study found that two consecutive weeks of amphetamine treatment does not cause significant positive bias (Rygula et al., 2015b). However, it is not clear whether acute administration of amphetamines induces a manic-like state or simply a state of hyperactivity (Minassian et al., 2016).

TABLE 2 | Cognitive bias in animal models of neuropsychiatric disorders.

Models of neuropsychiatric disorders	Animals	Gender	Paradigm	Bias
Depression				
Chronic psychosocial stress: daily social defeat for 3 weeks	Sprague Dawley rats	Male	Auditory judgment bias test	Negative; Papciak et al., 2013
Chronic restraint stress: 1-h daily immobilization for 3 weeks	Sprague Dawley rats	Male	Auditory judgment bias test	Negative; Rygula et al., 2013
Chronic unpredictable mild stress (CUMS): Both physical and social stressors were presented randomly across the light/dark cycle	Long-Evans rats	Male	Tactile judgment bias test	Negative; Chaby et al., 2013
Early life adversity: Maternal separation	Sprague Dawley rats	Male	Auditory judgment bias test; Affective bias test; Modified affective bias test	Non-significant; Stuart et al., 2019 More prone to corticosterone induced negative bias; A significant deficit in reward-associated positive bias; Stuart et al., 2019
Early life adversity: Early life competition	European starlings	Male and female	Visual judgment bias test	Negative; Bateson et al., 2015
Genetic model: 5-HTT knockout	Wildtype (+/+), heterozygous (+/−), and homozygous (−/−) 5-HTT knockout mice	Female	Spatial judgment bias test	No significant difference between the three groups; Kloke et al., 2014
Genetic model: Learned helpless model	Congenitally helpless (cLH) and congenitally non-helpless (cNLH) rats	Male	Spatial judgment bias test Auditory judgment bias test	More negative in cLH rats than that in cNLH rats; Enkel et al., 2010; Richter et al., 2012
5-HT depletion model: Para-chlorophenylalanine (pCPA) (50 mg/kg) for 6 days	German Landrace piglets	Female	Spatial judgment bias test	Negative; Stracke et al., 2017
Exposure to an isolation stressor of 60 min	Gallus	Male	Visual judgment bias test	Negative; Salmeto et al., 2011; Hymel and Sufka, 2012
Anxiety				
Change in light levels: Switch from low to high light levels	Lister-hooded rats	Male	Spatial judgment bias test	Negative; Burman et al., 2009
Light stimuli: Red or white light	BALB/c mice	Male	Olfactory judgment bias test	Negative bias in white light than in red light; Boleij et al., 2012
Anxiogenic drug FG7142 (3.0, 5.0 mg/kg)	Lister-hooded rats	Male	Auditory judgment bias test	Negative; Hales et al., 2016
FG7142 (1.0, 3.0, 5.0 mg/kg)	Lister-hooded rats	Male	Affective bias test	Negative in 3.0, 5.0 mg/kg and non-significant in 1.0 mg/kg; Stuart et al., 2013
FG7142 (5.0 mg/kg)	Lister-hooded rats	Male	Affective bias test	Negative; Stuart et al., 2015
FG7142 (3.0, 6.0 mg/kg)	Sprague Dawley rats	Male	Affective bias test	Negative; Hinchcliffe et al., 2017
Exposure to an isolation stressor of 5 min	Gallus	Male	Visual judgment bias test	Negative; Salmeto et al., 2011; Hymel and Sufka, 2012
1-methyl-chlorophenylpiperazine(m-CPP) (2 mg/kg)	Merino sheep	Female	Attention bias test	Negative; Lee et al., 2016
Bipolar disorder and Mania				
D-amphetamine (2 mg/kg) for 2 weeks	Sprague Dawley rats	Male	Auditory judgment bias test	Non-significant; Rygula et al., 2015b
*D-amphetamine (0.1, 0.5, 1 mg/kg)	Sprague Dawley rats	Male	Auditory judgment bias test	Positive in 1 mg/kg and non-significant in 0.1 and 0.5 mg/kg; Rygula et al., 2014
*Amphetamine (0.1, 0.3 mg/kg)	Lister-hooded rats	Male	Auditory judgment bias test	Positive in 0.3 mg/kg and non-significant in 0.1 mg/kg; Hales et al., 2017
Pain				
Chemotherapy-induced mucositis: Fluorouracil (5-FU) (150 mg/kg)	Sprague Dawley rats	Male	Tactile judgment bias test	Negative (72 h post 5-FU injection) and non-significant (120 h post 5-FU injection); George et al., 2018

(Continued)

TABLE 2 | (Continued)

Models of neuropsychiatric disorders	Animals	Gender	Paradigm	Bias
Partial saphenous nerve injury (PSNI)	Lister-hooded rats	Male	Affective bias test;	Negative bias was corrected by gabapentin; 50 mg/kg; Phelps et al., 2021
			Modified affective bias test	A significant deficit in reward-associated positive bias; Phelps et al., 2021
Postoperative pain: Hot-iron disbudding	Holstein calves	Male	Visual judgment bias test	Negative; Neave et al., 2013
Tumors transplantation	Nude mice	Male	Olfactory judgment bias test	Negative; Resasco et al., 2021
		Female		Non-significant; Resasco et al., 2021

**Acute administration of amphetamines may simply induce hyperactivity rather than strictly mania.*

In clinics, the mood stabilizers lithium and valproate are the most commonly used drugs to treat bipolar disorder (Geddes and Miklowitz, 2013). They can help patients find a balance between depression and mania (McIntyre et al., 2020). An animal study found that acute administration of lithium induced optimistic bias in rats that were generally pessimistic, while no significant bias was observed after injection of valproic acid in rats that were more neutral at baseline, which suggests that the effect direction of lithium may be affected by the valence of cognitive bias (Rygula et al., 2015a). Although such studies are rare, it still suggests that cognitive bias tests have the potential to be applied to the animal study of pharmacological mechanisms associated with bipolar disorder.

Pain

Pain includes not only physiological components but emotional and cognitive components as well (Price, 2000). Pain in humans can lead to decreased quality of life, anxiety, and depression (Kendig et al., 2000), while pain in animals can lead to reduced water and food intake and abnormal grooming, nesting, and burrowing behaviors (Jirkof, 2017). Previous studies have frequently used conditioned place avoidance (CPA) to examine emotion and avoidance associated with pain (Tappe-Theodor et al., 2019). However, the emotional and cognitive components of pain may be more complex. Cognitive bias tests, such as the JBT, focus on animals' interpretation of ambiguous information, while the ABT includes reward value. Therefore, cognitive bias tests will help to explore the emotion-motivation and cognition-evaluation dimensions of pain from diverse perspectives.

Dairy calves experiencing postoperative pain associated with hot-iron disbudding to prevent horn growth exhibited a negative interpretation of ambiguous cues (Neave et al., 2013). A study on rats with chronic inflammatory pain as a result of 5-fluorouracil (5-FU) injection to simulate chemotherapy-induced intestinal mucositis, found that 72 h after injection, optimistic decision-making was significantly reduced (George et al., 2018), while 120 h after injection, optimistic decision-making increased as the damaged intestine gradually recovered (George et al., 2018). Chronic neuropathic pain caused by saphenous nerve injury leads to a negative bias which can be corrected by gabapentin as tested by the ABT, and a reward deficit in developing value-based memory bias in the mABT (Phelps et al., 2021), suggesting that rats with chronic neuropathic pain experience negative emotions and deficits in sensitivity to reward value. In addition, a study using the JBT to examine cancer pain and discomfort in mice with tumors found that tumor-bearing male mice were more pessimistic than healthy controls (Resasco et al., 2021). In sum, these studies indicate that cognitive bias tests can effectively measure the negative emotional state caused by pain in animals from acute pain to chronic pain and that analgesics can partially correct this state, therefore can be used in the validation of therapeutic approaches.

Cognitive Bias Tests in Assessing the Effect of Drugs on Affective State

Cognitive bias tests have shown good validity in the assessment of drug-induced affective changes (Robinson, 2018), providing a new approach for preclinical drug screening. Studies using the ABT found that acute administration of the antidepressants such as fluoxetine, reboxetine, venlafaxine, and mirtazapine induced positive biases in animals (Hales et al., 2017). However, one problem with the ABT and other preclinical testing methods, such as forced swimming, is the inability to distinguish between acute and delayed onset of antidepressant action. For example, fluoxetine was found to act quickly in preclinical trials using forced swimming, but with delayed clinical onset (Cryan and Holmes, 2005). The JBT can help to resolve this issue. Acute administration of the conventional antidepressants fluoxetine, reboxetine, or venlafaxine did not cause an interpretation bias in animals compared to the clinical fast-acting antidepressant ketamine, and only long-term use of fluoxetine resulted in a positive bias (Hales et al., 2017). These data indicate that the JBT better reflects the time course of antidepressant effects and effectively screens out fast-acting drugs at the preclinical stage.

Negative emotional side effects caused by drugs can greatly reduce a patient's quality of life, affect medication compliance, and even cause the original therapeutic regimen to be broken down (George et al., 2018). Therefore, it is critical to assess potential emotional side effects of medication during preclinical studies. Cognitive bias tests have been used to study the emotional side effects of medications. One study used ABT to test some drugs that can increase the risk of depression in clinical patients and found that lipopolysaccharides (LPS), interferons-alpha (IFN-α), and tetrabenazine (a drug for the treatment of chorea in Huntington's disease) (Frank, 2010) can induce negative deviation in rats, but varenicline (a smoking cessation drug) (Tonstad et al., 2020), carbamazepine (an anticonvulsant) (Israel and Beaudry, 1988), or montelukast (an anti-asthma drug) (Markham and Faulds, 1998) did not induce significant bias

(Stuart et al., 2017). At present, the JBT has not been widely used in the preclinical screening of emotional side effects of drugs due to its long training time and complexity. It is necessary to further develop a more sensitive, fast, and simple animal experimental paradigm for cognitive bias in future research.

DISCUSSION

An important interpretation for the behavioral results of cognitive bias test is to reflect the emotional state of animals and its effectiveness has been widely verified (Nguyen et al., 2020), indicating potential application in animal studies associated with neuropsychiatric disorders. Compared to the forced swimming test, the JBT is more sensitive to the clinical onset time of antidepressants, while the ABT is more sensitive in the assessment of reward deficits than the sucrose preference test. Therefore, cognitive bias tests may be used for the early identification of neuropsychiatric disorders and validation of their therapies.

It should be mentioned that in addition to the change of emotional state, motivation factors can also affect cognitive bias. For example, Enkel et al. (2010) noticed that in different depression-like states, a pessimistic judgment bias toward ambiguous cues could result from a decrease in positive response rate coupled with either (1) an increase in negative response rate or (2) an increase in omission rate. The former may reflect increased motivation to avoid potential punishment, whereas the latter may reflect decreased motivation to approach potential reward. This indicates that even in similar affective states, different motivational mechanisms may underlie the formation of bias. Due to the length of the min-review, we cannot discuss more, but we refer interested readers to the review by Lewis et al. (2019) and a recent paper by Neville et al. (2020), both of which provide an in-depth discussion on this topic.

The psychological mechanisms underlying the emergence and transition of cognitive bias remain unclear. One theory explains the emergence of cognitive bias from the perspective of biological evolution and adaptation (Durisko et al., 2015). In everyday life, most information is ambiguous with few explicit cues. Therefore, individuals must use prior experiences to interpret the meaning of current situation ambiguous cues (Norbury et al., 2018). This cognitive process is vital to animal survival and is an adaptive behavior that can be influenced by cognitive bias, which can be advantageous in limiting cognitive resources for faster and more efficient decision-making (Enkel et al., 2010). However, in some disorders, cognitive bias may remain constant, leading to non-adaptive behaviors. For example, negative cognitive biases associated with depression are developed by exposure to persistent stress and other adverse factors. These negative cognitive biases lead to risk-avoidance and loss-reducing behavioral strategies (Durisko et al., 2015) which can be advantageous in an unsafe environment. However, in a safe environment, these behaviors can be non-adaptive. A depressed individual may not have the capacity to alter negative biases in different situations. The ability to alter biases to appropriately address the presented situation needs further research.

Precision medicine is a hot spot in clinical research in recent years (Manchia et al., 2020). The detection of individual emotional characteristics will help to formulate an individualized treatment plan for emotional diseases. Prior studies have shown that the effects of acute manipulation of the dopamine and serotonin systems on cognitive bias may depend on cognitive bias baseline. After acute administration of haloperidol, a dopamine D2 receptor antagonist, or escitalopram, a 5-HT reuptake inhibitor, "optimistic" rats became more pessimistic, while "pessimistic" rats became more optimistic (Golebiowska and Rygula, 2017a). Therefore, cognitive bias tests may serve to formulate therapeutic regimens based on individual patient characteristics and, as such, should be included in future neuropsychiatric drug research.

Finally, the neural mechanisms of cognitive biases are understudied. The prefrontal area plays an important role in decision-making under ambiguity and risk (Rouault et al., 2019). A study in rats found that lesions to the orbitofrontal cortex (OFC) but not to the medial PFC (mPFC) decreased the proportion of positive lever presses and increased the proportion of negative lever presses in response to ambiguous tones, indicating increased pessimism (Golebiowska and Rygula, 2017b). The basolateral amygdala is closely associated with prefrontal regions and is also involved in the assessment of ambiguity and uncertainty (Davis and Whalen, 2001). One study found that unpredictability increased c-Fos expression in the lateral amygdala of mice (Herry et al., 2007). Likewise, the lateral septum is an important area for the integration of cognitive and affective information that compares known information with unknown and inferred ambiguous cues (Wirtshafter and Wilson, 2021). A study has shown a decrease in c-Fos expression in the lateral septum in response to ambiguous cues (Boleij et al., 2012). Further research using surgery, electrophysiology, optogenetics, *in vivo* calcium imaging, and other techniques to study the neural correlates of cognitive bias is necessary to identify key brain regions and molecular targets of potential therapeutics.

AUTHOR CONTRIBUTIONS

NW, J-YW, and FL contributed to the conception of the review. Y-HZ wrote the original draft of the manuscript. NW and X-XL wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

FUNDING

This work was supported by the National Natural Science Foundation of China (NNSF) grants to NW (31671140), FL (31970926), and J-YW (31271092).

REFERENCES

Armstrong, T., and Olatunji, B. O. (2012). Eye tracking of attention in the affective disorders: a meta-analytic review and synthesis. *Clin. Psychol. Rev.* 32, 704–723. doi: 10.1016/j.cpr.2012.09.004

Aue, T., and Okon-Singer, H. (2015). Expectancy biases in fear and anxiety and their link to biases in attention. *Clin. Psychol. Rev.* 42, 83–95. doi: 10.1016/j.cpr.2015.08.005

Bateson, M., Emmerson, M., Ergün, G., Monaghan, P., and Nettle, D. (2015). Opposite Effects of Early-Life Competition and Developmental Telomere Attrition on Cognitive Biases in Juvenile European Starlings. *PLoS One* 10:e0132602. doi: 10.1371/journal.pone.0132602

Beck, A. T. (1967). *Depression: Clinical, Experimental, and Theoretical Aspects*. New York, NY: Harper and Row.

Bianchi, R., Laurent, E., Schonfeld, I. S., Bietti, L. M., and Mayor, E. (2020). Memory bias toward emotional information in burnout and depression. *J. Health Psychol.* 25, 1567–1575. doi: 10.1177/1359105318765621

Boleij, H., van't Klooster, J., Lavrijsen, M., Kirchhoff, S., Arndt, S. S., and Ohl, F. (2012). A test to identify judgement bias in mice. *Behav. Brain Res.* 233, 45–54. doi: 10.1016/j.bbr.2012.04.039

Bower, G. H. (1981). Mood and memory. *Am. Psychol.* 36, 129–148.

Brydges, N. M., Hall, L., Nicolson, R., Holmes, M. C., and Hall, J. (2012). The effects of juvenile stress on anxiety, cognitive bias and decision making in adulthood: a rat model. *PLoS One* 7:e48143. doi: 10.1371/journal.pone.0048143

Burman, O. H., Parker, R. M., Paul, E. S., and Mendl, M. T. (2009). Anxiety-induced cognitive bias in non-human animals. *Physiol. Behav.* 98, 345–350. doi: 10.1016/j.physbeh.2009.06.012

Chaby, L. E., Cavigelli, S. A., White, A., Wang, K., and Braithwaite, V. A. (2013). Long-term changes in cognitive bias and coping response as a result of chronic unpredictable stress during adolescence. *Front. Hum. Neurosci.* 7:328. doi: 10.3389/fnhum.2013.00328

Cryan, J. F., and Holmes, A. (2005). The ascent of mouse: advances in modelling human depression and anxiety. *Nat. Rev. Drug Discov.* 4, 775–790. doi: 10.1038/nrd1825

Czéh, B., Fuchs, E., Wiborg, O., and Simon, M. (2016). Animal models of major depression and their clinical implications. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 64, 293–310. doi: 10.1016/j.pnpbp.2015.04.004

Dainer-Best, J., Lee, H. Y., Shumake, J. D., Yeager, D. S., and Beevers, C. G. (2018). Determining optimal parameters of the self-referent encoding task: a large-scale examination of self-referent cognition and depression. *Psychol. Assess.* 30, 1527–1540. doi: 10.1037/pas0000602

Davis, M., and Whalen, P. J. (2001). The amygdala: vigilance and emotion. *Mol. Psychiatry* 6, 13–34. doi: 10.1038/sj.mp.4000812

Durisko, Z., Mulsant, B. H., and Andrews, P. W. (2015). An adaptationist perspective on the etiology of depression. *J. Affect. Disord.* 172, 315–323. doi: 10.1016/j.jad.2014.09.032

Enkel, T., Gholizadeh, D., von Bohlen und Halbach, O., Sanchis-Segura, C., Hurlemann, R., Spanagel, R., et al. (2010). Ambiguous-Cue Interpretation is Biased Under Stress- and Depression-Like States in Rats. *Neuropsychopharmacology* 35, 1008–1015. doi: 10.1038/npp.2009.204

Everaert, J. (2021). Interpretation of ambiguity in depression. *Curr. Opin. Psychol.* 41, 9–14. doi: 10.1016/j.copsyc.2021.01.003

Everaert, J., Koster, E. H. W., and Derakshan, N. (2012). The combined cognitive bias hypothesis in depression. *Clin. Psychol. Rev.* 32, 413–424. doi: 10.1016/j.cpr.2012.04.003

Frank, S. (2010). Tetrabenazine: the first approved drug for the treatment of chorea in US patients with Huntington disease. *Neuropsychiatr. Dis. Treat.* 6, 657–665. doi: 10.2147/ndt.S6430

Geddes, J. R., and Miklowitz, D. J. (2013). Treatment of bipolar disorder. *Lancet* 381, 1672–1682. doi: 10.1016/s0140-6736(13)60857-0

George, R. P., Barker, T. H., Lymn, K. A., Bigatton, D. A., Howarth, G. S., and Whittaker, A. L. (2018). A Judgement Bias Test to Assess Affective State and Potential Therapeutics in a Rat Model of Chemotherapy-Induced Mucositis. *Sci. Rep.* 8:8193. doi: 10.1038/s41598-018-26403-7

Golebiowska, J., and Rygula, R. (2017a). Effects of acute dopaminergic and serotonergic manipulations in the ACI paradigm depend on the basal valence of cognitive judgement bias in rats. *Behav. Brain Res.* 327, 133–143. doi: 10.1016/j.bbr.2017.02.013

Golebiowska, J., and Rygula, R. (2017b). Lesions of the Orbitofrontal but Not Medial Prefrontal Cortex Affect Cognitive Judgment Bias in Rats. *Front. Behav. Neurosci.* 11:51. doi: 10.3389/fnbeh.2017.00051

Hales, C. A., Bartlett, J. M., Arban, R., Hengerer, B., and Robinson, E. S. J. (2020). Role of the medial prefrontal cortex in the effects of rapid acting antidepressants on decision-making biases in rodents. *Neuropsychopharmacology* 45, 2278–2288. doi: 10.1038/s41386-020-00797-3

Hales, C. A., Houghton, C. J., and Robinson, E. S. J. (2017). Behavioural and computational methods reveal differential effects for how delayed and rapid onset antidepressants effect decision making in rats. *Eur. Neuropsychopharmacol.* 27, 1268–1280. doi: 10.1016/j.euroneuro.2017.09.008

Hales, C. A., Robinson, E. S., and Houghton, C. J. (2016). Diffusion Modelling Reveals the Decision Making Processes Underlying Negative Judgement Bias in Rats. *PLoS One* 11:e0152592. doi: 10.1371/journal.pone.0152592

Harding, E. J., Paul, E. S., and Mendl, M. (2004). Cognitive bias and affective state. *Nature* 427, 312–312. doi: 10.1038/427312a

Herry, C., Bach, D. R., Esposito, F., Di Salle, F., Perrig, W. J., Scheffler, K., et al. (2007). Processing of temporal unpredictability in human and animal amygdala. *J. Neurosci.* 27, 5958–5966. doi: 10.1523/JNEUROSCI.5218-06.2007

Hinchcliffe, J. K., Stuart, S. A., Mendl, M., and Robinson, E. S. J. (2017). Further validation of the affective bias test for predicting antidepressant and pro-depressant risk: effects of pharmacological and social manipulations in male and female rats. *Psychopharmacology* 234, 3105–3116. doi: 10.1007/s00213-017-4687-5

Hu, C., Luo, Y., Wang, H., Kuang, S., Liang, G., Yang, Y., et al. (2017). Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress. *PLoS One* 12:e0185129. doi: 10.1371/journal.pone.0185129

Hymel, K. A., and Sufka, K. J. (2012). Pharmacological reversal of cognitive bias in the chick anxiety-depression model. *Neuropharmacology* 62, 161–166. doi: 10.1016/j.neuropharm.2011.06.009

Israel, M., and Beaudry, P. (1988). Carbamazepine in psychiatry: a review. *Can. J. Psychiatry* 33, 577–584. doi: 10.1177/070674378803300701

Jirkof, P. (2017). Side effects of pain and analgesia in animal experimentation. *Lab. Anim.* 46, 123–128. doi: 10.1038/laban.1216

Jones, S., Neville, V., Higgs, L., Paul, E. S., Dayan, P., Robinson, E. S. J., et al. (2018). Assessing animal affect: an automated and self-initiated judgement bias task based on natural investigative behaviour. *Sci. Rep.* 8:12400. doi: 10.1038/s41598-018-30571-x

Kendig, H., Browning, C. J., and Young, A. E. (2000). Impacts of illness and disability on the well-being of older people. *Disabil. Rehabil.* 22, 15–22. doi: 10.1080/096382800297088

Kloke, V., Schreiber, R. S., Bodden, C., Mollers, J., Ruhmann, H., Kaiser, S., et al. (2014). Hope for the best or prepare for the worst? Towards a spatial cognitive bias test for mice. *PLoS One* 9:e105431. doi: 10.1371/journal.pone.0105431

Krakenberg, V., Woigk, I., Garcia Rodriguez, L., Kastner, N., Kaiser, S., Sachser, N., et al. (2019). Technology or ecology? New tools to assess cognitive judgement bias in mice. *Behav. Brain Res.* 362, 279–287. doi: 10.1016/j.bbr.2019.01.021

Lee, C., Verbeek, E., Doyle, R., and Bateson, M. (2016). Attention bias to threat indicates anxiety differences in sheep. *Biol. Lett.* 12:20150977. doi: 10.1098/rsbl.2015.0977

Lewis, L. R., Benn, A., Dwyer, D. M., and Robinson, E. S. J. (2019). Affective biases and their interaction with other reward-related deficits in rodent models of psychiatric disorders. *Behav. Brain Res.* 372:112051. doi: 10.1016/j.bbr.2019.112051

Lovibond, P. F., and Lovibond, S. H. (1995). The structure of negative emotional states: comparison of the Depression Anxiety Stress Scales (DASS) with the Beck Depression and Anxiety Inventories. *Behav. Res. Ther.* 33, 335–343. doi: 10.1016/0005-7967(94)00075-u

Luo, L., Reimert, I., de Haas, E. N., Kemp, B., and Bolhuis, J. E. (2019). Effects of early and later life environmental enrichment and personality on attention bias in pigs (*Sus scrofa* domesticus). *Anim. Cogn.* 22, 959–972. doi: 10.1007/s10071-019-01287-w

Manchia, M., Pisanu, C., Squassina, A., and Carpiniello, B. (2020). Challenges and Future Prospects of Precision Medicine in Psychiatry. *Pharmgenomics Pers. Med.* 13, 127–140. doi: 10.2147/pgpm.S198225

Markham, A., and Faulds, D. (1998). Montelukast. *Drugs* 56, 251–256. doi: 10.2165/00003495-199856020-00010

McIntyre, R. S., Berk, M., Brietzke, E., Goldstein, B. I., López-Jaramillo, C., Kessing, L. V., et al. (2020). Bipolar disorders. *Lancet* 396, 1841–1856. doi: 10.1016/s0140-6736(20)31544-0

Mennen, A. C., Norman, K. A., and Turk-Browne, N. B. (2019). Attentional bias in depression: understanding mechanisms to improve training and treatment. *Curr. Opin. Psychol.* 29, 266–273. doi: 10.1016/j.copsyc.2019.07.036

Minassian, A., Young, J. W., Cope, Z. A., Henry, B. L., Geyer, M. A., and Perry, W. (2016). Amphetamine increases activity but not exploration in humans and mice. *Psychopharmacology* 233, 225–233. doi: 10.1007/s00213-015-4098-4

Mitte, K. (2008). Memory bias for threatening information in anxiety and anxiety disorders: a meta-analytic review. *Psychol. Bull.* 134, 886–911. doi: 10.1037/a0013343

Monroe, S. M., and Anderson, S. F. (2015). Depression: the shroud of heterogeneity. *Curr. Dir. Psychol. Sci.* 24, 227–231. doi: 10.1177/0963721414568342

Neave, H. W., Daros, R. R., Costa, J. H. C., von Keyserlingk, M. A. G., and Weary, D. M. (2013). Pain and pessimism: dairy calves exhibit negative judgement bias following hot-iron disbudding. *PLoS One* 8:e80556. doi: 10.1371/journal.pone.0080556

Neville, V., King, J., Gilchrist, I. D., Dayan, P., Paul, E. S., and Mendl, M. (2020). Reward and punisher experience alter rodent decision-making in a judgement bias task. *Sci. Rep.* 10:11839. doi: 10.1038/s41598-020-68737-1

Nguyen, H. A. T., Guo, C., and Homberg, J. R. (2020). Cognitive Bias Under Adverse and Rewarding Conditions: A Systematic Review of Rodent Studies. *Front. Behav. Neurosci.* 14:14. doi: 10.3389/fnbeh.2020.00014

Norbury, A., Robbins, T. W., and Seymour, B. (2018). Value generalization in human avoidance learning. *eLife* 7:e34779. doi: 10.7554/eLife.34779

Novak, J., Bailoo, J. D., Melotti, L., Rommen, J., and Wurbel, H. (2015). An Exploration Based Cognitive Bias Test for Mice: Effects of Handling Method and Stereotypic Behaviour. *PLoS One* 10:e0130718. doi: 10.1371/journal.pone.0130718

Papciak, J., Popik, P., Fuchs, E., and Rygula, R. (2013). Chronic psychosocial stress makes rats more 'pessimistic' in the ambiguous-cue interpretation paradigm. *Behav. Brain Res.* 256, 305–310. doi: 10.1016/j.bbr.2013.08.036

Phelps, C. E., Lumb, B. M., Donaldson, L. F., and Robinson, E. S. (2021). The partial saphenous nerve injury model of pain impairs reward-related learning but not reward sensitivity or motivation. *Pain* 162, 956–966. doi: 10.1097/j.pain.0000000000002177

Price, D. D. (2000). Psychological and neural mechanisms of the affective dimension of pain. *Science* 288, 1769–1772. doi: 10.1126/science.288.5472.1769

Resasco, A., MacLellan, A., Ayala, M. A., Kitchenham, L., Edwards, A. M., Lam, S., et al. (2021). Cancer blues? A promising judgment bias task indicates pessimism in nude mice with tumors. *Physiol. Behav.* 238:113465. doi: 10.1016/j.physbeh.2021.113465

Richter, S. H., Schick, A., Hoyer, C., Lankisch, K., Gass, P., and Vollmayr, B. (2012). A glass full of optimism: enrichment effects on cognitive bias in a rat model of depression. *Cogn. Affect. Behav. Neurosci.* 12, 527–542. doi: 10.3758/s13415-012-0101-2

Robinson, E. S. J. (2018). Translational new approaches for investigating mood disorders in rodents and what they may reveal about the underlying neurobiology of major depressive disorder. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 373:20170036. doi: 10.1098/rstb.2017.0036

Rouault, M., Drugowitsch, J., and Koechlin, E. (2019). Prefrontal mechanisms combining rewards and beliefs in human decision-making. *Nat. Commun.* 10:301. doi: 10.1038/s41467-018-08121-w

Rygula, R., Szczech, E., Kregiel, J., Golebiowska, J., Kubik, J., and Popik, P. (2015b). Cognitive judgment bias in the psychostimulant-induced model of mania in rats. *Psychopharmacology* 232, 651–660. doi: 10.1007/s00213-014-3707-y

Rygula, R., Golebiowska, J., Kregiel, J., Holuj, M., and Popik, P. (2015a). Acute administration of lithium, but not valproate, modulates cognitive judgment bias in rats. *Psychopharmacology* 232, 2149–2156. doi: 10.1007/s00213-014-3847-0

Rygula, R., Papciak, J., and Popik, P. (2013). Trait pessimism predicts vulnerability to stress-induced anhedonia in rats. *Neuropsychopharmacology* 38, 2188–2196. doi: 10.1038/npp.2013.116

Rygula, R., Papciak, J., and Popik, P. (2014). The effects of acute pharmacological stimulation of the 5-HT, NA and DA systems on the cognitive judgement bias of rats in the ambiguous-cue interpretation paradigm. *Eur. Neuropsychopharmacol.* 24, 1103–1111. doi: 10.1016/j.euroneuro.2014.01.012

Salmeto, A. L., Hymel, K. A., Carpenter, E. C., Brilot, B. O., Bateson, M., and Sufka, K. J. (2011). Cognitive bias in the chick anxiety–depression model. *Brain Res.* 1373, 124–130. doi: 10.1016/j.brainres.2010.12.007

Segal, Z. V. (1988). Appraisal of the self-schema construct in cognitive models of depression. *Psychol. Bull.* 103:147.

Stracke, J., Otten, W., Tuchscherer, A., Puppe, B., and Düpjan, S. (2017). Serotonin depletion induces pessimistic-like behavior in a cognitive bias paradigm in pigs. *Physiol. Behav.* 174, 18–26. doi: 10.1016/j.physbeh.2017.02.036

Stuart, S. A., Butler, P., Munafo, M. R., Nutt, D. J., and Robinson, E. S. (2013). A translational rodent assay of affective biases in depression and antidepressant therapy. *Neuropsychopharmacology* 38, 1625–1635. doi: 10.1038/npp.2013.69

Stuart, S. A., Butler, P., Munafo, M. R., Nutt, D. J., and Robinson, E. S. (2015). Distinct Neuropsychological Mechanisms May Explain Delayed- Versus Rapid-Onset Antidepressant Efficacy. *Neuropsychopharmacology* 40, 2165–2174. doi: 10.1038/npp.2015.59

Stuart, S. A., Hinchcliffe, J. K., and Robinson, E. S. J. (2019). Evidence that neuropsychological deficits following early life adversity may underlie vulnerability to depression. *Neuropsychopharmacology* 44, 1623–1630. doi: 10.1038/s41386-019-0388-6

Stuart, S. A., Wood, C. M., and Robinson, E. S. J. (2017). Using the affective bias test to predict drug-induced negative affect: implications for drug safety. *Br. J. Pharmacol.* 174, 3200–3210. doi: 10.1111/bph.13972

Tappe-Theodor, A., King, T., and Morgan, M. M. (2019). Pros and Cons of Clinically Relevant Methods to Assess Pain in Rodents. *Neurosci. Biobehav. Rev.* 100, 335–343. doi: 10.1016/j.neubiorev.2019.03.009

Tonstad, S., Arons, C., Rollema, H., Berlin, I., Hajek, P., Fagerström, K., et al. (2020). Varenicline: mode of action, efficacy, safety and accumulated experience salient for clinical populations. *Curr. Med. Res. Opin.* 36, 713–730. doi: 10.1080/03007995.2020.1729708

Valvassori, S. S., Mariot, E., Varela, R. B., Bavaresco, D. V., Dal-Pont, G. C., Ferreira, C. L., et al. (2019). The role of neurotrophic factors in manic-, anxious- and depressive-like behaviors induced by amphetamine sensitization: implications to the animal model of bipolar disorder. *J. Affect. Disord.* 245, 1106–1113. doi: 10.1016/j.jad.2018.10.370

Wirtshafter, H. S., and Wilson, M. A. (2021). Lateral septum as a nexus for mood, motivation, and movement. *Neurosci. Biobehav. Rev.* 126, 544–559. doi: 10.1016/j.neubiorev.2021.03.029

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's Note: All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

frontiers | Frontiers in Behavioral Neuroscience

TYPE Mini Review
PUBLISHED 18 August 2022
DOI 10.3389/fnbeh.2022.995614

OPEN ACCESS

EDITED BY
Dongdong Qin,
Yunnan University of Chinese Medicine, China

REVIEWED BY
Na Wang,
Fudan University, China
Wu Shi Hao,
Kunming Institute of Zoology (CAS), China

*CORRESPONDENCE
Hongling Shi
kmshl1@126.com
Mei Zhang
1211909047@qq.com
Jianhong Hou
hjhjyy@126.com

†These authors have contributed equally to this work

SPECIALTY SECTION
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

RECEIVED 16 July 2022
ACCEPTED 29 July 2022
PUBLISHED 18 August 2022

CITATION
Li Y, Chen K, Wang J, Lu H, Li X, Yang L, Zhang W, Ning S, Wang J, Sun Y, Song Y, Zhang M, Hou J and Shi H (2022) Research progress on transcranial magnetic stimulation for post-stroke dysphagia. *Front. Behav. Neurosci.* 16:995614. doi: 10.3389/fnbeh.2022.995614

Research progress on transcranial magnetic stimulation for post-stroke dysphagia

Yi Li[1†], Kerong Chen[1†], Jiapu Wang[1†], Hanmei Lu[1], Xiaoyu Li[1], Lei Yang[1], Wenlu Zhang[1], Shujuan Ning[1], Juan Wang[1], Yi Sun[1], Yu Song[1], Mei Zhang[1*], Jianhong Hou[2*] and Hongling Shi[1*]

[1]Department of Rehabilitation Medicine, Third People's Hospital of Yunnan Province, Kunming, China, [2]Department of Orthopedics, Third People's Hospital of Yunnan Province, Kunming, China

Dysphagia is one of the most common manifestations of stroke, which can affect as many as 50–81% of acute stroke patients. Despite the development of diverse treatment approaches, the precise mechanisms underlying therapeutic efficacy remain controversial. Earlier studies have revealed that the onset of dysphagia is associated with neurological damage. Neuroplasticity-based transcranial magnetic stimulation (TMS), a recently introduced technique, is widely used in the treatment of post-stroke dysphagia (PSD) by increasing changes in neurological pathways through synaptogenesis, reorganization, network strengthening, and inhibition. The main objective of this review is to discuss the effectiveness, mechanisms, potential limitations, and prospects of TMS for clinical application in PSD rehabilitation, with a view to provide a reference for future research and clinical practice.

KEYWORDS

post-stroke dysphagia, neuroplasticity, transcranial magnetic stimulation, effectiveness, therapeutic mechanism

Introduction

Dysphagia, defined as "difficulty swallowing," is one of the most important clinical manifestations of stroke and a common consequence of neurological damage caused by a range of diseases (Fung et al., 2004). Studies have confirmed that 50–81% of acute stroke patients may experience swallowing problems (Hamdy, 2010). In most cases, the post-stroke dysphagia (PSD) will improve spontaneously. However, approximately 11–50% of patients may have long-term disability (Kumar et al., 2010; Cohen et al., 2016).

Although dysphagia gradually resolves spontaneously in the early stages of disease in most cases, severe and persistent forms of dysphagia remain prevalent in about 13% of stroke patients (Mann et al., 1999). The presence of dysphagia is linked to increased physical and psychological stress in

patients, families, and caregivers, along with reduced quality of life (Eslick and Talley, 2008). In addition, dysphagia may cause various life-threatening complications, such as aspiration pneumonia, asphyxia, dehydration, and malnutrition (Smithard et al., 1996). In particular, aspiration pneumonia can trigger various complications, the most acute being infection and sepsis (Kalita et al., 2015). These complications increase the risk of prolonged hospital stays, high medical expenses and significant mortality, causing a major negative impact at both the individual and society level. Therefore, rehabilitation therapy of PSD remains a significant clinical issue that needs to be urgently addressed.

Studies have demonstrated that central causes of dysphagia in stroke patients include cortical or brain-stem damages, and peripheral causes include damages to the nerves or muscles involved in swallowing. The brain-stem lesions are more commonly associated with dysphagia (Balcerak et al., 2022). Notably, dysphagia is usually caused by infratentorial lesions, while sensory afferent disturbances usually cause dysphagia in supratentorial stroke. However, the exact mechanism of PSD is not well understood.

The treatment options of PSD include behavioral therapy, oral care, pharmacology, neurostimulation, and dietary interventions. Various physical therapies and preventive measures can avoid dysphagia-related complications. However, there is a lack of medical or electrophysiological interventions to facilitate recovery from dysphagia after acute or subacute stroke.

Existing treatments for PSD include postural training (Hägg and Larsson, 2004), dietary modification (Hägg and Anniko, 2008; McCullough et al., 2012), swallowing movements (Hägg and Anniko, 2008), compensation techniques (Lin et al., 2003), drug therapy, oral motor stimulation (Kang et al., 2012), music therapy (Kim, 2010), facial sensory stimulation, pharyngeal electrical stimulation, neuromuscular electrical stimulation, non-invasive brain stimulation, botulinum toxin injection, and acupuncture therapy (Terré et al., 2013; Yang et al., 2015). Nevertheless, these treatment strategies cannot change the physiology of impaired swallowing biomechanics as well as cannot promote the recovery of impaired swallowing neural networks in stroke patients (Speyer et al., 2010).

According to a previous study, the pathogenic cascade of dysphagia is as follows: after peripheral or central (corticobulbar tract) impairment of the cranial nerves innervating the swallowing muscles, tongue movement is limited, with soft palate paralysis. Consequently, intraoral and pharyngeal pressure cannot be fully increased, movement of food from the oral cavity to the pharynx and esophagus is weak, and transit time is significantly prolonged. The retention increases hyperreflexia or spasm of sphincter and cricopharyngeal muscle in the esophageal inlet of patients with supraglomerular damage (pseudobulbar palsy) and movement of the swallowing muscles is uncoordinated, resulting in accidental ingestion of food into the trachea (Ertekin et al., 2000). In recent years, accumulating evidence has shown that transcranial magnetic stimulation (TMS) can induce changes in the excitability of the cerebral cortex, promote plastic alterations in nerves, control the release of neurotransmitters (Lanza et al., 2015), and manage dysphagia through regulating neuroplasticity. The main objective of this review is to synthesize clinical studies and investigate the effectiveness, mechanisms of action, advantages, and disadvantages of TMS in clinical practice.

Transcranial magnetic stimulation

Transcranial magnetic stimulation is a non-invasive stimulation technique based on the principles of neuroplasticity that induces changes in neurological pathways by altering neurons in target cortical areas through synaptogenesis, reorganization, network strengthening, and inhibition, causing local depolarization of the magnetic field below the skull and activation or inhibition of activity in cortical areas (Hallett, 2000; Koerselman et al., 2004). It was also reported that the feasibility of using external magnetism to stimulate the nerves and brain (Barker et al., 1985). The group described TMS as a non-invasive technique to stimulate the human motor cortex. At present, TMS is widely used as a routine diagnostic tool in neurophysiological studies owing to its safe and technical characteristics (Rossi et al., 2009). This approach is based on speech, language, and swallowing disorders of the nervous system (Naeser et al., 2005; Khedr et al., 2009; Verin and Leroi, 2009; Barwood et al., 2011a,b,c). TMS exerts therapeutic effects by directly modulating specific pathways in the brain, which may ultimately affect longer-term communication and swallowing outcomes. Recent advances in TMS technology facilitate its application in clinical neurorehabilitation programs for patients with brain injury. Earlier reports have also demonstrated positive therapeutic effects on swallowing function after TMS, highlighting its potential as a treatment modality for dysphagia (Ridding and Rothwell, 2007). Multiple systematic reviews and meta-analyses have confirmed the beneficial effects of TMS on PSD (Yang et al., 2015; Pisegna et al., 2016; Liao et al., 2017; Chiang et al., 2019; Marchina et al., 2021) and swallow-related outcomes in patients. Moreover, the most intense effects of peripheral and cortical neurostimulation, including those of TMS, occur during the first 2 weeks after stroke (Yang et al., 2015). The efficacy of TMS for PSD from clinical trials and meta-analyses were illustrated in **Table 1**.

Mechanism of action of transcranial magnetic stimulation

Transcranial magnetic stimulation, a tool for high-pressure brain stimulation, presents an alternative method for treatment of dysphagia *via* modulation of neuroplasticity. The procedure

TABLE 1 Summary of studies on the efficacy of TMS for PSD from clinical trials and meta-analyses.

Stimulation mode and intensity	Stimulation target	Sample	Treatment cycle	Test method	Main results	References
rTMS (3 Hz)	Target cortical representation in ipsilateral pharyngeal region	21	5 days	WST	rTMS > basic rehabilitation training; improvement rates of the control and rTMS groups were 31.0 and 65.6%, respectively; WST score; the standard, improvement of dysphagia in the rTMS group was significantly higher than that in the control group ($p < 0.05$)	Yang et al., 2015; Jiao et al., 2022
rTMS (10 Hz)	Bilateral irritation	35	3 weeks	CDS, DOSS, PAS, VDS	CDS, DOSS, PAS, and VDS scores in both groups; scores in the bilateral group > scores in the unilateral group ($p < 0.05$)	Park et al., 2017
rTMS (10 Hz)	Ipsilateral motor cortex	35	3 weeks	CDS, DOSS, PAS, VDS	CDS, DOSS, PAS, and VDS scores in both groups; scores in the bilateral group > scores in the unilateral group ($p < 0.05$)	Park et al., 2017
TMS (5 Hz)	Lingual cortical motor area	15	10 days	VFSS, SAPP	No significant difference in VFSS or SAPP were observed between the two groups	Cheng et al., 2017
TMS (3 Hz)	Ipsilateral	15	5 days	WST, DD, cortical excitability	Both WST and DD were improved as well as cortical excitability in the affected hemisphere	Du et al., 2016
TMS (1 Hz)	Contralateral	13	5 days	WST, DD, cortical excitability	Both WST and DD were improved as well as cortical excitability in the unaffected hemisphere and cortical excitability in the affected hemisphere	Du et al., 2016
rTMS (10 Hz)	Ipsilateral	16	10 days	SSA, DD, cortical excitability	Cortical excitability in the affected or unaffected hemisphere were improved; significant improvement in SSA score; no change in DD score	Zhang et al., 2019
rTMS (1 Hz)	Contralateral	16	10 days	SSA, DD, cortical excitability	Cortical excitability in the affected or unaffected hemisphere were improved; significant improvement in SSA score; no change in DD score	Zhang et al., 2019
rTMS (1 Hz)	Epilepsy	16	10 days	SSA, DD, cortical excitability	Cortical excitability in the affected or unaffected hemisphere were improved; SSA score in the bilateral group > SSA score in the unilateral group; no change in DD score	Zhang et al., 2019
rTMS (1 Hz)	Contralateral	6	15 days	MASA and Functional Oral Intake Scale	MASA and functional oral intake scale scores were improved	Tarameshlu et al., 2019
TMS (3 Hz)	Ipsilateral esophageal cortical area	14	5 days	DD	Improvement in DD score	Khedr et al., 2009
rTMS (10 Hz)	Contralateral motor cortex of bilateral mylohyoid muscles	11	2 weeks	CDS, DOSS, PAS, VDS	DOSS, PAS and VDS scores in the bilateral group > scores in the unilateral group	Park et al., 2017
rTMS (10 Hz)	Ipsilateral motor cortex of mylohyoid muscle	12	2 weeks	CDS, DOSS, PAS, VDS	DOSS, PAS and VDS scores in the bilateral group > scores in the unilateral group	Park et al., 2017
rTMS (1 Hz)	Ipsilateral	4	5 days	MASA	MASA scores were improved	Ghelichi et al., 2016
rTMS (5 Hz)	Ipsilateral pharyngeal motor hotspot	8	2 weeks	PAS, VDS	VDS score: significant improvement in pharyngeal motor function. Activation of bilateral primary motor cortices, anterior motor cortex, and right prefrontal cortex	Park et al., 2019

(Continued)

TABLE 1 Continued

Stimulation mode and intensity	Stimulation target	Sample	Treatment cycle	Test method	Main results	References
rTMS (5 Hz)	Lingual motor cortex	2	2 weeks	MASA and swallowing-related quality of life	MASA and swallowing-related quality of life were improved	Cheng et al., 2015
rTMS (10 Hz)	Cerebellum	1	/	PMEP, cPAS	Improvement in PMEP amplitude (55% above baseline) and swallowing safety (17% below baseline)	Vasant et al., 2019
rTMS (1 Hz)	Contralateral	14	4 weeks	MASA and quality of life assessments	Improvement in quality of life; no significant change in MASA	Ünlüer et al., 2019

TMS, transcranial magnetic stimulation; PSD, post-stroke dysphagia; WST, water-swallowing test; CDS, Clinical Dysphagia Scale; DOSS, Dysphagia Outcome and Severity Scale; PAS, Permeation Aspiration Scale; MASA, Mann Assessment of Swallowing Ability; VDS, Videofluoroscopic Dysphagia Scale; PMEP, representative pharyngeal motor evoked potential amplitude; VFSS, videofluoroscopic swallowing study; SAPP, swallowing activity and participation profile; cPAS, cumulative penetration-aspiration score; DD, degree of dysphagia; SSA, standardized swallowing assessment.

is based on the principle of inductance and non-invasively transmits electrical energy to the brain through the scalp and skull (Wassermann, 1998). A large current pulse generator is employed to release high currents thousands of amperes greater than that flowing through the coil, up to several kilowatts in power. These short magnetic pulses cause a sustained increase or decrease in cortical excitability. A brief but intense current is passed through a TMS coil placed on the scalp, creating a magnetic field that penetrates the skull to a depth of about 1.5–2 cm and induces a sufficiently strong electric field to depolarize surface axons and activate cortical neural networks (Lefaucheur et al., 2014). In addition, an electromyographic response to the target musculature is produced, known as motor-evoked potential (MEP) (Fitzgerald et al., 2006). Subsequently, descending motor shooting along the corticospinal tracts from the cortex to peripheral muscles is elicited to adjust the excitability of the cerebral cortex. TMS can be divided into high frequency (≥1 Hz) TMS and low frequency (≤1 Hz) stimulation processes (Wassermann, 1998). High frequency tends to enhance the excitability of the cerebral cortex while low frequency exerts the opposite effect (Hamdy et al., 1998; Fitzgerald et al., 2006). In stroke patients recovering from dysphagia, functional recovery was found to be associated with increased cortical representation of the intact hemisphere, highlighting the importance of reorganization of intact neural networks in PSD recovery (Pascual-Leone et al., 1998). Repetitively applied TMS, also known repetitive TMS (rTMS), can induce changes in synaptic plasticity similar to long-term potentiation (LTP) or long-term depression (LTD), that is, increased or decreased synaptic strength (Stefan et al., 2002; Hoogendam et al., 2010). The precise mechanism remains unknown but is thought to be mediated by the activity of *N*-methyl-D-aspartate (NMDA) receptors, as revealed by studies using NMDA antagonists (Fitzgerald et al., 2006; Huang et al., 2007). Other known rTMS modalities include intermittent (excitatory) theta burst stimulation (iTBS) and continuous (inhibitory) TBS (cTBS) (Ridding and Rothwell, 2007). However, recent reports suggest that the ability to respond to these protocols varies on an individual basis (Ridding and Rothwell, 2007).

Studies have confirmed that the damages to subcortical white matter (the internal capsule and within the brainstem) caused dysphagia, possibly due to disruption in the sensorimotor pathways of the corticobulbar tract. TMS may exert effects on PSD by regulating sensorimotor pathways in the brain. However, the details of how TMS change the communication and connection of cortical neural networks to achieve the therapeutic effect remain largely unexplored. The advantages and disadvantages of TMS were shown in **Table 2**.

Advantages of transcranial magnetic stimulation

Transcranial magnetic stimulation is widely regarded as a safe and non-invasive form of nerve stimulation that can be used to directly manipulate cerebral cortex activity. In recent years, this innovative neuromodulation technology has been widely applied in neuroscience and countless cognitive fields (Barwood et al., 2011b) and shown to exert therapeutic effects by directly regulating specific pathways in the brain, which could ultimately affect longer-term communication and swallowing disorder prognosis (Naeser et al., 2005; Cotelli et al., 2008; Khedr et al., 2009; Verin and Leroi, 2009; Barwood et al., 2011a,c; Geeganage et al., 2012; Murdoch et al., 2012). The potential nerve priming effect induced by TMS is reported to effectively improve performance. Recent progress in TMS technology facilitates

TABLE 2 Advantages and disadvantages of transcranial magnetic stimulation (TMS).

Advantages	Disadvantages
① Good safety and non-invasiveness	① No significant beneficial effects on genetic factors, death, dependence, disability, prognosis and length of hospital stay
② Long-term impact on communication and swallowing disorder prognosis	② The effect of nerve stimulation therapy was not analyzed separately
③ Potentially improves performance after administration	③ The number of studies is limited, with small sample sizes, uneven case quality, and heterogeneity among studies
④ TMS can enhance muscle control of swallowing after stroke	
⑤ Shorter course of treatment	
⑥ TMS induces alterations in the functional status of local cerebral cortex, enhances synaptic function, and regulates neuronal function in the brain	
⑦ Accurate and optimal balance in the excitatory and inhibitory control functions of the cerebral cortex	

its application in clinical neurorehabilitation programs for patients with brain injury and the existing evidence shows that high-frequency TMS can enhance the muscle control of swallowing after stroke. For instance, in a study by Verin and Leroi (Geeganage et al., 2012) using TMS to stimulate the musculohyoid cortical area, the swallowing function of the patient improved at 3 days after stimulation. In a review by Cochrane (Zhai et al., 2020) on management of PSD, cortical rTMS reduced the need for physical or cognitive engagement in complex cases and had the potential to shorten the course of treatment. Previous studies have demonstrated that this non-reduced magnetic signal can reach the target area of brain tissue through the skull, thereby changing the functional status of the local cerebral cortex, enhancing synaptic function, and regulating neuronal function in the brain (Bath et al., 2018). Moreover, TMS has different intensities, frequencies and stimulation areas and can modulate the relationships and interactions among neural networks, thus affecting the functions of different regions. TMS promotes accurate and optimal balance of excitatory and inhibitory control functions in the cerebral cortex.

Current limitations

Despite the considerable benefits of TMS, lots of limitations restrict its use in clinical practice in terms of effectiveness, safety, and clinical study design. First, no significant beneficial effects of TMS on genetic factors, death, dependence, disability, prognosis, or length of hospital stay have been reported (Hoshi and Tamura, 1993; Wiethoff et al., 2014; Horvath et al., 2016). Second, patients in a few of earlier trials received traditional rehabilitation training, which made it impossible to separately analyze the effects of nerve stimulation therapy. In treatment of PSD with TMS, the optimal choice of stimulation site (unaffected hemisphere, affected hemisphere, or bilateral hemispheres) has not yet been determined. Based on different viewpoints on the recovery mechanism of PSD, the choice of excitatory stimulation (high frequency) or inhibitory stimulation (low frequency) at the corresponding site (involved, affected, or bilateral side) is controversial. In additions, the number of reported studies is limited, with small sample sizes, uneven case quality and significant heterogeneity among studies. Therefore, the available data are insufficient draw accurate conclusions on the recommended optimal treatment regimen.

Future prospects

This review provides a summary of the efficacy and underlying mechanisms of TMS activity in patients with PSD. A large majority of studies to date has used water-swallowing test (WST), clinical dysphagia scale (CDS), Dysphagia Outcome and Severity Scale (DOSS), Permeation Aspiration Scale (PAS), Mann Assessment of Swallowing Ability (MASA), Videofluoroscopic Dysphagia Scale (VDS), representative pharyngeal motor evoked potential (PMEP) amplitude, cumulative penetration-aspiration score (cPAS), and degree of dysphagia (DD) to evaluate the significance of the results. However, given the evidence for the validity of the results, it may be possible to incorporate more credible tests to draw strong conclusions in future studies. In 1993, Hoshi and Tamura demonstrated the validity of measuring different cortical regions with functional near-infrared spectroscopy (fNIRS). For the first time, the potential of fNIRS imaging brain activation sequences were reported (Ehlis et al., 2009). fNIRS is a neuroimaging technique used to map the function of the human cerebral cortex that utilizes the principle of near-infrared (NIR) spectroscopy (NIRS). Changes in optical properties of the human cerebral cortex are detected simultaneously from multiple measurement sites and the results displayed in the form of maps or images in specific areas. Over the years, fNIRS has emerged as a key neuroimaging technique that has contributed significantly to advances in understanding human brain function. In recent years, the validity of fNIRS measurements

has been repeatedly demonstrated by simultaneous functional magnetic resonance imaging (fMRI) measurements, with widely recognized applications in newborn/child and adult language processing in cognitive neuroscience. Although TMS demonstrate great potential to accelerate the improvement of swallowing function in patients with PSD, there is currently a lack of real-time assessment tool for brain function to optimize TMS parameters. As an assessment tool of brain activity, fNIRS can be used to measure the changes in hemoglobin (Hb) concentrations within the brain, which can not only evaluate the effect of TMS treatment, but also can guide the optimization of TMS treatment regimen during the PSD rehabilitation. In the future, we should combine the TMS and fNIRS to serve as a reference for upcoming clinical and laboratory research.

Author contributions

All authors listed have made a substantial, direct, and intellectual contribution to the work, and approved it for publication.

Funding

This work was supported by the Yunnan Provincial Academician and Expert Workstation (202005AF150073).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Balcerak, P., Corbiere, S., Zubal, R., and Kägi, G. (2022). Post-stroke Dysphagia: Prognosis and Treatment–A Systematic Review of RCT on Interventional Treatments for Dysphagia Following Subacute Stroke. *Front. Neurol.* 13:823189. doi: 10.3389/fneur.2022.823189

Barker, A. T., Jalinous, R., and Freeston, I. L. (1985). Non-invasive magnetic stimulation of human motor cortex. *Lancet* 1, 1106–1107. doi: 10.1016/S0140-6736(85)92413-4

Barwood, C. H., Murdoch, B. E., Whelan, B. M., Lloyd, D., Riek, S., Jd, O. S., et al. (2011a). Improved language performance subsequent to low-frequency rTMS in patients with chronic non-fluent aphasia post-stroke. *Eur. J. Neurol.* 18, 935–943. doi: 10.1111/j.1468-1331.2010.03284.x

Barwood, C. H., Murdoch, B. E., Whelan, B. M., Lloyd, D., Riek, S., O'Sullivan, J., et al. (2011b). The effects of low frequency Repetitive Transcranial Magnetic Stimulation (rTMS) and sham condition rTMS on behavioural language in chronic non-fluent aphasia: Short term outcomes. *NeuroRehabilitation* 28, 113–128. doi: 10.3233/NRE-2011-0640

Barwood, C. H., Murdoch, B. E., Whelan, B. M., Lloyd, D., Riek, S., O'Sullivan, J. D., et al. (2011c). Modulation of N400 in chronic non-fluent aphasia using low frequency Repetitive Transcranial Magnetic Stimulation (rTMS). *Brain Lang.* 116, 125–135. doi: 10.1016/j.bandl.2010.07.004

Bath, P. M., Lee, H. S., and Everton, L. F. (2018). Swallowing therapy for dysphagia in acute and subacute stroke. *Cochrane Database Syst. Rev.* 10:CD000323. doi: 10.1002/14651858.CD000323.pub3

Cheng, I. K. Y., Chan, K. M. K., Wong, C. S., Li, L. S. W., Chiu, K. M. Y., Cheung, R. T. F., et al. (2017). Neuronavigated high-frequency repetitive transcranial magnetic stimulation for chronic post-stroke dysphagia: A randomized controlled study. *J. Rehabil. Med.* 49, 475–481. doi: 10.2340/16501977-2235

Cheng, I. K., Chan, K. M., Wong, C. S., and Cheung, R. T. (2015). Preliminary evidence of the effects of high-frequency repetitive transcranial magnetic stimulation (rTMS) on swallowing functions in post-stroke individuals with chronic dysphagia. *Int. J. Lang. Commun. Disord.* 50, 389–396. doi: 10.1111/1460-6984.12144

Chiang, C. F., Lin, M. T., Hsiao, M. Y., Yeh, Y. C., Liang, Y. C., and Wang, T. G. (2019). Comparative Efficacy of Noninvasive Neurostimulation Therapies for Acute and Subacute Poststroke Dysphagia: A Systematic Review and Network Meta-analysis. *Arch. Phys. Med. Rehabil.* 100, 739–750.e4. doi: 10.1016/j.apmr.2018.09.117

Cohen, D. L., Roffe, C., Beavan, J., Blackett, B., Fairfield, C. A., Hamdy, S., et al. (2016). Post-stroke dysphagia: A review and design considerations for future trials. *Int. J. Stroke* 11, 399–411. doi: 10.1177/1747493016639057

Cotelli, M., Manenti, R., Cappa, S. F., Zanetti, O., and Miniussi, C. (2008). Transcranial magnetic stimulation improves naming in Alzheimer disease patients at different stages of cognitive decline. *Eur. J. Neurol.* 15, 1286–1292. doi: 10.1111/j.1468-1331.2008.02202.x

Du, J., Yang, F., Liu, L., Hu, J., Cai, B., Liu, W., et al. (2016). Repetitive transcranial magnetic stimulation for rehabilitation of poststroke dysphagia: A randomized, double-blind clinical trial. *Clin. Neurophysiol.* 127, 1907–1913. doi: 10.1016/j.clinph.2015.11.045

Ehlis, A. C., Ringel, T. M., Plichta, M. M., Richter, M. M., Herrmann, M. J., and Fallgatter, A. J. (2009). Cortical correlates of auditory sensory gating: a simultaneous near-infrared spectroscopy event-related potential study. *Neuroscience* 159, 1032–1043. doi: 10.1016/j.neuroscience.2009.01.015

Ertekin, C., Aydogdu, I., Tarlaci, S., Turman, A. B., and Kiylioglu, N. (2000). Mechanisms of dysphagia in suprabulbar palsy with lacunar infarct. *Stroke* 31, 1370–1376. doi: 10.1161/01.STR.31.6.1370

Eslick, G. D., and Talley, N. J. (2008). Dysphagia: epidemiology, risk factors and impact on quality of life–a population-based study. *Aliment. Pharmacol. Ther.* 27, 971–979. doi: 10.1111/j.1365-2036.2008.03664.x

Fitzgerald, P. B., Fountain, S., and Daskalakis, Z. J. (2006). A comprehensive review of the effects of rTMS on motor cortical excitability and inhibition. *Clin. Neurophysiol.* 117, 2584–2596. doi: 10.1016/j.clinph.2006.06.712

Fung, C. W., Khong, P. L., To, R., Goh, W., and Wong, V. (2004). Video-fluoroscopic study of swallowing in children with neurodevelopmental disorders. *Pediatr. Int.* 46, 26–30. doi: 10.1111/j.1442-200X.2004.t01-1-.x

Geeganage, C., Beavan, J., Ellender, S., and Bath, P. M. (2012). Interventions for dysphagia and nutritional support in acute and subacute stroke. *Cochrane Database Syst. Rev.* 10:CD000323. doi: 10.1002/14651858.CD000323.pub2

Ghelichi, L., Joghataei, M. T., Jalaie, S., Nakhostin-Ansari, N., Forogh, B., and Mehrpour, M. (2016). A single-subject study to evaluate the inhibitory repetitive

transcranial magnetic stimulation combined with traditional dysphagia therapy in patients with post-stroke dysphagia. *Iran. J. Neurol.* 15, 140–145.

Hägg, M., and Anniko, M. (2008). Lip muscle training in stroke patients with dysphagia. *Acta Otolaryngol.* 128, 1027–1033. doi: 10.1080/00016480701813814

Hägg, M., and Larsson, B. (2004). Effects of motor and sensory stimulation in stroke patients with long-lasting dysphagia. *Dysphagia* 19, 219–230. doi: 10.1007/s00455-004-0016-3

Hallett, M. (2000). Transcranial magnetic stimulation and the human brain. *Nature* 406, 147–150. doi: 10.1038/35018000

Hamdy, S. (2010). Role of Neurostimulation and Neuroplasticity in the Rehabilitation of Dysphagia After Stroke. *Perspect. Swallow. Swallow. Disord.* 19, 3–9. doi: 10.1044/sasd19.1.3

Hamdy, S., Aziz, Q., Rothwell, J. C., Power, M., Singh, K. D., Nicholson, D. A., et al. (1998). Recovery of swallowing after dysphagic stroke relates to functional reorganization in the intact motor cortex. *Gastroenterology* 115, 1104–1112. doi: 10.1016/S0016-5085(98)70081-2

Hoogendam, J. M., Ramakers, G. M., and Di Lazzaro, V. (2010). Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimul.* 3, 95–118. doi: 10.1016/j.brs.2009.10.005

Horvath, J. C., Vogrin, S. J., Carter, O., Cook, M. J., and Forte, J. D. (2016). Effects of a common transcranial direct current stimulation (tDCS) protocol on motor evoked potentials found to be highly variable within individuals over 9 testing sessions. *Exp. Brain Res.* 234, 2629–2642. doi: 10.1007/s00221-016-4667-8

Hoshi, Y., and Tamura, M. (1993). Dynamic multichannel near-infrared optical imaging of human brain activity. *J. Appl. Physiol.* 75, 1842–1846. doi: 10.1152/jappl.1993.75.4.1842

Huang, Y. Z., Chen, R. S., Rothwell, J. C., and Wen, H. Y. (2007). The after-effect of human theta burst stimulation is NMDA receptor dependent. *Clin. Neurophysiol.* 118, 1028–1032. doi: 10.1016/j.clinph.2007.01.021

Jiao, Y., Li, G., and Dai, Y. (2022). Clinical effect of repetitive transcranial magnetic stimulation on dysphagia due to stroke. *Neurol. Sci.* 43, 3139–3144. doi: 10.1007/s10072-021-05799-7

Kalita, M., Majewska, K., Gradowska, A., Karwowska, K., and Ławiński, M. (2015). Food, mechanic and septic complications in patients enterally nutritioned in home conditions. *Pol. Przegl. Chir.* 86, 466–472. doi: 10.2478/pjs-2014-0083

Kang, J. H., Park, R. Y., Lee, S. J., Kim, J. Y., Yoon, S. R., and Jung, K. I. (2012). The effect of bedside exercise program on stroke patients with Dysphagia. *Ann. Rehabil. Med.* 36, 512–520. doi: 10.5535/arm.2012.36.4.512

Khedr, E. M., Abo-Elfetoh, N., and Rothwell, J. C. (2009). Treatment of post-stroke dysphagia with repetitive transcranial magnetic stimulation. *Acta Neurol. Scand.* 119, 155–161. doi: 10.1111/j.1600-0404.2008.01093.x

Kim, S. J. (2010). Music therapy protocol development to enhance swallowing training for stroke patients with dysphagia. *J. Music Ther.* 47, 102–119. doi: 10.1093/jmt/47.2.102

Koerselman, F., Laman, D. M., van Duijn, H., van Duijn, M. A., and Willems, M. A. (2004). A 3-month, follow-up, randomized, placebo-controlled study of repetitive transcranial magnetic stimulation in depression. *J. Clin. Psychiatry* 65, 1323–1328. doi: 10.4088/JCP.v65n1005

Kumar, S., Selim, M. H., and Caplan, L. R. (2010). Medical complications after stroke. *Lancet. Neurol.* 9, 105–118. doi: 10.1016/S1474-4422(09)70266-2

Lanza, G., Cantone, M., Lanuzza, B., Pennisi, M., Bella, R., Pennisi, G., et al. (2015). Distinctive patterns of cortical excitability to transcranial magnetic stimulation in obstructive sleep apnea syndrome, restless legs syndrome, insomnia, and sleep deprivation. *Sleep Med. Rev.* 19, 39–50. doi: 10.1016/j.smrv.2014.04.001

Lefaucheur, J. P., André-Obadia, N., Antal, A., Ayache, S. S., Baeken, C., Benninger, D. H., et al. (2014). Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). *Clin. Neurophysiol.* 125, 2150–2206. doi: 10.1016/j.clinph.2014.05.021

Liao, X., Xing, G., Guo, Z., Jin, Y., Tang, Q., He, B., et al. (2017). Repetitive transcranial magnetic stimulation as an alternative therapy for dysphagia after stroke: a systematic review and meta-analysis. *Clin. Rehabil.* 31, 289–298. doi: 10.1177/0269215516644771

Lin, L. C., Wang, S. C., Chen, S. H., Wang, T. G., Chen, M. Y., and Wu, S. C. (2003). Efficacy of swallowing training for residents following stroke. *J. Adv. Nurs.* 44, 469–478. doi: 10.1046/j.0309-2402.2003.02830.x

Mann, G., Hankey, G. J., and Cameron, D. (1999). Swallowing function after stroke: prognosis and prognostic factors at 6 months. *Stroke* 30, 744–748. doi: 10.1161/01.STR.30.4.744

Marchina, S., Pisegna, J. M., Massaro, J. M., Langmore, S. E., McVey, C., Wang, J., et al. (2021). Transcranial direct current stimulation for post-stroke dysphagia: a systematic review and meta-analysis of randomized controlled trials. *J. Neurol.* 268, 293–304. doi: 10.1007/s00415-020-10142-9

McCullough, G. H., Kamarunas, E., Mann, G. C., Schmidley, J. W., Robbins, J. A., and Crary, M. A. (2012). Effects of Mendelsohn maneuver on measures of swallowing duration post stroke. *Top. Stroke Rehabil.* 19, 234–243. doi: 10.1310/tsr1903-234

Murdoch, B. E., Ng, M. L., and Barwood, C. H. (2012). Treatment of articulatory dysfunction in Parkinson's disease using repetitive transcranial magnetic stimulation. *Eur. J. Neurol.* 19, 340–347. doi: 10.1111/j.1468-1331.2011.03524.x

Naeser, M. A., Martin, P. I., Nicholas, M., Baker, E. H., Seekins, H., Kobayashi, M., et al. (2005). Improved picture naming in chronic aphasia after TMS to part of right Broca's area: an open-protocol study. *Brain Lang.* 93, 95–105. doi: 10.1016/j.bandl.2004.08.004

Park, E., Kim, M. S., Chang, W. H., Oh, S. M., Kim, Y. K., Lee, A., et al. (2017). Effects of Bilateral Repetitive Transcranial Magnetic Stimulation on Post-Stroke Dysphagia. *Brain Stimul.* 10, 75–82. doi: 10.1016/j.brs.2016.08.005

Park, J. W., Kim, H., Park, T., Yeo, J. S., Hong, H. J., and Oh, J. Y. (2019). A pilot study of the effects of high-frequency repetitive transcranial magnetic stimulation on dysphagia in the elderly. *Neurogastroenterol. Motil.* 31:e13561. doi: 10.1111/nmo.13561

Pascual-Leone, A., Tormos, J. M., Keenan, J., Tarazona, F., Cañete, C., and Catalá, M. D. (1998). Study and modulation of human cortical excitability with transcranial magnetic stimulation. *J. Clin. Neurophysiol.* 15, 333–343. doi: 10.1097/00004691-199807000-00005

Pisegna, J. M., Kaneoka, A., Pearson, W. G. Jr., Kumar, S., and Langmore, S. E. (2016). Effects of non-invasive brain stimulation on post-stroke dysphagia: A systematic review and meta-analysis of randomized controlled trials. *Clin. Neurophysiol.* 127, 956–968. doi: 10.1016/j.clinph.2015.04.069

Ridding, M. C., and Rothwell, J. C. (2007). Is there a future for therapeutic use of transcranial magnetic stimulation? *Nat. Rev. Neurosci.* 8, 559–567. doi: 10.1038/nrn2169

Rossi, S., Hallett, M., Rossini, P. M., and Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* 120, 2008–2039. doi: 10.1016/j.clinph.2009.08.016

Smithard, D. G., O'Neill, P. A., Parks, C., and Morris, J. (1996). Complications and outcome after acute stroke. Does dysphagia matter?. *Stroke* 27, 1200–1204. doi: 10.1161/01.STR.27.7.1200

Speyer, R., Baijens, L., Heijnen, M., and Zwijnenberg, I. (2010). Effects of therapy in oropharyngeal dysphagia by speech and language therapists: a systematic review. *Dysphagia* 25, 40–65. doi: 10.1007/s00455-009-9239-7

Stefan, K., Kunesch, E., Benecke, R., Cohen, L. G., and Classen, J. (2002). Mechanisms of enhancement of human motor cortex excitability induced by interventional paired associative stimulation. *J. Physiol.* 543, 699–708. doi: 10.1113/jphysiol.2002.023317

Tarameshlu, M., Ansari, N. N., Ghelichi, L., and Jalaei, S. (2019). The effect of repetitive transcranial magnetic stimulation combined with traditional dysphagia therapy on poststroke dysphagia: a pilot double-blinded randomized-controlled trial. *Int. J. Rehabil. Res.* 42, 133–138. doi: 10.1097/MRR.0000000000000336

Terré, R., Panadés, A., and Mearin, F. (2013). Botulinum toxin treatment for oropharyngeal dysphagia in patients with stroke. *Neurogastroenterol. Motil.* 25, 896–e702. doi: 10.1111/nmo.12213

Ünlüer, N., Temuçin, ÇM., Demir, N., Serel Arslan, S., and Karaduman, A. A. (2019). Effects of Low-Frequency Repetitive Transcranial Magnetic Stimulation on Swallowing Function and Quality of Life of Post-stroke Patients. *Dysphagia* 34, 360–371. doi: 10.1007/s00455-018-09965-6

Vasant, D. H., Sasegbon, A., Michou, E., Smith, C., and Hamdy, S. (2019). Rapid improvement in brain and swallowing behavior induced by cerebellar repetitive transcranial magnetic stimulation in poststroke dysphagia: A single patient case-controlled study. *Neurogastroenterol. Motility* 31:e13609. doi: 10.1111/nmo.13609

Verin, E., and Leroi, A. M. (2009). Poststroke dysphagia rehabilitation by repetitive transcranial magnetic stimulation: a noncontrolled pilot study. *Dysphagia* 24, 204–210. doi: 10.1007/s00455-008-9195-7

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic *Stimulation. June 5-7, 1996.*

Electroencephalogr. Clin. Neurophysiol. 108, 1–16. doi: 10.1016/S0168-5597(97)00096-8

Wiethoff, S., Hamada, M., and Rothwell, J. C. (2014). Variability in response to transcranial direct current stimulation of the motor cortex. *Brain Stimul.* 7, 468–475. doi: 10.1016/j.brs.2014.02.003

Yang, S. N., Pyun, S. B., Kim, H. J., Ahn, H. S., and Rhyu, B. J. (2015). Effectiveness of Non-invasive Brain Stimulation in Dysphagia Subsequent to Stroke: A Systemic Review and Meta-analysis. *Dysphagia* 30, 383–391. doi: 10.1007/s00455-015-9619-0

Zhai, B., Fu, J., Xiang, S., Shang, Y., Yan, Y., Yin, T., et al. (2020). Repetitive transcranial magnetic stimulation ameliorates recognition memory impairment induced by hindlimb unloading in mice associated with BDNF/TrkB signaling. *Neurosci. Res.* 153, 40–47. doi: 10.1016/j.neures.2019.04.002

Zhang, C., Zheng, X., Lu, R., Yun, W., Yun, H., and Zhou, X. (2019). Repetitive transcranial magnetic stimulation in combination with neuromuscular electrical stimulation for treatment of post-stroke dysphagia. *J. Int. Med. Res.* 47, 662–672. doi: 10.1177/0300060518807340

frontiers | Frontiers in Behavioral Neuroscience

TYPE Case Report
PUBLISHED 08 September 2022
DOI 10.3389/fnbeh.2022.987259

OPEN ACCESS

EDITED BY
Dongdong Qin,
Yunnan University of Chinese Medicine, China

REVIEWED BY
Haifeng Li,
Zhejiang University School of Medicine, China
Hongmei Tang,
Guangzhou Medical University, China

*CORRESPONDENCE
Jing Ma
majing@etyy.cn
Yun Liu
liuyun@etyy.cn

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

RECEIVED 06 July 2022
ACCEPTED 16 August 2022
PUBLISHED 08 September 2022

CITATION
He X, Ma X, Wang J, Zou Z, Huang H, Ren J, Liu C, Zheng N, Ma J and Liu Y (2022) Case report: Identification and clinical phenotypic analysis of novel mutation of the *PPP1CB* gene in NSLH2 syndrome.
Front. Behav. Neurosci. 16:987259.
doi: 10.3389/fnbeh.2022.987259

Case report: Identification and clinical phenotypic analysis of novel mutation of the *PPP1CB* gene in NSLH2 syndrome

Xuemei He[1†], Xiuli Ma[2†], Jing Wang[1†], Zhuo Zou[1], Haoyu Huang[1], Jian Ren[1], Chunming Liu[1], Nan Zheng[1], Jing Ma[2*] and Yun Liu[1*]

[1]Department of Rehabilitation, Kunming Children's Hospital, Kunming Medical University, Yunnan, China, [2]Department of Otolaryngology, Head and Neck Surgery, Kunming Children's Hospital, Kunming Medical University, Yunnan, China

Objective: To screen and analyze the genetic mutations in the *PPP1CB* gene in a patient with Noonan syndrome with loose anagen hair-2 (NSLH2) in Yunnan Province, China and explore the possible molecular pathogenesis.

Methods: After obtaining informed consent, we collected the patient's medical history and carried out physical and laboratory examinations for the NSLH2 proband and the family members. Genomic DNA was extracted from the peripheral blood of all individuals. The coding regions including all pathogenic exons, parts of introns, and promoters of genes were sequenced by next-generation sequencing. Pathogenic mutations, which were detected in the probands and their parents, were verified by Sanger sequencing.

Results: The clinical manifestations of NSLH2 included prominent forehead, yellowish hair, slightly wide eye distance, sparse eyebrows, bilateral auricle deformity, reduced muscle tension, and cardiac and visual abnormalities. The proband carried a c.371A>G mutation in exon 3 of *PPP1CB*, which is a missense mutation. This was a *de novo* mutation as the parents of the proband showed no mutation at this site.

Conclusion: In this study, we identified a novel mutation of *PPP1CB*, which enriched the mutation spectrum of the *PPP1CB* gene and provided a basis for the diagnosis of NSLH2.

KEYWORDS

***PPP1CB*, Noonan syndrome with loose anagen hair-2, developmental delay, next-novel mutation of the *PPP1CB* gene 2 generation sequencing, DNA mutation analysis**

Introduction

Noonan syndrome-like disorder with loose anagen hair-2 (NSLH2, OMIM: 617506) is an autosomal dominant inherited disorder. It is a class of RAS signaling pathway-related syndromes (RASopathies) resulting from genetic variations in the RAS/MAPK pathways (Mazzanti et al., 2003). The presently reported pathogenic genes are *SHOC2* and *PPP1CB*. The *SHOC2* gene mutation causes type 1 Noonan syndrome with

loose anagen hair (NSLH1) and the *PPP1CB* gene mutation causes NSLH2. Protein phosphatase 1 (PPP1), a major type 1 serine/threonine phosphatase, is widely expressed and regulates a variety of cellular functions, including metabolism, cell division, and muscle contraction (Barker et al., 1994). In addition to certain characteristics of Noonan syndrome, NSLH2 is characterized by chronic hair loss due to easily pulled out and thinning hair, slow growth, and pale color. Most NSLH2 patients are short in stature owing to lack of growth hormone (Cordeddu et al., 2009). This disease can also involve the skin and nervous, cardiovascular, and skeletal systems.

In this study, we identified and analyzed the genetic mutations of a pediatric patient with a clinical diagnosis of "general developmental delay, visual dysplasia, ventricular septal defect, nodal cause to be investigated" and his parents by using total exome sequencing technology. The sequencing results showed that the child carried the c.371A>G (exon 3) mutation in *PPP1CB*. This study enriched the mutation spectrum of the *PPP1CB* gene and provided a certain reference for the diagnosis of NSLH.

Case presentation

Case

The proband was a female patient aged 6 months and 14 days with general developmental delay, visual dysplasia, ventricular septal defect, and nodding of unknown cause who was admitted to our hospital in Yunnan Province in 2019. Clinical data of the patient and her families were collected, and comprehensive physical examination and intelligence assessment were carried out. Computed tomography (CT) of the temporal bone and magnetic resonance imaging (MRI) of the skull were performed. This study was approved by the Medical Ethics Committee of Kunming Children's Hospital. The genetic diagnosis was approved by the child's family members, and informed consent was signed.

Genetic testing methods

Peripheral blood of the patient and her family members was collected to construct a DNA extraction. Tissue genomic DNA was extracted using the Blood Genome Column Medium Volume Extraction Kit (Convoy Century), following the kit instructions. All exon sequences related to clinical diseases were captured and hybridized to enrich the target region sequences. Double-end sequencing with the aid of low depth whole genome sequencing based on the Illumina technology sequencing platform. The filtered sequences were aligned to the Human Genome Reference Sequence (UCSC, HG19) of the NCBI database using BWA software (http://bio-bwa.sourceforge.net/), and any redundant data in the PCR process were removed. The relevant information of single nucleotide polymorphism (SNP) and insertion-deletion mutation (Indels) was analyzed using GATK software. All SNPs and Indels were annotated by Annovar software. Mutant loci with a frequency of <0.05 were screened out from the standard databases including the 1000 Genome Project, Exome Variant Server, and Exec. SIFT. Polyphen-2 Mutation Taster and GERP++ software were used to predict the pathogenicity and conservatism of missense mutations. SPidex software was used to analyze the pathogenicity of shear site changes. Sanger sequencing verified the second-generation sequencing mutation sites. Mutation pathogenicity was analyzed according to the ACMG guidelines. The relation of genotype to phenotype was conducted.

Clinical data analysis

The patient was a girl aged 6 months and 14 days who was admitted to our hospital on July 14, 2020, for "developmental delay." The baby was delivered by cesarean section owing to uterine scarring at 38 weeks^{+6} of gestation, with a birth weight of 3,100 grams. Her mother denied any history of abnormal pregnancy or perinatal asphyxia and rescue; moreover, the patient's jaundice was not severe in the neonatal period. The child's psychomotor development was delayed since childhood, and the stability of the head in the vertical plane was poor when examined by the doctor. She could only turn over from the supine position to the lateral position, and her hands could not take objects actively. Her eyes followed the red ball $<90°$, and she was not good at following the sources of sound. She often sucked her fingers. Her family members complained that her facial expression was slightly tense when she changed from the lying position to the sitting position without the back chair, and occasionally exhibited double oblique vision. She was in good spirits and sleeping well, but she had poor appetite and indigestion often, milk flap in stool, and would often vomit milk before the age of 6 months. After 6 months, her symptoms began to ease. Family members reported that she exhibited frequent nodding behavior, but no symptoms of epileptic seizure such as mouth and face blue, hug shape, limb shaking, eyes staring, foaming etc.

The patient's elder sister was 3.5 years old, and her development was consistent with age. Her parents were in good health and were not closely related. There was no similar medical history in her family.

Physical examination analysis

The patient showed stable vital signs and the head circumference was 41 cm. The anterior fontanelle was flat and soft with a size of about 0.5 × 0.5 cm. As shown in Figure 1,

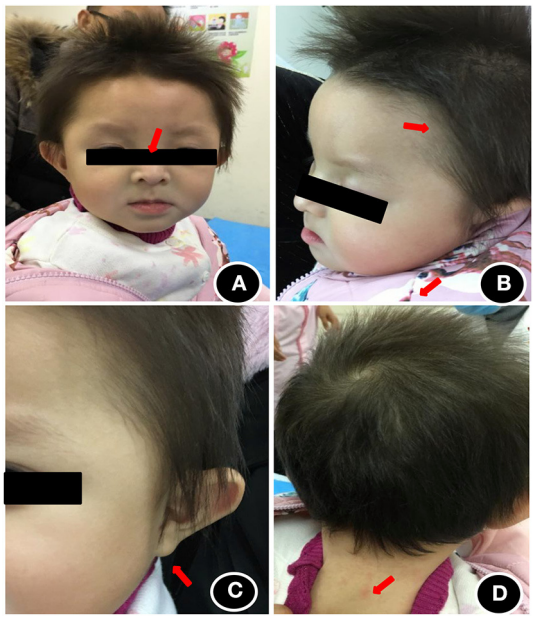


FIGURE 1
Head and facial features of the child at the age of 1 year. **(A)** Wide eye spacing, sparse eyebrows, light color; **(B)** Loose hair, sparse hair, light color; **(C)** abnormal auricle appearance; **(D)** dry and eczema-prone skin.

she exhibited some unique head and facial features, including prominent forehead, white skin, yellowish hair color, slightly wider eye distance, scant eyebrows, light complexion, bilateral auricle shape abnormity, Both pupils are equal in size and round, and dry skin that was prone to eczema (Figure 1). There was no desquamation, no light reflex, no congestion in the pharynx, no resistance in the neck, and no obvious abnormality in the heart, lung, and abdomen. Extremities did not exhibit the symptoms of edema and cyanosis. The muscle volume of the extremities was normal, but the muscle tone was reduced. The knee reflex was present. Her hands are less flexible for active picking and she has less movement in the midline position of her body. Prone position could be assumed with elbow support, but the patient could lean forward to sit independently only for some time. Standing double lower limbs will support, but she could not jump.

Auxiliary examination analysis

Blood biochemical indices, thyroid function, and vitamin D level were normal in July 2020. The electroencephalogram was also normal. Chromosomal karyotyping showed 46, XX. Ultrasonography of the heart revealed ventricular septal defect and pseudomonal tumor formation (rupture of about 2.5 mm and 1.8 mm) (Figure 2A). Radiography of the hip joint showed bilateral hip dysplasia (Figure 3). Brain MRI showed slightly wider extracerebral space in the bilateral frontotemporal region; no definite abnormality was found in the rest of the brain. The results of auditory brain stem response were Transient Evoked Otoacoustic Emission (TEOAE) in both ears. The auditory brain stem response was as follows: The click waveform of 70 dBnHL in both ears was well-differentiated, and the interval and latency were normal. The response threshold was 20 dBnHL in the right ear and 25 dBnHL in the left ear.

Griffiths developmental assessment

Motor:score 3.8 points, developmental age 3 month, developmental level percentile1–2.5%; Personal-society: score 3.4 points, developmental age 2–2.5m, developmental level percentile <1%; Language: score 2.1 points, developmental age 1 m, developmental level percentile <1%. Hand-eye coordination:score 2.4 points, developmental age 1.5–2m, developmental level percentile <1%; Performance:score 2.7 points, developmental age 2.5m, developmental level percentile <1%. These results indicate that the development of each energy region was delay. Fundus examination revealed retinal exudation around the right eye and no obvious abnormality in the left eye. Fundus examination in August 2020 showed no abnormality in either eye.

Follow-up: (i) The follow-up to October 1, 2020, 9 months of age 27 days, children from 7.8 kg weight increased to 8.5 kg, head circumference has no obvious change, will each hand holding a toy, can sit alone 10 s, the light can be caught and beat 180°, rattle loss will be looking for, the reaction is a bit slow, call names I will turn, slower reaction, will imitate poop-poop sound, cookies from hello, like to play with a rattle, fine motor, chase, chase before listening, language is improved. (ii) In the follow-up to December 24, 2020, the child could sit independently for 2–3 min, pick things with both hands, and laugh when communicating with others. Gross motor, fine motor, cognition, language, and social interaction were better than before. (iii) Follow-up was carried out until January 8, 2021. At that time, the child was 1 year and 9 days old. The child could sit independently for 7–8 min and could turn over, but not crawl; Will take the initiative, will hold their toys, model bell ringing, will tear paper, not the thumb and forefinger pinch pills; Listen to the sound will look for the sound source, will change hands; Made will laugh aloud, will unconsciously shout “mom”, will lift surface play peek-a-boo, can recognize a person, sometimes nod, spirit, poor diet, prone to indigestion, defecate have milk disc, stool stem node, to sleep, sometimes nod, no opening week and was blue, no hug, no limb jitter, unique eye gaze, no saliva and so on. Gross motor, fine motor, cognition, language, and social skills showed progress compared to before. The follow-up to January 2021, her EEG was normal.

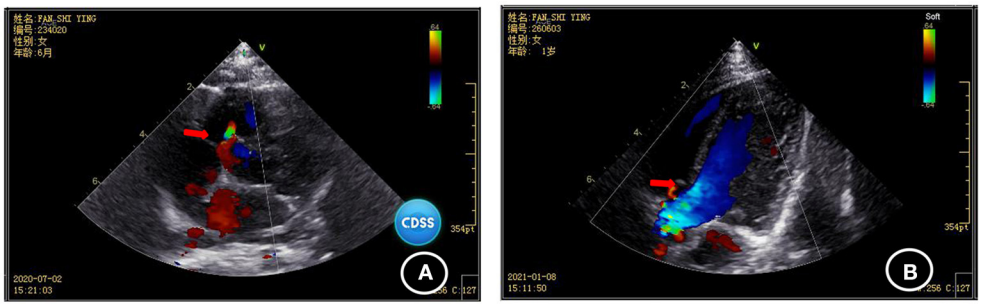


FIGURE 2
Comparison of the results of two cardiac ultrasonographies of the child **(A)** ventricular septal defect and pseudomonal tumor formation (rupture of about 2.5 mm and 1.8 mm) were found in July 2020; **(B)** Ventricular septal defect and pseudo membrane tumor formation (1.3 mm rupture) were found by heart color doppler ultrasound in January 2021.

Cardiac ultrasonography in January 2021 revealed ventricular septal defect and pseudo membrane neoplasm (1.3 mm rupture) (Figure 2B). The GDF showed the following results:

Exercise: total bare 7, developmental age 6.5–7 m, percentile 1–2.5%; Personal-society: total bare value 6.5, developmental age 5–5.5 m, percentile <1%; Language: total bare 6.5, developmental age 6–6.5 m, percentile 1–2.5%; Hand-eye coordination: total bare 6.5, developmental age 6.5–7 m, percentile 1–2.5%; and Performance: Total bare 6.5, developmental age 6.5–7 m, percentile 1–2.5%. The results of her development evaluation indicate that the development of each area is deficient, but the overall development level was slightly improved. Evaluation of bone age indicated that her bone age was equivalent to 2 years. Radiographs of the hip joint showed that the bilateral acetabular fossa was slightly flat and shallower, and the bilateral acetabular angle was enlarged.

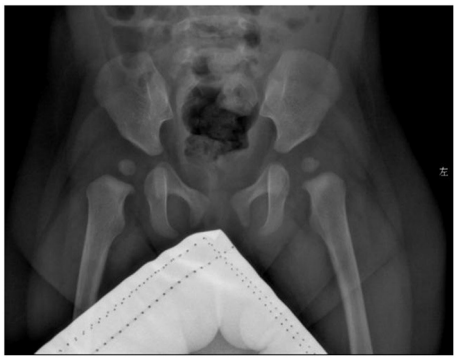


FIGURE 3
Radiographs of the child's hip joint show slightly flat and shallow bilateral acetabular fossa and enlarged bilateral acetabular angle.

Genetic test results

The patient had a novel heterozygous mutation in exon 3 of the coding region of *PPP1CB* (CHR2-29001861), and the nucleotide 371 changed from A to G (C. 371A→ G) (Figure 4A), resulting in the mutation of the amino acid at the 124th position from histidine to arginine (p.H124R). This is a missense mutation and predicts a change of protein function. Sanger sequencing verified that the child's parents did not carry the mutation at the site, which was a spontaneous mutation (Figure 4B), according to the ACMG guidelines. The mutation is classified as "class 2 - possibly pathogenic."

The missense mutation c.371A>G was predicted to result in a histidine to arginine substitution at codon 124. The alignment of *PPP1CB* from different genera namely *Homo, Pan, Macaca, Canis, Bos, Mus, Rattus, Gallus, Danio, Drosophila,* and *Anopheles* is shown in Figure 5A. The three-dimensional structures of wild-type *PPP1CB* was simulated according to the crystal structure (Figure 5B). The missense variant (p.H124R) was predicted to perturb protein structure because of the substitution of histidine by arginine. In this respect, our prediction study revealed that the novel mutation possibly lead to protein dysfunction (Figures 5C,D).

Discussion

Ras/mitogen-activated protein Kinase (RAS/MAPK) is one of the most important signaling pathways involved in the regulation of cell proliferation, survival, apoptosis, differentiation, immune response, and nervous system function (Zhang and Zeng, 2020). RAS signaling pathway-related syndromes are a group of syndromes caused by germline mutations in genes affecting the RAS mitogen-activated protein kinase (MAPK) pathway (Schubbert et al., 2007; Matozaki et al., 2009; Motta et al., 2020). RAS signaling pathway-related

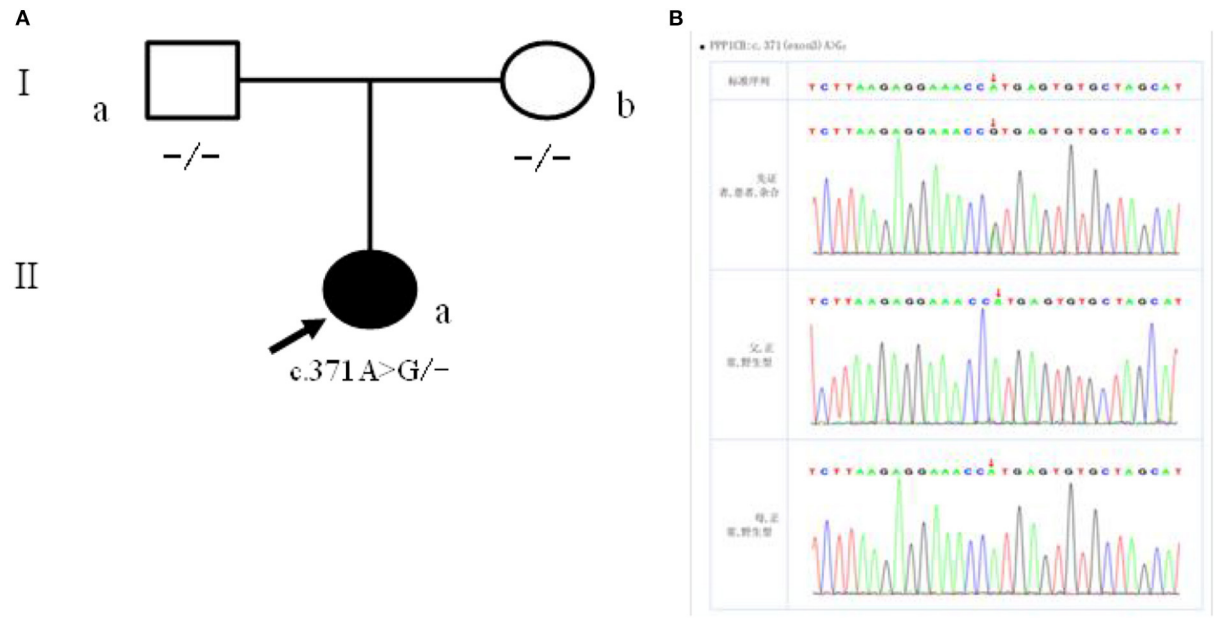


FIGURE 4
(A) Children with family figure for NSLH2 clinical phenotype. Her father (I-a) and mother (I-b) had normal clinical manifestations; **(B)** children with children and parents Sanger sequencing diagram (II-a) for c. 371 A>G heterozygous mutations. Her father (I-a) and mother (I-b) the site are for the wild type.

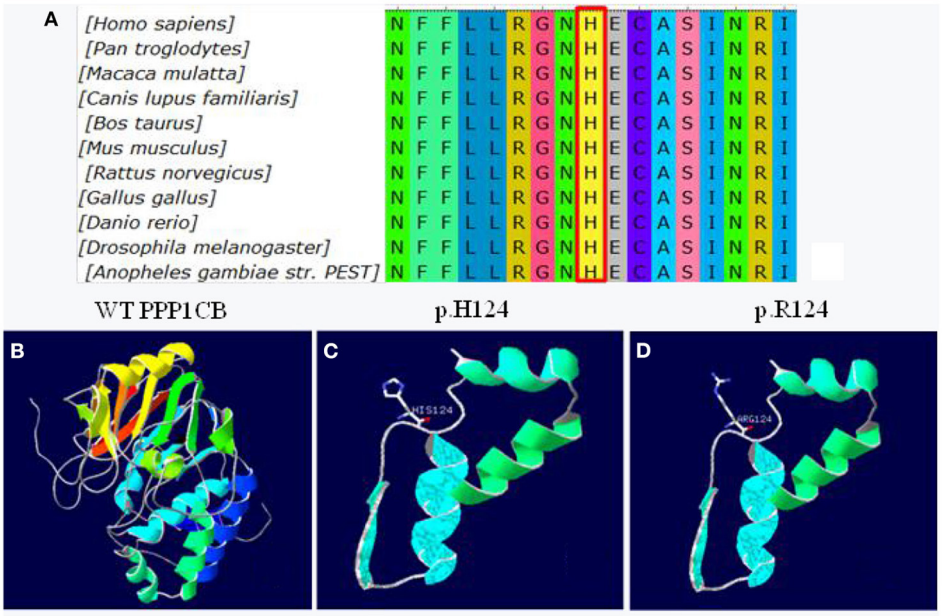


FIGURE 5
Conservation and 3D molecular model of *PPP1CB* mutations. **(A)** Protein alignment showing *PPP1CB* p.H124 occurred at evolutionarily conserved amino acids (in red box) across 11 genera; **(B)** Wild type of *PPP1CB*; **(C)** Three-dimensional molecular models of wild-type protein at site p.H124; **(D)** Abnormal structure of mutant-type protein.

syndromes are a group of diseases caused by gene mutations in the RAS/MAPK pathway, including Noonan syndrome (NS), Noonan-like syndrome with multiple lentigines, NSML), NSLH, Capillary malformation-arteriovenous malformation (CM-AVM), Costello syndrome (CS), Cardio-facio-cutaneous syndrome (CFCS), neurofibromatosis type 1 (NF1), and

TABLE 1 Basic information of the 17 cases of NSLH2 caused by *PPP1CB* gene mutation reported in literature.

SN	Sex	Nationality	Base sequence	Amino acid	PMID
1	Female	China	c.146C>G	p.Pro49Arg	PMID: 32476286
2	Male	China	c.548A>C	p.Glu183Ala	PMID: 30236064
3	Male	America	c.146G>C	p.Pro49Arg	PMID: 27264673
4	Male	America	c.166G>C	p.Ala56Pro	PMID: 27264673
5	Female	America	c.146G>C	p.Pro49Arg	PMID: 27264673
6	Female	America	c.146G>C	p.Pro49Arg	PMID: 27264673
7	Male	Brazil	c.146G>C	p.Pro49Arg	PMID: 28211982
8	Male	America	c.146C>G	p.Pro49Arg	PMID: 27868344
9	Male	America	c.146C>G	p.Pro49Arg	PMID: 27681385
10	Female	America	c.146C>G	p.Pro49Arg	PMID: 27681385
11	Male	America	c.146C>G	p.Pro49Arg	PMID: 27681385
12	Male	America	c.146C>G	p.Pro49Arg	PMID: 27681385
13	Male	America	c.548A>C	p.Glu183Ala	PMID: 27681385
14	Male	America	c.548A>T	p.Glu183Val	PMID: 27681385
15	Male	America	c.754G>T	p.Asp252Tyr	PMID: 27681385
16	Male	America	c.820G>A	p.Glu274Lys	PMID: 27681385
17	Female	America	c.371A>G	p.His124Arg	This case

Legius syndrome (LS). The overall incidence of RAS signal-associated syndromes in live births has been reported to be as high as 1 in 1250-700 (Wright and Kerr, 2010). In addition, the clinical manifestations of these diseases such as cardiovascular abnormalities, skin abnormalities, special facial features, neurological abnormalities, and varying degrees of intellectual disability overlap to a certain extent. It is difficult to diagnose these diseases only through clinical manifestations. Currently, whole exome sequencing has played a role in identifying the genetic etiology of RAS signaling pathway-related syndromes (Gripp et al., 2016).

To date, 23 genes related to RAS signaling pathway-related syndrome have been reported in literature; these are *PTPN11, SOS1, RAF1, NRAS, CBL, NF1, RASA1, HRAS, BRAF, MAP2K1, MAP2K2, KRAS, SPRED1, SHOC2, RRAS, RIT1, RASA2, SOS2, MAP3K8, SPRY1, MYST4, LZTR1,* and *A2ML1* (Schubbert et al., 2007; Matozaki et al., 2009; Wright and Kerr, 2010; Gripp et al., 2016; Umeki et al., 2018; Motta et al., 2020; Zhang and Zeng, 2020). Among them, 16 genes are related to Noonan syndrome (Li et al., 2020), and two genes, namely *SHOC2* and *PPP1CB*, are related to Noonan syndrome with birth hair loosening (Haverfield et al., 2018).

Protein phosphatase 1 (PPP1), a major type 1 serine/threonine phosphatase, is widely expressed and regulates a variety of cellular functions including metabolism, cell division, and muscle contraction (Barker et al., 1994). PPP1 is a serine/threonine specific phosphatase that balances serine/threonine kinases to regulate the activation of signaling proteins such as mitogen-activated protein kinases in the RAS/MAPK pathway. PPP1 is a holoenzyme composed of a catalytic subunit (PPP1C) and a regulatory subunit (PPP1R). PPP1 has three catalytic subunits (PPP1C), namely alpha subunit (encoded by *PPP1Ca*), beta subunit (encoded by *PPP1CB*), and gamma subunit (encoded by *PPP1Cc*), and selective splicing within each site produces multiple PPP1 holoenzymes and leads to different functions. Differential expression of the PPP1c subtype depends on cell type or tissue or even cell location, but they have similar functional properties *in vitro*. Many studies have demonstrated the multiple roles of PPP1c subtype in the regulation of cell function. PPP1CA has been widely studied for its role in the cell cycle and apoptosis of immune cells. The PPP1CB subtype is muscle specific and involved in glycogen metabolism and muscle contraction.

Recently, the role of *PPP1CB* in cardiomyocytes has also been demonstrated: it is a myosin light chain phosphatase responsible for transient Ca2+ increase and the increase of cell shortening, and *PPP1CC*'s role in the regulation of mitosis and metabolic glutamate receptor inactivation has recently received attention (Dard et al., 2018; Degirmenci et al., 2020). *SHOC2* is a widely expressed protein that is rich in leucine repeats, which interacts with the catalytic subunit of PPP1. PPP1c plays an important role in the regulation of Ras /MAPK pathway; moreover, it also forms a complex with *SHOC2*, which is stimulated by MRAS and dephosphorylates RAFs at a serine inhibition site, thereby activating the signaling cascade. Although some studies have speculated that mutations in *PPP1CB* may lead to the activation of MAPK through the activation of RAF, further studies are needed to confirm this conclusion, e.g., amino acid changes resulting from *PPP1CB* mutation may result in enhanced substrate binding of the

PP1/SHOC2 complex or prolonged activation after stimulation (Young et al., 2018).

NSLH2 is also known as Mazzanti syndrome, and its main clinical features are loose hair, relative giant, growth hormone deficiency, and low intelligence (Li et al., 2020). Clinical reports are based on this syndrome's unique hair manifestations, combined with the findings of growth hormone deficiency and other typical Noonan-syndrome features. Loose growing hair is characterized by easily pluckable, sparse, thin, slow-growing, and irregularly textured hair, which is caused by abnormal hair bulbs lacking internal and external root sheaths. Most patients with NSLH2 have short stature due to growth hormone deficiency, but our patient has not yet shown this clinical symptom. Special facial features include big head; prominent forehead; wide eye spacing; drooping eyelids; flappy and low-set ears with oval helices often accompanied by thickening; short nose with a low bridge; short neck; and neck webbing. The facial features of patients of different ages are different, which are more prominent in infancy and early and middle childhood; these characteristic facial features tend to become increasingly untypical with age. The disease can also involve other ectodermal tissue such as the skin, and some patients have eczema, ichthyosis, and hair keratosis. Some other patients have congenital heart disease. Cardiovascular system involvement may be manifested as loss of the atrial or ventricular septum, mitral/tricuspid valve dysplasia, pulmonary artery stenosis, and/or cardiac hypertrophy. Some patients may also show abnormal skeletal development such as shield chest, chicken chest, funnel chest, and elbow valgus. Central nervous system involvement may be manifested as intellectual disability, often accompanied by attention deficit/hyperactivity disorder (Zarbo and Shwayder, 2018). Feeding difficulties are common in infants and young children.

At present, 16 cases of *PPP1CB* gene mutation type 2 Noonan-like syndrome associated with birth hair loosening have been reported worldwide. Of these, two cases are reported in China (Zhou et al., 2020); ours is the third reported case in China. As shown in Table 1, there are seven types of gene mutations reported in 16 patients (Matozaki et al., 2009; Zambrano et al., 2017; Lin et al., 2018; Zhou et al., 2020; Maruwaka, 2022; 21). c.146C>G (p.Pro49Arg) is the hot spot of *PPP1CB* gene mutations (Zambrano et al., 2017). The mutation in this case was c.371A>G (p.His124Arg), which is a novel mutation to the best of our knowledge (Table 1). The main clinical manifestation of the 17 NSLH2 patients reported thus far (including our patient) includes unusual facial features, short stature, chest deformity, and congenital heart disease. Among them, 76.5% patients had short stature and low ear position; 70% patients had loose hair; 58% had forehead protrusion; and 47% had heart disease, wide-set eyes, and feeding difficulties (Figure 6).

The diagnosis of NSLH2 was confirmed by combining the results of genetic testing. Our patient had hip dysplasia, which

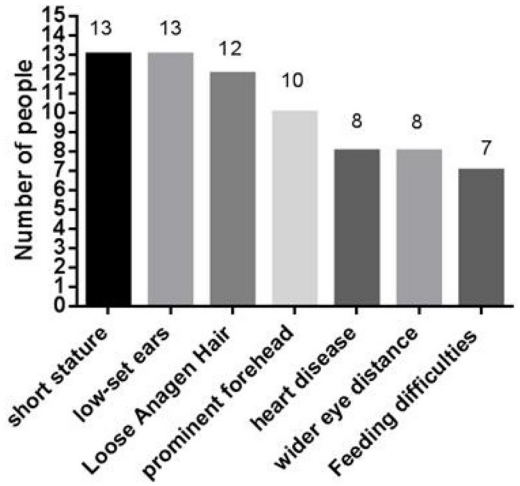


FIGURE 6
The type of NSLH2 clinical manifestations in 17 cases caused by PPP1CB gene mutation.

was not found in previous reports. Further, most patients with NSLH2 had low ear position, but our patient did not, rather only bilateral auricle shape abnormality. Until now, no symptoms such as short stature, slow growth, and thoracic deformity have been found in the patient. Cerebellar tonsil hernia, Chiari malformation, or changes in corpus callosum have not been seen by cranial NMR. This may be because the patient is still young and has not yet shown other possible symptoms of the disease; hence, continued follow-up and preventive treatment for some of the predicted dysfunction may be required.

The diagnosis of NSLH2 is based on the patient's history, physical examination, genetic testing, and other auxiliary tests. After the definite diagnosis of NSLH2, the evaluation of cardiovascular system, endocrine system, skeletal system, digestive system, nerve, vision, skin, hearing and growth and development should be improved (Li et al., 2020). At present, there is no specific treatment for NSLH2, and symptomatic treatment is still the first choice of therapy. Cardiovascular system involvement may manifest as atrial or ventricular septal defects, mitral/tricuspid valve dysplasia, pulmonary artery stenosis, and cardiac hypertrophy. Regular follow-up, drug therapy, interventional therapy, or surgical operation should be selected according to the condition and severity (Maruwaka, 2022). Because most NSLH2 patients have problems such as overall stunting and short stature, the nutritional status and feeding conditions should be followed-up long-term, and regular nutritional assessments and timely interventions should be carried out. In 2007, the guidelines of the US Food and Drug Administration and the Pediatric Endocrinology, Genetics, and Metabolism Group of the Chinese Medical Association used recombinant human growth hormone (rhGH) for the treatment

of short stature caused by Noonan syndrome (Degirmenci et al., 2020). In 2020, a study used rhGH to treat a patient with NSLH2 type 2 caused by *PPP1CB* mutation, and the results showed that the linear growth of the patient had improved (Zhou et al., 2020).

Among the 16 patients reported, one patient developed severe intractable epileptic convulsions due to a mutation in the *PPP1CB* gene C.548A>C (P.Glu183Ala). The patient's seizures were barely controlled by conventional antiepileptic drugs, but were eventually relatively controlled by a ketogenic diet. It is suggested that NSLH2 may cause RAS/MAPK-related epilepsy, and ketogenic diet may have a certain effect on *PPP1CB*-associated Noonan syndrome manifested as infantile spasm (Lin et al., 2018). The family members of the patient in this study complained that the patient had frequent nodding, and regular follow-up was needed to recheck the video electroencephalogram to exclude epilepsy. In future follow-up, attention should be paid to the electroencephalogram results, to ensure early detection, early diagnosis, and early treatment.

At present, there are no long-term follow-up reports of patients with type 2 Noonan syndrome with hair loosening. Through regular follow-up, we found that active comprehensive rehabilitation therapy could improve the patient's motor, language, social, and cognitive skills. Comprehensive rehabilitation training including physical therapy, occupational therapy, speech therapy, sensory integration training, and special education can improve the level of intellectual function, social life ability, and self-care ability of children, and reduce the degree of treatment barriers and limited participation. Although no serious complications have occurred in our patient up to the time of writing, it is still necessary to follow-up the organ function of each system and carry out Multidisciplinary teamwork of the children are normal, and the risk of rebearing children with NSLH2 is small. However, we still need to be alert regarding reproductive chimerism. Genotypes and phenotypes caused by RASopathies are better understood now than before and hence, it is relatively easy for families for obtain a prenatal diagnosis. However, the new variation of prenatal diagnosis still has certain limitations, thus, clinicians should increase alertness, carefully check when prenatal diagnosis fetal face, once found, wide eyes, short nose forehead the special features such as, low set ears, should be on high alert, genetic tests in a timely manner.

In conclusion, we report a novel variant c.371A>G in exon 3 of the *PPP1CB* gene in a young pediatric patient with NSLH2. This study enriched the gene mutation spectrum of *PPP1CB*, and provided some reference for the diagnosis of this syndrome. To our knowledge, this is the first Chinese study to report this novel mutation in *PPP1CB* in NSLH2; therefore, further functional studies are needed to confirm the underlying pathogenic mechanism.

Ethics statement

Written informed consent was obtained from the individual' legal guardian/next of kin, for the publication of any potentially identifiable images or data included in this article.

Author contributions

XH, XM, JW, JM, and YL conceived this study, contributed to the study design, and attributed to project management. XH, XM, and JW wrote this manuscript and performed data collection. ZZ and CL generated the figures and tables. HH and JR contributed to guidance on English writing and performed data analysis. NZ carried out the literature search. XH and JW revised the manuscript. All authors have read and approved the content of the manuscript.

Funding

This study was supported by the Kunming Medical University Joint Project-the Science and Technology Planning Project of the Yunnan Provincial Department of Science and Technology (Nos. 202101AY070001-215 and 202201AY070001-205), the Scientific Research Fund of Yunnan Provincial Education Department (No. 2022J0202), and Kunming Health Science and Technology Talent Training Project [No. 2022-SW (back-up)-005].

Acknowledgments

We gratefully acknowledge the extraordinary generosity of the study participants.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Barker, H. M., Brewis, N. D., Street, A. J., Spurr, N. K., and Cohen, P. T. (1994). Three genes for protein phosphatase 1 map to different human chromosomes: sequence, expression and gene localisation of protein serine/threonine phosphatase 1 beta (PPP1CB). *Biochim. Biophys. Acta*. 1220, 212–218. doi: 10.1016/0167-4889(94)90138-4

Cordeddu, V., Di Schiavi, E., Pennacchio, L. A., Ma'ayan, A., Sarkozy, A., and Fodale, V. (2009). Mutation of SHOC2 promotes aberrant protein N-myristoylation and causes Noonan-like syndrome with loose anagen hair. *Nat Genet*. 41, 1022–1026. doi: 10.1038/ng.425

Dard, L., Bellance, N., Lacombe, D., and Rossignol, R. (2018). RAS signalling in energy metabolism and rare human diseases. *Biochim. Biophys. Acta Bioenerg*. 1859, 845–867. doi: 10.1016/j.bbabio.2018.05.003

Degirmenci, U., Wang, M., and Hu, J. (2020). Targeting aberrant RAS/RAF/MEK/ERK signaling for cancer therapy. *Cells*. 9, 198. doi: 10.3390/cells9010198

Gripp, K. W., Aldinger, K. A., Bennett, J. T., Baker, L., Tusi, J., Powell-Hamilton, N., et al. (2016). A novel rasopathy caused by recurrent de novo missense mutations in PPP1CB closely resembles Noonan syndrome with loose anagen hair. *Am. J. Med. Genet. A*. 170, 2237–2247. doi: 10.1002/ajmg.a.37781

Haverfield, E., Masood, M. M., Henin, M., and Aylsworth, A. S. (2018). Cleft palate and hypopituitarism in a patient with Noonan-like syndrome with loose anagen hair-1. *Am. J. Med. Genet. A*. 176, 2024–2027. doi: 10.1002/ajmg.a.40432

Li, X., Wang, X., Wang, J., Fu, L., Luo, X., Fu, J., et al. (2020). Clinical practice guidelines for Noonan syndrome. *Chin. J. Med. Genet*. 3, 324–328. doi: 10.3760/cma.j.issn.1003-9406.2020.03.017

Lin, C. H., Lin, W. D., Chou, I. C., Lee, I. C., Fan, H. C., and Hong, S. Y. (2018). Epileptic spasms in PPP1CB-associated Noonan-like syndrome: a case report with clinical and therapeutic implications. *BMC Neurol*. 18, 150. doi: 10.1186/s12883-018-1157-6

Maruwaka, K., Nakajima, Y., Yamada, T., Tanaka, T., Kosaki, R., Inagaki, H., et al. (2022). Two Japanese patients with Noonan syndrome-like disorder with loose anagen hair 2. *Am. J. Med. Genet. A*. 188, 2246–2250. doi: 10.1002/ajmg.a.62733

Matozaki, T., Murata, Y., Saito,Y., Okazawa, H., and Ohnishi, H. (2009). Protein tyrosine phosphatase SHP-2: a proto-oncogene product that promotes Ras activation. *Cancer Sci*. 100, 1786–1793. doi: 10.1111/j.1349-7006.2009.01257.x

Mazzanti, L., Cacciari, E., Cicognani, A., Bergamaschi, R., Scarano, E., and Forabosco, A. (2003). Noonan-like syndrome with loose anagen hair: a new syndrome? *Am. J. Med. Genet. A*. 118A, 279–286. doi: 10.1002/ajmg.a.10923

Motta, M., Sagi-Dain, L., Krumbach, O. H. F., Hahn, A., Peleg, A., German, A., et al. (2020). Activating MRAS mutations cause Noonan syndrome associated with hypertrophic cardiomyopathy. *Hum. Mol. Genet*. 29, 1772–1783. doi: 10.1093/hmg/ddz108

Schubbert, S., Shannon, K., and Bollag, G. (2007). Hyperactive Ras in developmental disorders and cancer. *Nat. Rev. Cancer*. 7, 295–308. doi: 10.1038/nrc2109

Umeki, I., Niihori, T., Abe, T., Kanno, S. I., Okamoto, N., Mizuno, S., et al. (2018). Delineation of LZTR1 mutation-positive patients with Noonan syndrome and identification of LZTR1 binding to RAF1-PPP1CB complexes. *Hum. Genet*. 138, 21–35. doi: 10.1007/s00439-018-1951-7

Wright, E. M., and Kerr, B. (2010). RAS-MAPK pathway disorders: important causes of congenital heart disease, feeding difficulties, developmental delay and short stature. *Arch. Dis. Child*. 95, 724–730. doi: 10.1136/adc.2009.160069

Young, L. C., Hartig, N., Boned Del Río, I., Sari, S., Ringham-Terry, B., and Wainwright, J. R. (2018). SHOC2-MRAS-PP1 complex positively regulates RAF activity and contributes to Noonan syndrome pathogenesis. *Proc. Natl. Acad. Sci. U S A*. 115, E10576–E10585. doi: 10.1073/pnas.1720352115

Zambrano, R. M., Marble, M., Chalew, S. A., Lilje, C., Vargas, A., and Lacassie, Y. (2017). Further evidence that variants in PPP1CB cause a rasopathy similar to Noonan syndrome with loose anagen hair. *Am. J. Med. Genet. A*. 173, 565–567. doi: 10.1002/ajmg.a.38056

Zarbo, A., and Shwayder, T. (2018). Loose anagen hair syndrome. *J. Pediatr*. 199, 282–282. doi: 10.1016/j.jpeds.2018.03.005

Zhang, J. J., and Zeng, Y. (2020). Prenatal diagnosis of RAS signaling pathway associated syndrome. *Chin. J. Med. Genet*. 37, 1291–1294. doi: 10.3760/cma.j.cn511374-20190717-00354

Zhou, P., Zhu, L., Fan, Q., Liu, Y., Zhang, T., and Yang, T. (2020). A case report of Noonan syndrome-like disorder with loose anagen hair 2 treated with recombinant human growth hormone. *Am. J. Med. Genet. Part A*. 182, 1–5. doi: 10.1002/ajmg.a.61638

frontiers | Frontiers in Behavioral Neuroscience

TYPE Original Research
PUBLISHED 09 September 2022
DOI 10.3389/fnbeh.2022.925122

OPEN ACCESS

EDITED BY
Dongdong Qin,
Yunnan University of Chinese Medicine, China

REVIEWED BY
Samuel Nemanich,
Marquette University, United States
Dinesh Bhatia,
North Eastern Hill University, India

*CORRESPONDENCE
Kaishou Xu
xksyi@126.com
Hongmei Tang
gdthm@126.com

†These authors share first authorship

SPECIALTY SECTION
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

RECEIVED 21 April 2022
ACCEPTED 16 August 2022
PUBLISHED 09 September 2022

CITATION
He W, Huang Y, He L, Liu L, Zeng P, Qiu H, Wang X, Zhou H, Chen Z, Xu Y, Zhao J, Wang W, Tang H and Xu K (2022) Safety and effects of transcranial direct current stimulation on hand function in preschool children with hemiplegic cerebral palsy: A pilot study.
Front. Behav. Neurosci. 16:925122.
doi: 10.3389/fnbeh.2022.925122

Safety and effects of transcranial direct current stimulation on hand function in preschool children with hemiplegic cerebral palsy: A pilot study

Wenjie He[1†], Yuan Huang[1,2†], Lu He[1†], Liru Liu[1], Peishan Zeng[1], Huiying Qiu[1], Xiaoyue Wang[1], Hongyu Zhou[1], Zhaofang Chen[1], Yi Xu[1], Jingyi Zhao[1], Wenda Wang[1], Hongmei Tang[1*] and Kaishou Xu[1*]

[1]Department of Rehabilitation, Guangzhou Women and Children's Medical Center, Guangzhou Medical University, Guangzhou, China, [2]School of Medicine, South China University of Technology, Guangzhou, China

Transcranial direct current stimulation (tDCS) has shown a promising prospect in improving function and spasticity in school-aged children with cerebral palsy, but little is known in preschool children. The aim of this study was to explore the safety and effects of tDCS on hand function in preschool children (aged 3–6 years) with hemiplegic cerebral palsy (HCP). We designed a crossover, single-blind, sham-controlled study in 30 preschool children with HCP, who were recruited to receive one session of sham and one session of active anodal tDCS (1.5 mA, 20 min) on the primary motor cortex of the affected hemisphere, with a 24-h interval between the two sessions. Questionnaire was completed by each participant and their attendants immediately, 90 min, and 24 h after each session to monitor common adverse events of tDCS, such as skin irritation, skin erythema, burning sensation, headache, dizziness, etc. Box and Block Test, Selective Control of the Upper Extremity Scale, Modified Ashworth Scale, and Melbourne Assessment 2 were conducted at baseline, immediately, and 90 min after each session. No severe adverse event occurred during the study and only a few of them felt transient and slight discomfort. Results also showed that all participants performed better at Box and Block Test of the hemiplegic hand immediately after a single anodal tDCS ($P < 0.05$) and this improvement lasted at least 90 min and more than 24 h. However, there was no significant improvement in Selective Control of the Upper Extremity Scale of both hands, Box and Block Test of the non-hemiplegic hand, Modified Ashworth Scale, and Melbourne Assessment 2 of the hemiplegic upper limb ($P > 0.05$). Shortly, this study supported the safety and effects of a single anodal tDCS on improving the manual dexterity of the hemiplegic hand for preschool children with HCP. Further researches with larger samples about the optimal dose and treatment cycle of tDCS for preschool children with

HCP are warranted. This study gained the approval of ethics committee of the organization and was registered at chictr.org (ChiCTR2000031141).

KEYWORDS

hemiplegic cerebral palsy, transcranial direct current stimulation, safety, hand function, preschool children

Introduction

Cerebral palsy is characterized as movement and posture disorders with complicated etiology and stable prevalence of 2–3.5 cases per 1,000 live births (Colver et al., 2014; Li et al., 2022). Hemiplegic cerebral palsy (HCP) is the most common type of cerebral palsy, accounting for 44% (Zelnik et al., 2016). Hemiplegic cerebral palsy affects one side of the body and the upper limb is more involved, which causes unimanual dysfunction, impaired dexterity, and poor bimanual coordination, exerting serious negative effects on daily activities throughout their lifetime.

Several types of interventions have been successfully employed to improve hand function for children with HCP in recent years, such as constraint-induced movement therapy and bimanual intensive therapy, which aim at improving motor function by specific upper limb tasks and may facilitate the brain plasticity in a way from the periphery to the center (Gordon et al., 2011; Novak et al., 2020). Meanwhile, the effect was still unsatisfactory for some children and there were a few new techniques, such as transcranial magnetic stimulation and transcranial direct current stimulation (tDCS), combined with intensive therapy to enhance the effect (Duarte Nde et al., 2014; Wu et al., 2022). Transcranial direct current stimulation, a simple and portable non-invasive brain stimulation which works by means of delivering low-level direct current to facilitate or inhibit cortical spontaneous neuronal activity (DaSilva et al., 2011; Brunoni et al., 2012; Marquez et al., 2015), has attracted more and more attention in healthy humans and clinical populations (Shin et al., 2015; Lefaucheur et al., 2017; O'Leary et al., 2021). In healthy volunteers, interesting findings that tDCS could safely enhance memory, emotional regulation, language, attention, and learning processes have been reported (Shin et al., 2015; Ciechanski and Kirton, 2017). In clinical studies, previous findings demonstrated that anodal tDCS was effective for limb-kinetic apraxia in Parkinson's disease, for motor function in stroke patients, for control functions in children with attention deficit/hyperactivity disorder and for symptom reduction in autism spectrum disorder (Kang et al., 2016; Osorio and Brunoni, 2019; Nejati et al., 2020; Park et al., 2022).

For children with HCP, the majority of tDCS researches focused on improving spasticity and lower limb function and only a few researches investigated the tDCS effects on hand function in HCP (Fleming et al., 2018; O'Leary et al., 2021). Most studies showed the improvement in spasticity, gait velocity and cadence, body sway velocity and balance after single or continuous anodal tDCS combined with other therapy (Collange Grecco et al., 2015; Auvichayapat et al., 2017; Grecco et al., 2017). But two studies showed no significant effect in hand function after serial sessions of cathodal tDCS over the contralesional primary motor cortex (Kirton et al., 2017; Gillick et al., 2018). Meanwhile, anodal tDCS, usually applied separately or combined with other traditional therapies, unilaterally over the primary motor cortex (M1) of the affected or more affected hemisphere, safely improved hand function for school-aged children with HCP without serious adverse event reported (Auvichayapat et al., 2017; Moura et al., 2017; Inguaggiato et al., 2019). All of the above researches were primarily conducted in school-aged children and young adults with HCP and there was a paucity of researches about the safety and effects of tDCS on preschool children (aged 3–6 years old). However, preschool children are in a developing stage of cortical excitability and corticospinal excitability (Säisänen et al., 2018). Given the potential mechanism of tDCS, this period might be more critical for its application and rehabilitation of hand function.

The evidence about optimal tDCS current and duration for HCP is still insufficient but some studies have shown that the safety and effects of tDCS could be influenced by density (Krishnan et al., 2015). Current intensities in most studies about tDCS in pediatric populations have ranged from 0.3 to 2.0 mA and the most frequently used intensity in HCP was 1 mA with a duration of 20 min (Krishnan et al., 2015; O'Leary et al., 2021). Notably, relevant researches have indicated that low current (0.7 mA) was too weak to produce measurable corticospinal excitability changes and behavioral effects for individuals with HCP (Gillick et al., 2018; Nemanich et al., 2019). Another pilot study first explored the safety and effects of anodal tDCS at 1.5 mA for 20 min in school-aged children with HCP, whose parameters were on the basis of evidence from stroke in adults (Inguaggiato et al., 2019). The safety and effects of these tDCS parameters (1.5 mA, 20 min) remain unknown in preschool children with HCP.

To fill this gap, we designed this study to investigate the safety and effects of a single anodal tDCS (1.5 mA, 20 min) over the M1 on hand function in preschool children with HCP.

Methods

Our study was a crossover, single-blind, sham controlled trial, which gained the approval of ethics committee of the organization and was registered at chictr.org (ChiCTR2000031141). All legal guardians of participants signed the informed consent before enrollment.

Participants

Thirty participants were recruited in the rehabilitation department of Guangzhou Women and Children's Medical Center from September 2019 to February 2020. We screened children (3–6 years old) diagnosed as HCP according to published criteria (Rosenbaum et al., 2007) and categorized as Manual Ability Classification System or Mini-Manual Ability Classification System levels I to II. The exclusion criteria were as follows: (i) other severe illness such as congenital heart disease, uncontrolled epilepsy, leukemia, severe sensory disturbance, and visual problem; (ii) contraindications for tDCS including children with metal or electronic implants, with local skin injury or inflammation, with significantly increased intracranial pressure, with hyperalgesia in the stimulated area, with convulsions or uncontrolled seizure and those who suffered from serious adverse events after tDCS (Antal et al., 2017); (iii) previous botulinum toxin treatment over the past 6 months or preparation for receiving botulinum toxin treatment during trial; (iv) previous surgery of the impaired upper limb. Thirty children completed the entire study. The flow chart of this study was shown in Figure 1.

Design

All recruited children were randomized into two groups in a 1:1 ratio using a random number table produced by Statistical Product and Service Solutions for Windows (release 26.0, SPSS), and each group received a single session of active anodal tDCS or a single sham tDCS over M1 first and the stimulation was switched after 24 h (crossover phase). Participants and guardians were blind to tDCS assignment. Safety questionnaire was completed immediately (T1), 90 min (T2), and 24 h after tDCS (T3). Assessments of hand function were performed by two independent and occupational therapists at baseline (T0), immediately (T1), and 90 min (T2) after each session. The device was produced by Wuhan Yimai Medical Technology Co., Ltd. and the model was EM8060.

Interventions

Two 5.5 × 4.0 cm electrodes were placed on the scalp with the anode positioned in the region over the M1 of the affected or more affected hemisphere according to the 10–20 electroencephalogram system, with the cathode electrode placed over the contralateral supraorbital area. The rationale of unilateral stimulation was based on a concept that stimulating the injured brain could enhance motor learning. During active anodal tDCS, a constant current of 1.5 mA was applied for 20 min (with 30 s for ramping up at the beginning and down at the end). The same stimulation protocol was applied in sham tDCS but the current lasted only 30 s. This protocol was proposed by previous tDCS investigation on HCP (Inguaggiato et al., 2019).

Outcome assessments

Safety

All participants and their attendants completed an adverse events questionnaire at T1, T2, T3. The questionnaire consisted of eight commonly-reported adverse events (i.e., dizziness, headache, scalp pain, burning sensation, tingling, drowsiness, itching, skin redness) as well as an "other" category that allowed them to describe uncovered experiences/sensations (Brunoni et al., 2011; Krishnan et al., 2015; Reckow et al., 2018). The intensity of adverse events was rated verbally by one of the occupational therapists (i.e., 0, absent; 1, mild; 2, moderate; 3, severe).

Hand function

The gross motor function of all children was measured by Gross Motor Function Classification System (GMFCS) (Paulson and Vargus-Adams, 2017). The manual abilities were classified by the Manual Ability Classification System (for children over 4 years old) or Mini-Manual Ability Classification System (for children aged 3–4 years old) (Eliasson et al., 2017; Paulson and Vargus-Adams, 2017).

All assessments of hand function were based on the dimensions of international classification of functioning, disability, and health (ICF) (Cieza et al., 2019; Madden and Bundy, 2019) and conducted at T0, T1, and T2. Box and Block Test was used to measure the gross manual dexterity for adults with upper limb paresis and also to determine therapeutic efficiency for children with HCP in clinical rehabilitation, which features advantages of simplicity of operator, reliability, and repeatable measurement (Platz et al., 2005; Jongbloed-Pereboom et al., 2013; Araneda et al., 2019). Melbourne Assessment 2 mainly assessed the movement quality of the upper limb for children with neurological impairment aged 2 years and 6 months to 15 years (Randall et al., 2012, 2014; Elvrum et al., 2016). Selective Control of the Upper Extremity Scale was a method that could be used to evaluate the selective motor control of the upper extremity in children aged 3–18 years

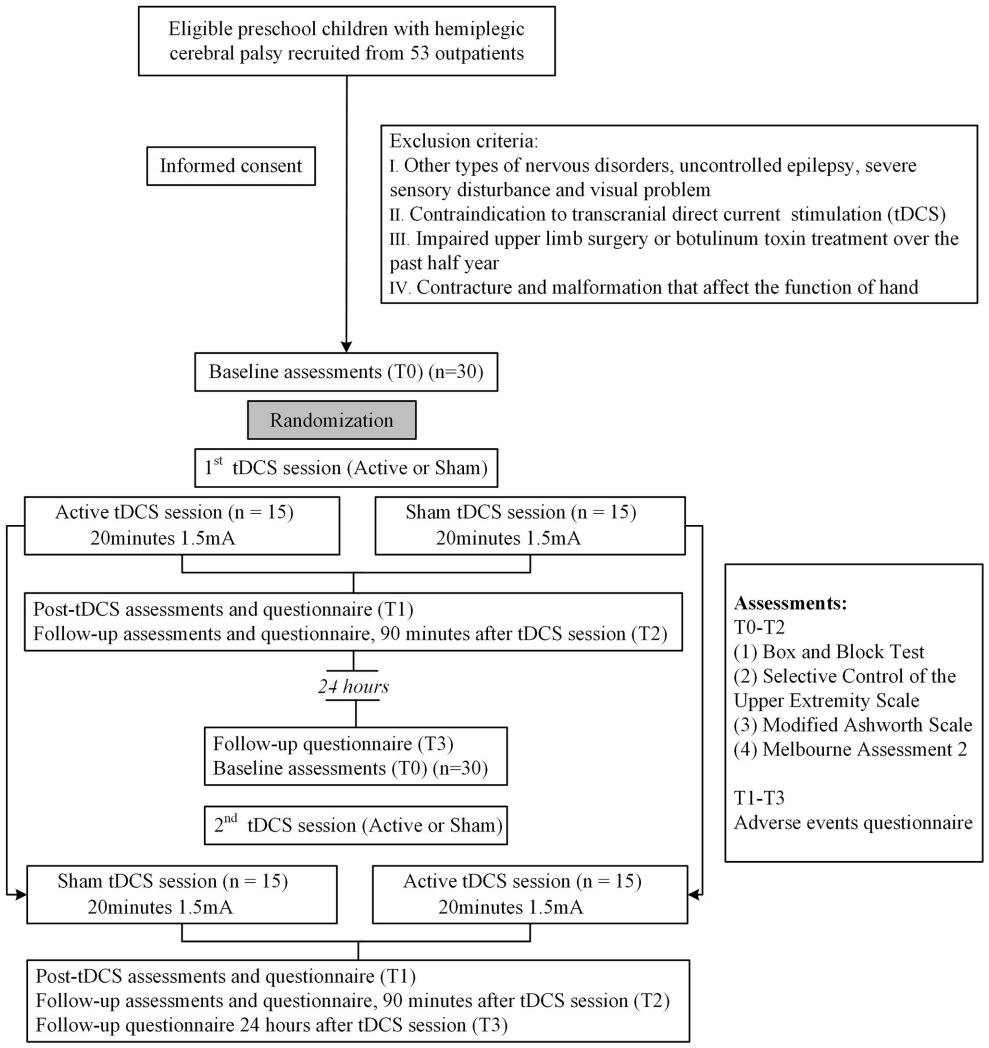


FIGURE 1
Consort diagram and study flow.

with cerebral palsy, whose content validity, reliability, construct validity, intra-, and interrater reliability have been determined (Wagner et al., 2016; Yildiz et al., 2020). The Modified Ashworth Scale was the most prevalent tool to measure the tone of specific muscles in children with cerebral palsy (Meseguer-Henarejos et al., 2018).

Statistical analysis

SPSS 26.0 (IBM, Armonk, New York, USA) was used to carry out statistical analysis. We adopted repeated-measures analysis of variance [tDCS (active vs. sham) or Day (Day 1 vs. Day 2) × time] for all assessments of hand function. Follow-up one-way repeated-measures ANOVA was used for significant interactions and single effects for time, whereas one-way between-factor ANOVA was used for tDCS and day and corrected for multiple comparisons (Bonferroni).

The normality of data was examined by the Shapiro-Wilk test. Moreover, before running the analysis, the sphericity test for repeated measures analysis of variance was assessed by Mauchly's test; whenever assumptions were not met, Greenhouse-Geisser correction was used for violations of sphericity.

Results

In total, 15 boys and 15 girls were recruited into this trial (mean age ± SD: 47.53 ± 11.23 months, range: 36–72 months). The ratio of damaged hemispheres on the left and right was 13:17. For MACS, 28 and 2 children were at level I and level II,

TABLE 1 Characteristics of the participants.

No.	tDCS order	Gender	Age (months)	HCP side	MACS	GMFCS	High risk factors	MRI
1	AS	F	38	L	I	I	Premature birth	White matter maldevelopment
2	AS	F	37	L	I	I	NA	NA
3	AS	M	44	L	I	I	NA	NA
4	AS	F	70	R	I	I	Premature birth	NA
5	AS	F	42	R	I	II	Premature birth	NA
6	AS	F	40	R	I	II	Premature birth	NA
7	AS	M	46	L	I	I	Premature birth	Left ventricle semicovoid patch
8	AS	M	63	L	I	I	NA	NA
9	AS	F	68	R	I	I	NA	White matter maldevelopment
10	AS	F	38	L	I	I	Jaundice, hypoxia	NA
11	AS	M	51	L	I	I	Premature birth	NA
12	AS	M	53	R	I	I	NA	NA
13	AS	M	44	L	I	I	NA	NA
14	AS	M	36	R	II	I	Premature birth	NA
15	AS	M	43	R	I	I	Hypoxia	Left brain patchy lesion
16	SA	F	41	L	I	I	Premature birth	NA
17	SA	F	45	R	I	I	Meconium aspiration	NA
18	SA	M	40	R	I	I	NA	NA
19	SA	M	43	R	I	I	NA	NA
20	SA	F	61	R	I	I	NA	NA
21	SA	M	40	L	I	II	Cerebral hemorrhage	Left ventricular dilation
22	SA	F	67	R	I	I	NA	NA
23	SA	M	49	L	I	I	NA	NA
24	SA	F	41	R	I	I	NA	NA
25	SA	M	38	R	II	II	NA	NA
26	SA	M	50	R	I	I	Hypoxia	NA
27	SA	F	42	L	I	I	NA	White matter maldevelopment
28	SA	M	41	L	I	I	NA	NA
29	SA	F	72	R	I	I	NA	NA
30	SA	F	41	R	I	I	NA	Left ventricular dilation

HCP, hemiplegic cerebral palsy; No., number; M, male; F, female; A, active; S, sham; R, right; L, left; MACS, manual ability classification system; GMFCS, Gross Motor Function Classification System; NA, not available.

respectively. For GMFCS, 26 and 4 children were at level I and level II, respectively. The characteristics of all participants were shown in Table 1.

Safety

No severe adverse event occurred among the 30 participants and only a few of them felt transient and slight discomfort (tingling, itching, burning sensation, dizziness, etc.). With respect to the self-report questionnaire assessing tDCS adverse events, as shown in Table 2, only a limited number of participants reported transient and slight discomfort after both active (the proportion of dizziness, burning sensation, tingling, and itching were 1/30, 1/30, 2/30, and 1/30, respectively) and sham stimulation (the proportion of tingling is 2/30). All adverse events were mild.

Improvement of unimanual function

The affected hand of all participants performed better after accepting active tDCS at T1 and T2 compared to baseline in Box and Block Test. There was significant interaction of "tDCS × time" ($P < 0.01$) and no significant interaction of "Day × time" ($P = 0.465$). There were significant simple effect for tDCS ($P < 0.01$) and time ($P < 0.01$), whereas no significant main effect for day ($P = 0.229$). Follow-up one-way repeated-measures ANOVA was used for significant interactions and single effects for time and *post-hoc* pairwise comparison revealed significantly

TABLE 2 Adverse events of participants during the study.

Adverse events	T1 No. (Mean intensity)		T2 No. (Mean intensity)		T3 No. (Mean intensity)	
	Active	Sham	Active	Sham	Active	Sham
Dizziness	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Headache	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Scalp pain	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Burning sensation	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tingling	2 (1)	2 (1)	0 (0)	0 (0)	0 (0)	0 (0)
Drowsiness	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Itching	1 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Skin redness	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other	Absent	Absent	Absent	Absent	Absent	Absent

T1, immediately after the end of two transcranial direct current stimulation sessions (tDCS); T2, 90 min after two tDCS sessions; T3, 24 h after two tDCS sessions; No., number; Mean intensity, mean intensity range of the events (0, absent; 1, mild; 2, moderate; 3, severe).

more at T1 ($P < 0.01$) and T2 ($P < 0.01$) compared to baseline after active tDCS and no main effect of time after sham tDCS ($P = 0.114$; Figure 2A). We also separately analyzed the Box and Block Test data of each day, the outcome showed that the affected hand performed better only after active tDCS at both Day 1 and Day 2 ($T1_{Day1}$ vs. $T0_{Day1}$, $P < 0.01$ and $T2_{Day1}$ vs. $T0_{Day1}$, $P < 0.01$; $T1_{Day2}$ vs. $T0_{Day2}$, $P < 0.01$; and $T2_{Day2}$ vs. $T0_{Day2}$, $P < 0.01$; Figures 3A,C).

In addition, we applied a pair T-test to assess the baseline of Box and Block Test of the affected hand for the group who received active tDCS first and significant difference was found ($T0_{Day1} = 19.93$ vs. $T0_{Day2} = 21.00$, $P = 0.02$), while there was no significant difference between $T0_{Day1}$ and $T0_{Day2}$ in the group who received sham tDCS first ($P = 0.262$).

As for the unaffected hand, there was no significant interaction of "tDCS × time" ($P = 0.098$) and no significant interaction of "day × time" ($P = 0.244$) in Box and Block Test between active and sham tDCS. There was no significant main effect of time ($P = 0.091$), Day ($P = 0.37$), and tDCS ($P = 0.058$) (Figure 2A and Table 3). The separate analysis of the Box and Block Test data showed no significant difference in T1 and T2 compared baseline in both Day 1 and Day 2 (all $P > 0.05$, Figures 3B,D). All children showed no difference in Selective Control of the Upper Extremity Scale of both hands after active or sham tDCS (Figure 2B).

Affected upper extremity performance

There was no difference for the four sub-scales of Melbourne Assessment 2 (range of motion, level of grasp and release, accuracy, and fluency; Figure 2C). As for muscular tone, nearly all children showed no difference in the outcome of Modified Ashworth Scale (biceps brachii, forearm pronator, flexor carpi radialis, adductor pollicis, and digiti flexor; Figure 2D).

Discussion

This study aimed to investigate the safety as well as the immediate and short-term effects of a single anodal tDCS (1.5 mA) over M1 on the upper limb function for preschool children. No serious adverse event occurred during this study. The outcomes also showed that a single anodal tDCS (1.5 mA, 20 min) over the affected M1 improved dexterity of the affected hand for preschool children with HCP. These results complemented the existing evidence on the safety and effects of tDCS (1.5 mA, 20 min) in preschool children with HCP.

Consistent with previous studies (Ciechanski and Kirton, 2017; Gillick et al., 2018), no serious adverse event occurred among all children during and after our experiment. Only a few of participants felt transient and slight discomfort (tingling, itching, burning sensation, dizziness, etc.), which occurred in both active tDCS stimulation and sham tDCS stimulation. And these adverse effects also occurred in similar investigations with higher incidence (Mutlu et al., 2008; Inguaggiato et al., 2019). The recorded lower frequency of adverse events in our study might attribute to the parameters of tDCS and the poorer ability to describe uncomfortable feelings for preschool children. When adverse events occurred, we would stop the intervention as soon as possible and activated code blue if needed and we had a professional medical team to track every participant all the way. Our study provided evidence for safety of a single anodal tDCS over M1 at used parameter (1.5 mA, 20 min) for preschool children with HCP.

A previous study indicated that younger children obtained lower scores than older children with HCP in Box and Block Test which means poorer hand dexterity (Jongbloed-Pereboom

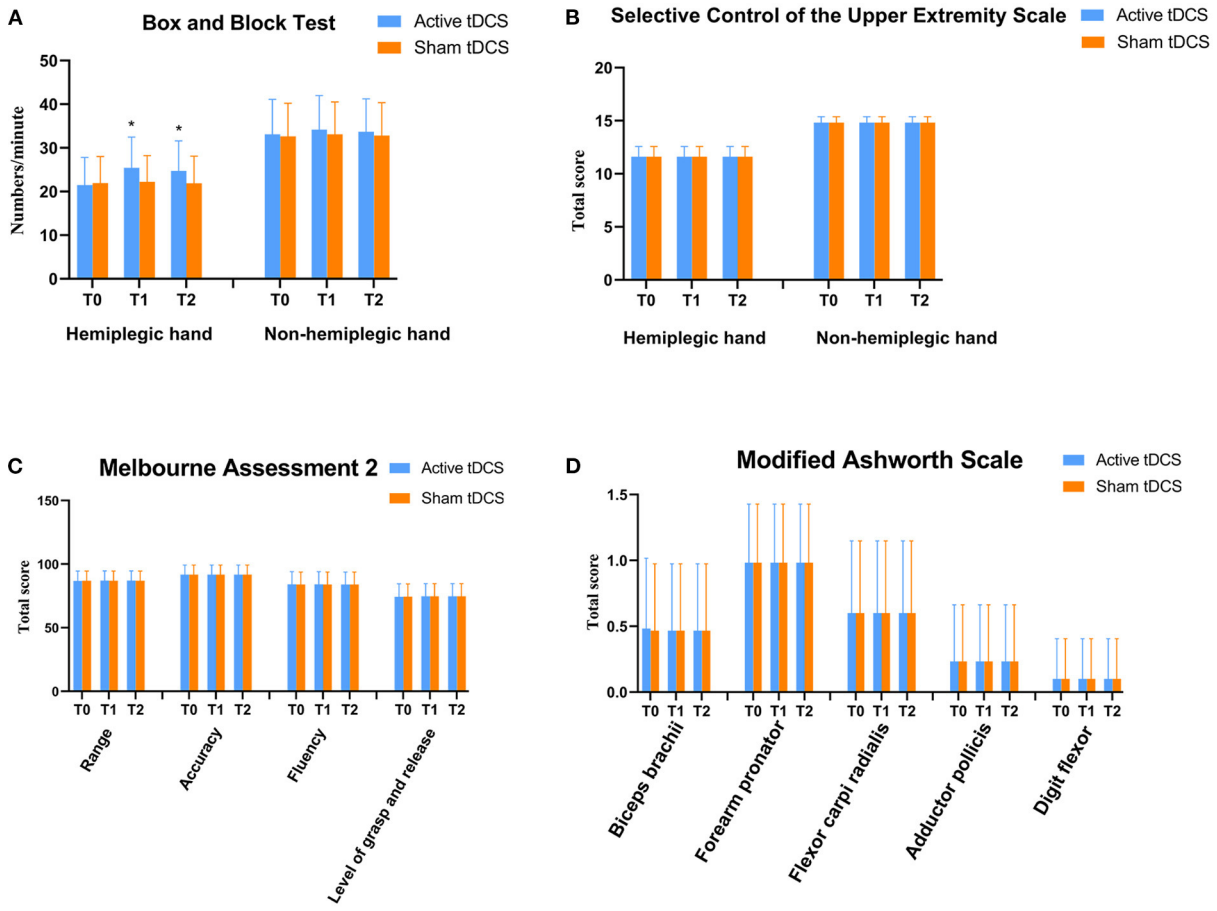


FIGURE 2
Changes of hand function in the two transcranial direct current stimulation (tDCS) sessions at T0, T1, T2. **(A)** Box and Block Test scores of both hands. **(B)** Selective Control of the Upper Extremity Scale scores of both hands. **(C)** Melbourne Assessment 2 scores of the hemiplegic hand. **(D)** Modified Ashworth Scale scores of the hemiplegic hand. T0, baseline; T1, immediately after two tDCS sessions; T2, 90 min after two tDCS sessions. *Significant difference compared to baseline ($P < 0.05$).

et al., 2013). According to a research about the reliability and responsiveness of the Box and Block Test for children with cerebral palsy, the clinical significant difference for the Box and Block Test was 1.9 (blocks) on the more affected hand (Araneda et al., 2019). The results of this study showed a change in the Box and Block Test of the affected hand at T1 (3.73 blocks) and T2 (3.14 blocks) compared to T0, which indicated that preschool children with HCP performed better in Box and Block Test of the affected hand after a single anodal tDCS (1.5 mA, 20 min) over the affected M1; improvement was found immediately after stimulation and lasted for at least 90 min. Meanwhile, the significant difference of $T0_{Day1}$ and $T0_{Day2}$ for the group who received a single active tDCS firstly indicated that this positive effect might last over 24 h, which differed from a similar study (Inguaggiato et al., 2019). According to a study about the impact of age on tDCS (Saldanha et al., 2020), we considered that the difference of age in the focused population (preschool children vs. individuals aged 10–28 years old) might account for this inconsistent result. The plasticity-dependent effects induced by tDCS indicated that the brain of preschool children featured with developing cortical and corticospinal excitability might benefit more from this tool than school-aged children and adult individuals with HCP. In short, this improvement was temporary rather than long-term, which was consistent with previous study. For example, a single session of anodal tDCS over the primary motor cortex of the hemisphere ipsilateral to the brain lesion led to momentary motor improvements in both upper limbs of the children with spastic hemiparetic CP in a study (Moura et al., 2017). Another study also indicated that a single anodal tDCS temporarily improved hand dexterity skills for patients in the subacute phase of stroke (Fusco et al., 2014). Contrast to the hemiplegic hand, the dexterity of non-hemiplegic one was not weaken by stimulation, which was in line with a previous study (Inguaggiato et al., 2019). Although there

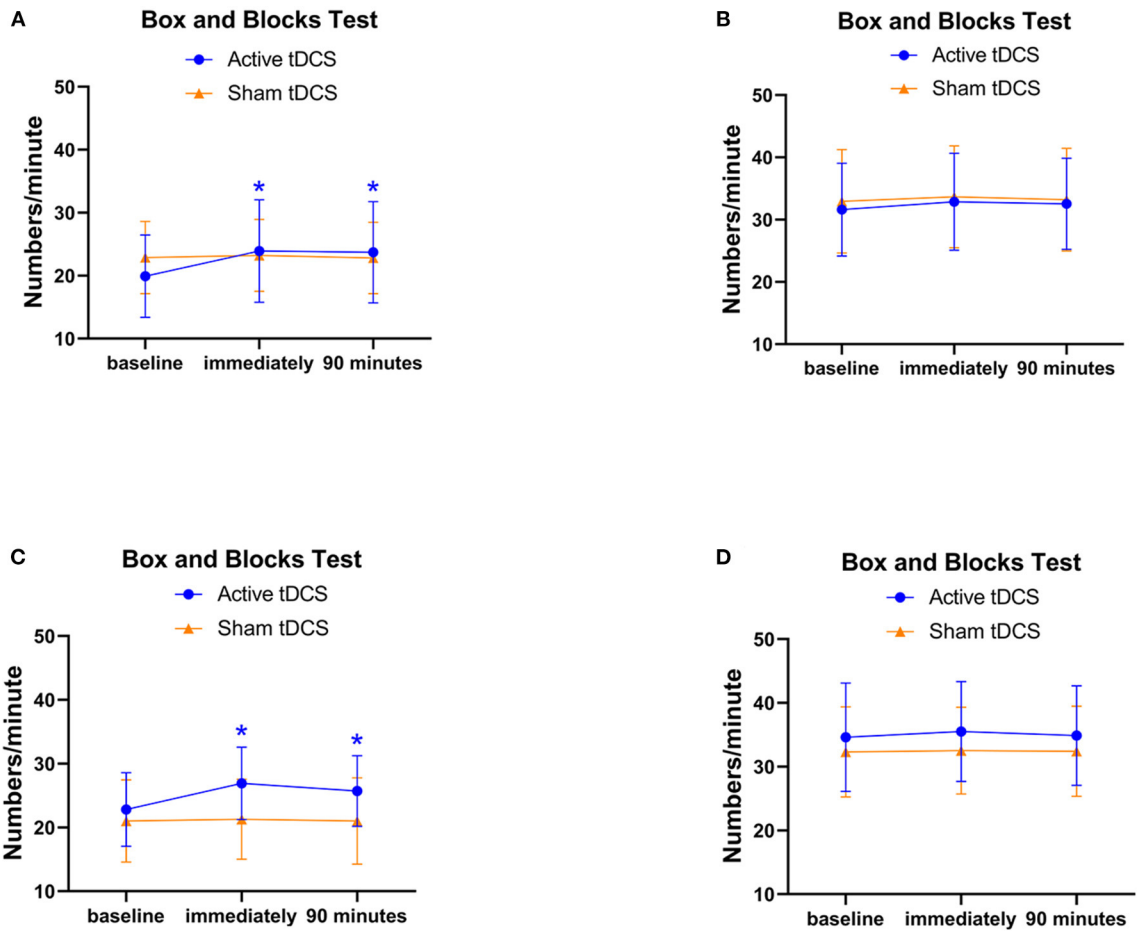


FIGURE 3
(A) The Box and Block Test of the affected hand in Day 1; **(B)** The Box and Block Test of the unaffected hand in Day 1; **(C)** The Box and Block Test of the affected hand in Day 2; **(D)** The Box and Block Test of the unaffected hand in Day 2; *significant difference compared to baseline ($P < 0.05$).

was no significant difference, we noticed that some participants got higher scores at the Box and Block Test of the non-hemiplegic hand after active tDCS rather than sham tDCS. Because of the interhemispheric competition and inhibition, the loss of inhibition over the unaffected hemisphere from the affected hemisphere caused the increased excitability of the unaffected hemisphere for individuals with HCP. Based on that anodal tDCS might improve hand dexterity by upregulating the excitability of the lesioned motor cortex, the different pattern of interhemispheric competition, and inhibition might contribute to the outcome of non-paretic hand. According to previous study, greater hemisphere excitation was associated with greater gains in motor function (Cunningham et al., 2015).

With regard to Melbourne Assessment 2, Selective Control of the Upper Extremity Scale, Modified Ashworth Scale, no positive effect emerged after a single anodal tDCS, which might be due to the following reasons. For one thing, the Box and Block Test was to test the hand dexterity featuring advantages of simplicity of operator, high reliability, and responsiveness (Araneda et al., 2019). The Melbourne Assessment 2 tested the affected hand function and motor quality and the Selective Control of the Upper Extremity Scale tested selective motor control of the upper extremity but their responsiveness to determine whether it could assess therapy-induced improvements remains to be determined (Elvrum et al., 2016; Lieber et al., 2021). The Modified Ashworth Scale mainly tested the muscle tone. The Box and Block Test was sensitive to changes produced by a single anodal tDCS in hands due to its high reliability and responsiveness. Conversely, the Melbourne Assessment 2 and the Selective Control of the Upper Extremity Scale might be less sensitive. Secondly, for the Modified Ashworth Scale, a single anodal tDCS might produce no effect on muscle tone, which was consistent with previous study (Comino-Suárez et al., 2021).

Limitations of the present study were that the washout period of 24 h was not long, which might lead to significant

TABLE 3 Comparison of Box and Block Test between the two treatment groups.

Assessments	Intervention point	Active tDCS ($n = 30$)	Sham tDCS ($n = 30$)	*P*-value
Box and Block Test (affected hand)	T0	20.93 (5.70)	21.45 (5.57)	0.095
	T1	24.66 (5.73)	21.76 (5.49)	<0.001
	T2	24.07 (5.93)	21.34 (5.52)	<0.001
Box and Block Test (unaffected hand)	T0	33.10 (8.01)	32.63 (7.60)	0.098*
	T1	34.20 (7.79)	33.10 (7.41)	
	T2	33.70 (7.53)	32.80 (7.57)	

P-value represents between-group differences. Data shown are means (SD). tDCS, transcrainal direct current stimulation; T0, baseline; T1, immediately after the end of two tDCS sessions; T2, 90 min after the two tDCS sessions. *Interaction "tDCS × time" showed no significant difference in this index.

difference on $T0_{Day1}$ and $T0_{Day2}$ for the group who received active tDCS first. Also, this study was not double blind. It was indicated that the wash-out time should be longer in future similar study. At the same time, relevant clinical information on brain lesions and injuries for the part of participants was incomplete. Lastly, according to the available MRI information, there were variety of findings related to white matter injuries or malformations, but the anodal tDCS only applied to presumed M1, which might neglect the relationship of lesions/injuries and stimulation area.

Studies with larger samples about the optimal dose, duration, and treatment cycle of tDCS for preschool children with HCP are warranted. On the other hand, there are some views that the timing, severity of brain lesion and the individual corticospinal tracts projections in HCP might exert influence on tDCS efficacy (Gillick et al., 2018). Further researches are needed to focus on these points, thus providing more help for applying tDCS into neurodevelopmental rehabilitation in pediatric population.

Conclusion

A single application of anodal tDCS (1.5 mA, 20 min) over M1 safely and tolerably improved the affected hand dexterity for preschool children with HCP.

Data availability statement

The original contributions presented in the study were included in the article/supplementary material, further inquiries can be directed to the corresponding author/s.

Ethics statement

The studies involving human participants were reviewed and approved by Guangzhou Women and Children's Medical Center Research Ethics Committee. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

Author contributions

KX conceived this work, contributed to study design, project management, and fund procurement. WH and YH wrote this manuscript and performed data collection and analysis. LH and LL generated the figures and tables. PZ contributed to guidance on English writing. HZ, ZC, YX, JZ, and WW carried out literature search. HQ and XW contributed to participant recruitment. KX and HT revised the manuscript. All authors have read and approved the content of the manuscript.

Funding

This work was supported by the National Natural Science Foundation of China (81902309, 81672253), the Natural Science Foundation of Guangdong Province (2019A1515010420, 2021A1515011303, 2021A1515012543), the Basic and Applied Basic Foundation of Guangdong Province (2020A1515110435), and funds from the Guangzhou Municipal Science and Technology Project (202102010205, 202102020581) and the Featured Clinical Technique of Guangzhou (2019TS55). The funders played no role in the design, conduct, or reporting of this study.

Acknowledgments

The authors gratefully acknowledge the study participants for their generosity and the assessors for professional measurements. The authors also thank Dr. Jinhua Lu who helped with statistical analysis.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

Antal, A., Alekseichuk, I., Bikson, M., Brockmöller, J., Brunoni, A. R., Chen, R., et al. (2017). Low intensity transcranial electric stimulation: safety, ethical, legal regulatory and application guidelines. *Clin. Neurophysiol.* 128, 1774–1809. doi: 10.1016/j.clinph.2017.06.001

Araneda, R., Ebner-Karestinos, D., Paradis, J., Saussez, G., Friel, K. M., Gordon, A. M., et al. (2019). Reliability and responsiveness of the Jebsen-Taylor Test of Hand Function and the Box and Block Test for children with cerebral palsy. *Dev. Med. Child Neurol.* 61, 1182–1188. doi: 10.1111/dmcn.14184

Auvichayapat, P., Aree-Uea, B., Auvichayapat, N., Phuttharak, W., Janyacharoen, T., Tunkamnerdthai, O., et al. (2017). Transient changes in brain metabolites after transcranial direct current stimulation in spastic cerebral palsy: a pilot study. *Front. Neurol.* 8:366. doi: 10.3389/fneur.2017.00366

Brunoni, A. R., Amadera, J., Berbel, B., Volz, M. S., Rizzerio, B. G., and Fregni, F. (2011). A systematic review on reporting and assessment of adverse effects associated with transcranial direct current stimulation. *Int. J. Neuropsychopharmacol.* 14, 1133–1145. doi: 10.1017/s1461145710001690

Brunoni, A. R., Nitsche, M. A., Bolognini, N., Bikson, M., Wagner, T., Merabet, L., et al. (2012). Clinical research with transcranial direct current stimulation (tDCS): challenges and future directions. *Brain Stimul.* 5, 175–195. doi: 10.1016/j.brs.2011.03.002

Ciechanski, P., and Kirton, A. (2017). Transcranial direct-current stimulation can enhance motor learning in children. *Cereb. Cortex* 27, 2758–2767. doi: 10.1093/cercor/bhw114

Cieza, A., Fayed, N., Bickenbach, J., and Prodinger, B. (2019). Refinements of the ICF linking rules to strengthen their potential for establishing comparability of health information. *Disabil. Rehabil.* 41, 574–583. doi: 10.3109/09638288.2016.1145258

Collange Grecco, L. A., de Almeida Carvalho Duarte, N., Mendonça, M. E., Galli, M., Fregni, F., and Oliveira, C. S. (2015). Effects of anodal transcranial direct current stimulation combined with virtual reality for improving gait in children with spastic diparetic cerebral palsy: a pilot, randomized, controlled, double-blind, clinical trial. *Clin. Rehabil.* 29, 1212–1223. doi: 10.1177/0269215514566997

Colver, A., Fairhurst, C., and Pharoah, P. O. D. (2014). Cerebral palsy. *Lancet* 383, 1240–1249. doi: 10.1016/S0140-6736(13)61835-8

Comino-Suárez, N., Moreno, J. C., Gómez-Soriano, J., Megía-García, Á., Serrano-Muñoz, D., Taylor, J., et al. (2021). Transcranial direct current stimulation combined with robotic therapy for upper and lower limb function after stroke: a systematic review and meta-analysis of randomized control trials. *J. Neuroeng. Rehabil.* 18:148. doi: 10.1186/s12984-021-00941-0

Cunningham, D. A., Varnerin, N., Machado, A., Bonnett, C., Janini, D., Roelle, S., et al. (2015). Stimulation targeting higher motor areas in stroke rehabilitation: a proof-of-concept, randomized, double-blinded placebo-controlled study of effectiveness and underlying mechanisms. *Restor. Neurol. Neurosci.* 33, 911–926. doi: 10.3233/rnn-150574

DaSilva, A. F., Volz, M. S., Bikson, M., and Fregni, F. (2011). Electrode positioning and montage in transcranial direct current stimulation. *J. Vis. Exp.* 2011:2744. doi: 10.3791/2744

Duarte Nde, A., Grecco, L. A., Galli, M., Fregni, F., and Oliveira, C. S. (2014). Effect of transcranial direct-current stimulation combined with treadmill training on balance and functional performance in children with cerebral palsy: a double-blind randomized controlled trial. *PLoS ONE* 9:e105777. doi: 10.1371/journal.pone.0105777

Eliasson, A. C., Ullenhag, A., Wahlström, U., and Krumlinde-Sundholm, L. (2017). Mini-MACS: development of the Manual Ability Classification System for children younger than 4 years of age with signs of cerebral palsy. *Dev. Med. Child Neurol.* 59, 72–78. doi: 10.1111/dmcn.13162

Elvrum, A.-K. G., S'Ther, R., Riphagen, I. I., and Vik, T. (2016). Outcome measures evaluating hand function in children with bilateral cerebral palsy: a systematic review. *Dev. Med. Child Neurol.* 58, 662–671. doi: 10.1111/dmcn.13119

Fleming, M. K., Theologis, T., Buckingham, R., and Johansen-Berg, H. (2018). Transcranial direct current stimulation for promoting motor function in cerebral palsy: a review. *J. Neuroeng. Rehabil.* 15:121. doi: 10.1186/s12984-018-0476-6

Fusco, A., Iosa, M., Venturiero, V., De Angelis, D., Morone, G., Maglione, L., et al. (2014). After vs. priming effects of anodal transcranial direct current stimulation on upper extremity motor recovery in patients with subacute stroke. *Restor. Neurol. Neurosci.* 32, 301–312. doi: 10.3233/rnn-130349

Gillick, B., Rich, T., Nemanich, S., Chen, C. Y., Menk, J., Mueller, B., et al. (2018). Transcranial direct current stimulation and constraint-induced therapy in cerebral palsy: a randomized, blinded, sham-controlled clinical trial. *Eur. J. Paediatr. Neurol.* 22, 358–368. doi: 10.1016/j.ejpn.2018.02.001

Gordon, A. M., Hung, Y.-C., Brandao, M., Ferre, C. L., Kuo, H.-C., Friel, K., et al. (2011). Bimanual training and constraint-induced movement therapy in children with hemiplegic cerebral palsy: a randomized trial. *Neurorehabil. Neural Repair.* 25, 692–702. doi: 10.1177/1545968311402508

Grecco, L. A., Oliveira, C. S., Duarte, N. A., Lima, V. L., Zanon, N., and Fregni, F. (2017). Cerebellar transcranial direct current stimulation in children with ataxic cerebral palsy: a sham-controlled, crossover, pilot study. *Dev. Neurorehabil.* 20, 142–148. doi: 10.3109/17518423.2016.1139639

Inguaggiato, E., Bolognini, N., Fiori, S., and Cioni, G. (2019). Transcranial Direct Current Stimulation (tDCS) in unilateral cerebral palsy: a pilot study of motor effect. *Neural Plast.* 2019:2184398. doi: 10.1155/2019/2184398

Jongbloed-Pereboom, M., Nijhuis-van der Sanden, M. W. G., and Steenbergen, B. (2013). Norm scores of the box and block test for children ages 3–10 years. *Am. J. Occup. Ther.* 67, 312–318. doi: 10.5014/ajot.2013.006643

Kang, N., Summers, J. J., and Cauraugh, J. H. (2016). Transcranial direct current stimulation facilitates motor learning post-stroke: a systematic review and meta-analysis. *J. Neurol. Neurosurg. Psychiatry* 87, 345–355. doi: 10.1136/jnnp-2015-311242

Kirton, A., Ciechanski, P., Zewdie, E., Andersen, J., Nettel-Aguirre, A., Carlson, H., et al. (2017). Transcranial direct current stimulation for children with perinatal stroke and hemiparesis. *Neurology* 88, 259–267. doi: 10.1212/wnl.0000000000003518

Krishnan, C., Santos, L., Peterson, M. D., and Ehinger, M. (2015). Safety of noninvasive brain stimulation in children and adolescents. *Brain Stimul.* 8, 76–87. doi: 10.1016/j.brs.2014.10.012

Lefaucheur, J. P., Antal, A., Ayache, S. S., Benninger, D. H., Brunelin, J., Cogiamanian, F., et al. (2017). Evidence-based guidelines on the therapeutic use of transcranial direct current stimulation (tDCS). *Clin. Neurophysiol.* 128, 56–92. doi: 10.1016/j.clinph.2016.10.087

Li, N., Zhou, P., Tang, H., He, L., Fang, X., Zhao, J., et al. (2022). In-depth analysis reveals complex molecular aetiology in a cohort of idiopathic cerebral palsy. *Brain* 145, 119–141. doi: 10.1093/brain/awab209

Lieber, J., Gartmann, T., Keller, J. W., and van Hedel, H. J. A. (2021). Validity and reliability of the Selective Control of the Upper Extremity Scale in children with upper motor neuron lesions. *Disabil. Rehabil.* 44, 3694–3700. doi: 10.1080/09638288.2021.1881171

Madden, R. H., and Bundy, A. (2019). The ICF has made a difference to functioning and disability measurement and statistics. *Disabil. Rehabil.* 41, 1450–1462. doi: 10.1080/09638288.2018.1431812

Marquez, J., Conley, A., Karayanidis, F., Lagopoulos, J., and Parsons, M. (2015). Anodal direct current stimulation in the healthy aged: effects determined by the hemisphere stimulated. *Restor. Neurol. Neurosci.* 33, 509–519. doi: 10.3233/rnn-140490

Meseguer-Henarejos, A.-B., Sanchez-Meca, J., Lopez-Pina, J.-A., and Carles-Hernandez, R. (2018). Inter- and intra-rater reliability of the Modified Ashworth Scale: a systematic review and meta-analysis. *Eur. J. Phys. Rehabil. Med.* 54, 576–590. doi: 10.23736/s1973-9087.17.04796-7

Moura, R. C. F., Santos, C., Collange Grecco, L., Albertini, G., Cimolin, V., Galli, M., et al. (2017). Effects of a single session of transcranial direct current stimulation on upper limb movements in children with cerebral palsy: a randomized, sham-controlled study. *Dev. Neurorehabil.* 20, 368–375. doi: 10.1080/17518423.2017.1282050

Mutlu, A., Livanelioglu, A., and Gunel, M. K. (2008). Reliability of ashworth and modified ashworth scales in children with spastic cerebral palsy. *BMC Musculoskelet. Disord.* 9:44. doi: 10.1186/1471-2474-9-44

Nejati, V., Salehinejad, M. A., Nitsche, M. A., Najian, A., and Javadi, A. H. (2020). Transcranial direct current stimulation improves executive dysfunctions in ADHD: implications for inhibitory control, interference control, working memory, and cognitive flexibility. *J. Atten. Disord.* 24, 1928–1943. doi: 10.1177/1087054717730611

Nemanich, S. T., Rich, T. L., Chen, C. Y., Menk, J., Rudser, K., Chen, M., et al. (2019). Influence of combined transcranial direct current stimulation and motor training on corticospinal excitability in children with unilateral cerebral palsy. *Front. Hum. Neurosci.* 13:137. doi: 10.3389/fnhum.2019.00137

Novak, I., Morgan, C., Fahey, M., Finch-Edmondson, M., Galea, C., Hines, A., et al. (2020). State of the evidence traffic lights 2019: systematic review of interventions for preventing and treating children with cerebral palsy. *Curr. Neurol. Neurosci. Rep.* 20, 3. doi: 10.1007/s11910-020-1022-z

O'Leary, G. H., Jenkins, D. D., Coker-Bolt, P., George, M. S., Kautz, S., Bikson, M., et al. (2021). From adults to pediatrics: a review noninvasive brain stimulation (NIBS) to facilitate recovery from brain injury. *Prog. Brain Res.* 264, 287–322. doi: 10.1016/bs.pbr.2021.01.019

Osorio, A. A. C., and Brunoni, A. R. (2019). Transcranial direct current stimulation in children with autism spectrum disorder: a systematic scoping review. *Dev. Med. Child Neurol.* 61, 298–304. doi: 10.1111/dmcn.14104

Park, J. E., Hallett, M., Jang, H. R., Kim, L. U., Park, K. J., Kim, S. K., et al. (2022). Effects of anodal stimulation and motor practice on limb-kinetic apraxia in Parkinson's disease. *Exp. Brain Res.* 240, 1249–1256. doi: 10.1007/s00221-021-06293-4

Paulson, A., and Vargus-Adams, J. (2017). Overview of four functional classification systems commonly used in cerebral palsy. *Children (Basel)* 4:30. doi: 10.3390/children4040030

Platz, T., Pinkowski, C., van Wijck, F., Kim, I. H., di Bella, P., and Johnson, G. (2005). Reliability and validity of arm function assessment with standardized guidelines for the Fugl-Meyer Test, Action Research Arm Test and Box and Block Test: a multicentre study. *Clin. Rehabil.* 19, 404–411. doi: 10.1191/0269215505cr832oa

Randall, M., Imms, C., and Carey, L. (2012). Further evidence of validity of the Modified Melbourne Assessment for neurologically impaired children aged 2 to 4 years. *Dev. Med. Child Neurol.* 54, 424–428. doi: 10.1111/j.1469-8749.2012.04252.x

Randall, M., Imms, C., Carey, L. M., and Pallant, J. F. (2014). Rasch analysis of The Melbourne assessment of unilateral upper limb function. *Dev. Med. Child Neurol.* 56, 665–672. doi: 10.1111/dmcn.12391

Reckow, J., Rahman-Filipiak, A., Garcia, S., Schlaefflin, S., Calhoun, O., DaSilva, A. F., et al. (2018). Tolerability and blinding of 4x1 high-definition transcranial direct current stimulation (HD-tDCS) at two and three milliamps. *Brain Stimul.* 11, 991–997. doi: 10.1016/j.brs.2018.04.022

Rosenbaum, P., Paneth, N., Leviton, A., Goldstein, M., Bax, M., Damiano, D., et al. (2007). A report: the definition and classification of cerebral palsy April 2006. *Dev. Med. Child Neurol. Suppl.* 109, 8–14.

Säisänen, L., Julkunen, P., Lakka, T., Lindi, V., Könönen, M., and Määttä, S. (2018). Development of corticospinal motor excitability and cortical silent period from mid-childhood to adulthood - a navigated TMS study. *Neurophysiol. Clin.* 48, 65–75. doi: 10.1016/j.neucli.2017.11.004

Saldanha, J. S., Zortea, M., Deliberali, C. B., Nitsche, M. A., Kuo, M. F., Torres, I., et al. (2020). Impact of age on tDCS effects on pain threshold and working memory: results of a proof of concept cross-over randomized controlled study. *Front. Aging Neurosci.* 12:189. doi: 10.3389/fnagi.2020.00189

Shin, Y. I., Foerster, Á., and Nitsche, M. A. (2015). Transcranial direct current stimulation (tDCS) - application in neuropsychology. *Neuropsychologia* 69, 154–175. doi: 10.1016/j.neuropsychologia.2015.02.002

Wagner, L. V., Davids, J. R., and Hardin, J. W. (2016). Selective control of the upper extremity scale: validation of a clinical assessment tool for children with hemiplegic cerebral palsy. *Dev. Med. Child Neurol.* 58, 612–617. doi: 10.1111/dmcn.12949

Wu, Q., Peng, T., Liu, L., Zeng, P., Xu, Y., Yang, X., et al. (2022). The effect of constraint-induced movement therapy combined with repetitive transcranial magnetic stimulation on hand function in preschool children with unilateral cerebral palsy: a randomized controlled preliminary study. *Front. Behav. Neurosci.* 16: 876567. doi: 10.3389/fnbeh.2022.876567

Yildiz, A., Yildiz, R., Celik, H. I., Manzak, O. F., and Elbasan, B. (2020). Construct and discriminative validity and reliability of the Selective Control of the Upper Extremity Scale (SCUES) in children with unilateral cerebral palsy. *Physiother. Theory Pract.* 38, 919–927. doi: 10.1080/09593985.2020.1805832

Zelnik, N., Lahat, E., Heyman, E., Livne, A., Schertz, M., Sagie, L., et al. (2016). The role of prematurity in patients with hemiplegic cerebral palsy. *J. Child Neurol.* 31, 678–682. doi: 10.1177/0883073815610430

frontiers | Frontiers in Behavioral Neuroscience

TYPE Methods
PUBLISHED 29 November 2022
DOI 10.3389/fnbeh.2022.948184

OPEN ACCESS

EDITED BY
Jiaojian Wang,
Kunming University of Science and Technology, China

REVIEWED BY
Dennis Dixon,
Center for Autism and Related Disorders, United States
Muhammad Naveed,
University of Minnesota Twin Cities, United States

*CORRESPONDENCE
Alberto Romano
alberto.romano01@ateneopv.it

SPECIALTY SECTION
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

RECEIVED 19 May 2022
ACCEPTED 10 November 2022
PUBLISHED 29 November 2022

CITATION
Zwilling M, Romano A, Hoffman H, Lotan M and Tesler R (2022) Development and validation of a system for the prediction of challenging behaviors of people with autism spectrum disorder based on a smart wearable shirt: A mixed-methods design. *Front. Behav. Neurosci.* 16:948184. doi: 10.3389/fnbeh.2022.948184

Development and validation of a system for the prediction of challenging behaviors of people with autism spectrum disorder based on a smart wearable shirt: A mixed-methods design

Moti Zwilling[1], Alberto Romano[2]*, Hay Hoffman[3], Meir Lotan[4] and Riki Tesler[2]

[1]Department of Economics and Business Administration, Ariel University, Ariel, Israel, [2]Department of Health System Management, Ariel University, Ariel, Israel, [3]Department of Computer Science, Ariel University, Ariel, Israel, [4]Department of Physical Therapy, School of Health Sciences, Ariel University, Ariel, Israel

Background: Most people with autism spectrum disorder (ASD) present at least one form of challenging behavior (CB), causing reduced life quality, social interactions, and community-based service inclusion.

Objectives: The current study had two objectives: (1) to assess the differences in physiological reaction to stressful stimuli between adults with and without high-functioning ASD; (2) to develop a system able to predict the incoming occurrence of a challenging behaviors (CBs) in real time and inform the caregiver that a CB is about to occur; (3) to evaluate the acceptability and usefulness of the developed system for users with ASD and their caregivers.

Methods: Comparison between physiological parameters will be conducted by enrolling two groups of 20 participants with and without ASD monitored while watching a relaxing and disturbing video. To understand the variations of the parameters that occur before the CB takes place, 10 participants with ASD who have aggressive or disruptive CBs will be monitored for 7 days. Then, an ML algorithm capable of predicting immediate CB occurrence based on physiological parameter variations is about to be developed. After developing the application-based algorithm, an efficient proof of concept (POC) will be carried out on one participant with ASD and CB. A focus group, including health professionals, will test the POC to identify the strengths and weaknesses of the developed system.

Results: Higher stress level is anticipated in the group of people with ASD looking at the disturbing video than in the typically developed peers. From the

obtained data, the developed algorithm is used to predict CBs that are about to occur in the upcoming 1 min. A high level of satisfaction with the proposed technology and useful consideration for further developments are expected to emerge from the focus group.

Clinical trial registration: [https://clinicaltrials.gov/], identifier [NCT05340608].

KEYWORDS

autism spectrum disorder, adult, problem behavior, wearable electronic devices, accident prevention, recurrent neural network

Introduction

Autism spectrum disorder (ASD) refers to a heterogeneous neurodevelopmental condition with symptoms that range from mild to severe. ASD is generally detected in childhood and is lifelong. It affects between 1 and 2% of the population (Baron-Cohen et al., 2009) and is characterized by social communication deficits, repetitive and unusual sensory-motor behaviors, and restricted and specific interests (American Psychiatric Association, 2013). The literature indicates that about 55% of the people diagnosed with ASD also exhibit intellectual disabilities (Knapp et al., 2009), and about 25% are non or minimally verbal (Arnold and Reed, 2016).

Challenging behaviors (CBs) (Murphy et al., 2005; Prizant and Wetherby, 2005; Reese et al., 2005; Baker et al., 2008; Chiang, 2008; O'Donnell et al., 2012) refer to a broad range of unusual behaviors expressed by individuals with ASD. Such behaviors might include aggression, destructiveness, self-injurious, and a range of other behaviors, such as unacceptable social and sexual conduct (Emerson, 2001; Holden and Gitlesen, 2006). Most studies reported high rates of CBs among individuals with ASD, with a prevalence of up to 94% presenting at least one type of challenging behavior (CB) (Matson et al., 2008; Jang et al., 2011). Other studies have reported on the appearance of CBs among 82% of participants, with 32.5% involving aggressive behavior toward themselves or others (Woodbury-Smith et al., 2006; Murphy et al., 2009). CBs may significantly impair the physical and mental health and the quality of life of the persons presenting such behaviors, those who care for them, those who manage them (therapists; teachers), and even their neighbors (Nissen and Haveman, 1997; Blacher and McIntyre, 2006; Mukaddes and Topcu, 2006; Felce et al., 2011).

Applying therapeutic strategies in such cases is strongly warranted to prevent the CBs from becoming a part of an individual's behavioral repertoire. In the absence of therapeutic strategies, such behaviors are unlikely to decrease and will typically remain or worsen without intervention (Berg et al., 2000). An effective intervention to reduce the outburst of CBs and one that could possibly lessen the severity of a CB could enhance the involvement of the person with ASD within society and reduce the financial and emotional burden of the family and caregivers while simultaneously decreasing the need for medications (Rose et al., 2004).

Several forms of intervention have been proposed for reducing CB in people with ASD, including medications (Malone et al., 2005; Blankenship et al., 2010; McPheeters et al., 2011; Sawyer et al., 2014), behavioral interventions (Scotti et al., 1996; Didden et al., 2006; Machalicek et al., 2007, 2016; Lydon et al., 2013; Fettig and Barton, 2014; Erturk et al., 2018; MacNaul and Neely, 2018; Weston et al., 2018; Inoue, 2019), cognitive/emotion-oriented interventions (Neal and Barton Wright, 2003; Zetteler, 2008; O'Neil et al., 2011; Cotelli et al., 2012; Subramaniam and Woods, 2012; Doyle et al., 2013), sensory stimulation/integration interventions (Lang et al., 2012; Barton et al., 2015; Case-Smith et al., 2015; Leong et al., 2015; Wan Yunus et al., 2015; Watling and Hauer, 2015), music therapy (Gold et al., 2006; Stephenson, 2006; Simpson and Keen, 2011; James et al., 2015; Fakhoury et al., 2017), psychosocial interventions (Seida et al., 2009; Reichow et al., 2013; Vanderkerken et al., 2013; Bishop-Fitzpatrick et al., 2014; Lim, 2019), communication training (Mirenda, 1997; Goldstein, 2002; Lequia et al., 2012; Walker and Snell, 2013; Gerow et al., 2018; Gregori et al., 2020), physical exercises (Eggermont and Scherder, 2006; Ogg-Groenendaal et al., 2014; Sorensen and Zarrett, 2014; Forbes et al., 2015; Bremer et al., 2016), and others (McDonnell et al., 2008; Tanner et al., 2015; Lindgren et al., 2016; Ferguson et al., 2019; Walker et al., 2021; Wahman et al., 2022). Despite the wide availability of intervention forms, no consensus has been reached concerning the global efficacy of any CB treatment in treating all the CBs types. As both desirable and undesirable behaviors are learned and maintained through interaction with the social and physical environment, the behavior-environment interaction can be described as positive or negative behavior contingencies. Experts agree in affirming that a better understanding of behavior-environment relations may lead to more effective interventions (Lloyd and Kennedy, 2014). Such knowledge can be obtained by analyzing the function of the behavior.

Functional behavior assessment (FBA) enables hypotheses about the relations among specific types of environmental events and behaviors. The idea behind FBA is that if these reinforcement contingencies can be identified, then interventions can be designed to decrease problem behavior and increase adaptive behavior by altering these contingencies (Cooper et al., 2020). Reinforcement contingencies maintaining CBs include positive and negative reinforcement. Positive reinforcements comprise social positive reinforcements (attention), tangible reinforcements (items or activities), and automatic positive reinforcements (engaging in the behavior itself, independently from the social environment). Negative reinforcements include social negative reinforcement (escape from socially mediated stimuli), and automatic negative reinforcement (escape from non-socially mediated stimuli such as pain) (Lloyd and Kennedy, 2014; Cooper et al., 2020). Evidence from the literature suggests that interventions based on functional assessment outcomes are more effective than those that are not function-based. Most interventions for CBs aim to prevent the occurrence of CBs themselves by guiding the person toward more adaptive behaviors while avoiding managing the consequences of CBs.

In line with the need for effective prevention strategies, the literature has been recently enriched with the proposal of using technological devices to predict CB occurrence based on the physiological parameters of the individual with ASD. The presence of atypical physiological arousal in people with ASD has been known for a long time, and the functional relation between homeostatic regulation and CB has already been hypothesized (Hutt and Hutt, 1965; Ornitz and Ritvo, 1968; Kinsbourne, 1980). More recently, atypical autonomic reactivity was reported as a common feature in people with ASD (Cohen et al., 2011; Levine et al., 2014; Klusek et al., 2015; Lydon et al., 2016). The use of physiological-biological signals such as the electrocardiogram, heart rate (HR), HR variability, respiratory rate (as well as changes in respiratory rate), and body movements are reiterated in several articles as markers for CB expressed in people with ASD (Goodwin et al., 2018, 2019; Ozdenizci et al., 2018; Taj-Eldin et al., 2018; Nuske et al., 2019).

Smart wearable shirts (SWS) are wearable medical devices that are considered to be a technological breakthrough, enabling continuous surveillance of human vital physiological signs without any disturbance to the activities of daily living. The SWS technology has been used in clinical research for the last two decades. In the previous few years, SWS have enabled the collection of varied physiological data outside the laboratory for a prolong time such as weeks at a time. The constant surveillance enabled by these devices allows for identifying physiological anomalies that deviate from the typical individual's behaviors that can be received, analyzed, and treated (Banaee et al., 2013). Furthermore, garments, such as t-shirts, have been found to be a highly preferred device to be used by individuals with ASD (Koo et al., 2018), with a moderate to high suitability index for this population (Taj-Eldin et al., 2018). Moreover, few studies have used SWS among individuals with ASD (Taj-Eldin et al., 2018; Black et al., 2020).

The analysis of physiological data can be achieved by machine learning (ML). ML is a method that provides automated approaches for data analysis (Murphy, 2012). It utilizes machine-constructed algorithms that detect specific patterns in the data through a training process (Gulshan et al., 2016). The use of ML approaches to predict CBs occurrence has increased in recent years (Francese and Yang, 2021). Masino et al. (2019) evaluated the accuracy of support vector machine (SVM) and logistic regression (LR) classifiers in differentiating physiological states associated with stressful and non-stressful scenarios in children with ASD in a controlled laboratory setting using wearables data. The authors reported on higher accuracy of the SVM classifier and suggested that ML models combined with wearables data may support real-time intervention in the population with ASD. Imbiriba et al. (2020) reported similar results when using an SVM combined with a principal component analysis (PCA) model to predict aggression in youth with ASD. The authors stated the adequacy of the model to predict aggression 3 min before their appearance. Moreover, higher prediction performance was reported for the SVM + PCA model than for the LR model. Consistently, Cantin-Garside et al. (2021) reported higher accuracy of SVM and k-nearest neighbor (kNN) algorithm in classifying self-injurious behavior in children with ASD compared to other methods [discriminant analysis (DA), decision trees (DT), Naïve Bayes (NB), and neural networks (NN)]. Furthermore, Zheng et al. (2021) proposed a multimodal data analysis to predict precursors of CBs of children with ASD through various ML algorithms. Their multimodal data capture platform is composed of wearable bio (peripheral physiological signals) and gesture (acceleration signals) sensors combined with Kinect cameras (facial expressions and head rotations). The study results pointed on a higher prediction accuracy for random forest (RF) and NN algorithms compared to SVM, DA, kNN, DT, and NB algorithms when looking for precursors of CBs. Although referred to the pediatric population only, these preliminary insights support using ML algorithms and wearable devices to predict CBs in people with ASD.

The current protocol consists of three phases, each with a specific goal. The first aim is to assess the differences in the measured physiological reaction between adults with high-functioning ASD and their typically developed peers utilizing SWS. The second goal is to create an *ad hoc* ML algorithm that will be utilized for real-time CB prediction and combined with a smartphone application that sends an alert when the CB is likely to occur. Finally, we aimed to test the developed system among people with ASD and assess its acceptability and usefulness for users with ASD and their caregivers.

Materials and methods

Study design

An observational study design will be implemented in the first two phases of the current research. In phase one, participants' (with and without ASD) physiological reactions to two visual stimuli (pleasant vs. disturbing) will be collected and analyzed. The physiological characteristics of the CBs presented by people with ASD will be collected in phase two, coupled with behavioral diaries filled out by the care providers. Finally, a single case study with a mixed-method design will be implemented in phase three, where the system validity proof of concept (POC) will be performed.

Ethics and safety issues

The research proposal was approved by the Ariel University Institutional Review Board (AU-HEA-ML-20201203), Asaf Harofe Institutional Review Board (0136-21-ASF), and the Israeli Ministry of Health (MOH_2022-01-25_010570). The implementation of the protocol was also approved by the head scientist from the Israeli Ministry of Social Affairs and Welfare. The trial protocol was registered in the World Health Organization Trial Registry (ClinicalTrials.gov ID: NCT05340608). Written consent was also given by the head of the residential centers hosting the second section of the proposed study. The study will be carried out following the Declaration of Helsinki principles. Written informed consent will be collected from all participants or their legal guardians at the recruitment stage. The SWSs planned to be used are non-invasive medical devices with sensors that collect physical signals from the participants. However, if a participant refuses to wear the SWS, he or she may withdraw from the study at any time without any repercussions.

Participants

According to the sample size calculation performed, a group of 20 subjects diagnosed with high-functioning ASD aged between 20 and 40 years residing at home [observation group (OG)], along with an age- and sex-matched control group (CG) of 20 typically developed peers, will be enrolled in the first protocol phase. In the second phase, 10 people with ASD presenting with intensive aggressive or disruptive CBs aged 20–40 years and their caregivers will be recruited. Finally, one participant with ASD aged 20–40 years exhibiting aggressive or disruptive CBs will participate in the third phase of the research as POC.

Outcome measures

Smart wearable shirt

The Hexoskin SWS (Hexoskin Inc., Montreal, QC, Canada) is a wearable device with several sensors to measure physiological signals. Its producer declares the SWS as a non-invasive SWS with textile-embedded sensors that allow the collection of multiple parameters. A detailed description of sensors equipped in the Hexoskin SWS is available on the producer's website.[1] The Hexoskin SWS will be used in all three phases of the research.

Behavioral diary

The care providers of participants enrolled in phase two will be asked to fill out a daily behavioral diary reporting the arousal level of each participant. Three arousal levels will be collected: quiet, agitated, and CB. The "quiet" state refers to a period in which the subject is relaxed or calmly going about his or her daily routine (e.g., resting on the sofa). Being "agitated" describes a behavioral activation state higher than "quiet". It can correspond to situations in which a physiological reaction is observed, such as redness, sweating, and increased respiratory rate, among others. It can occur in cases of euphoria (e.g., the subject is watching a show that he or she extremely enjoys); intense activity (e.g., doing a sport activity); or anger (e.g., the subject has been told that he or she cannot do an activity that he or she has requested and therefore vigorously protests), but cannot be defined as CB. "CB" state describes extreme agitation and an intense physiological response (redness, sweating, or increased respiratory rate). It can be accompanied by fierce anger (with or without aggressive or disruptive behaviors), strong states of anxiety, or a need to move intensely. In general, "CB" should correspond to reactions identified as exaggerated, excessive for the situation, or inadequate relative to the social context. Aggression behaviors will include self or other-directed physical or verbal aggression. In some cases, such behaviors may be uncontrolled by the participants. For each arousal level reported, caregivers will be asked to report on the following items: the beginning and ending time and date, arousal level, and operational definition of the accompanying behavior and activities. The behavioral diary will be collected within the second phase.

Quebec user evaluation of satisfaction with assistive technology

The Quebec User Evaluation of Satisfaction with Assistive Technology second edition (QUEST 2.0) (Demers et al., 2000) is a 12-item questionnaire designed to assess users' satisfaction with a wide range of assistive technology (Scherer, 2005). The 12

1 https://www.hexoskin.com

items are grouped into two areas representing user satisfaction with the assistive technologies related to the assistive device (eight items) and provided service (four items). A five-point Likert scale is given to each item, ranging from one ("not satisfied at all") to five ("very satisfied"). Strong psychometric proprieties have been published for the QUEST 2.0 (Demers et al., 2002). The QUEST 2.0 will be administered in phase three of the current protocol by participants' caregivers.

Focus group

A focus group is a qualitative data collection method often used in health research. The technique is used to produce a controlled discussion on specific issues within a group of people who share different experiences or relations with the focused topics (Kitzinger, 1994; Flores and Alonso, 1995). Under the focus group method, the group discussion is recorded, transcribed, and analyzed. In addition, a search for themes relevant to the investigated topic and the group agreement assessment is performed (Breen, 2006). Research questions that will be raised during the focus group include:

- Did wearing the SWS upset the participants?
- Was the system able to detect all relevant CBs?
- Was the system's operational speed sufficient to allow the in-time application of appropriate prevention strategies?
- Has the use of the system reduced the amount of CBs?
- What improvements can be applied to the system to increase its effectiveness?

Procedure

The research procedure is outlined in **Figure 1**. The protocol's expected start date is June 2022.

Phase one – Comparison of physiological outcomes between people with and without autism spectrum disorder

For the first phase, the physiological parameters of the people in the OG and CG will be acquired and recorded using the Hexoskin SWS while participants watch two different 5 min videos. One video will show relaxing images while emitting relaxing music (relaxing video). The second video will present human facial deformities accompanied by anxious music (disturbing video). Both videos will be presented to the participant when in a seated position. Before starting the relaxing video, the participant will be invited to relax and lean back onto the chair's backrest. The participant can close his eyes or keep them open at his or her discretion to promote relaxation. To watch the disturbing video, participants will be asked not to lean against the chair backrest and keep their eyes open for the duration of the video. The video viewed by each participant will be chosen randomly between the two videos. The entire session will be measured as lasting after approximately 20 min (including explaining the research protocol, putting on, and taking off the SWS).

Phase two – Classify the variations of the physiological parameters in people with autism spectrum disorder

Each participant enrolled in phase two will be asked to wear the Hexoskin SWS for seven consecutive days during waking hours while performing his or her usual daily activities. During the same 7 days, care providers will be asked to report the participants' status in the behavioral diary. Each evening the data collected by the Hexoskin SWS will be uploaded to an online cloud that is provided with the behavioral diary records of the day. Once the data from all the 10 participants

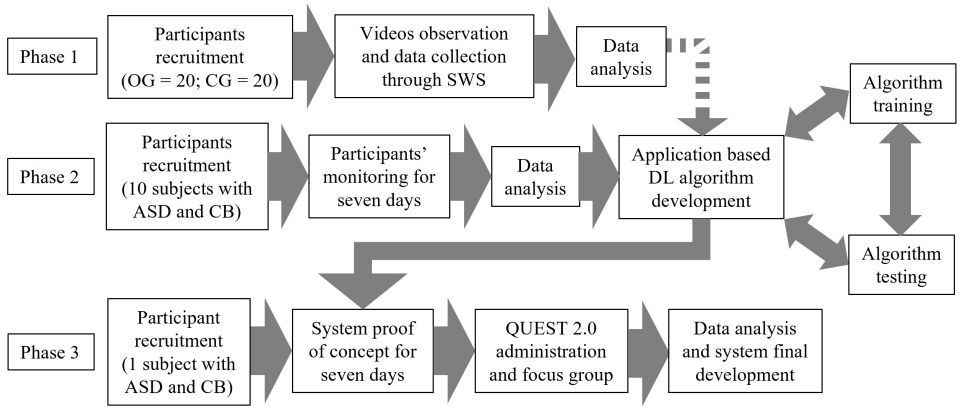


FIGURE 1
Protocol flowchart. The dotted arrow indicates that the integration of the data obtained from phase one will only occur if they bring added value to the development of the algorithm. OG, observation group; CG, control group; SWS, smart wearable shirt; ASD, autism spectrum disorder; DL, deep learning; CB, challenging behavior; QUEST 2.0, Quebec User Evaluation of Satisfaction with Assistive Technology 2nd edition.

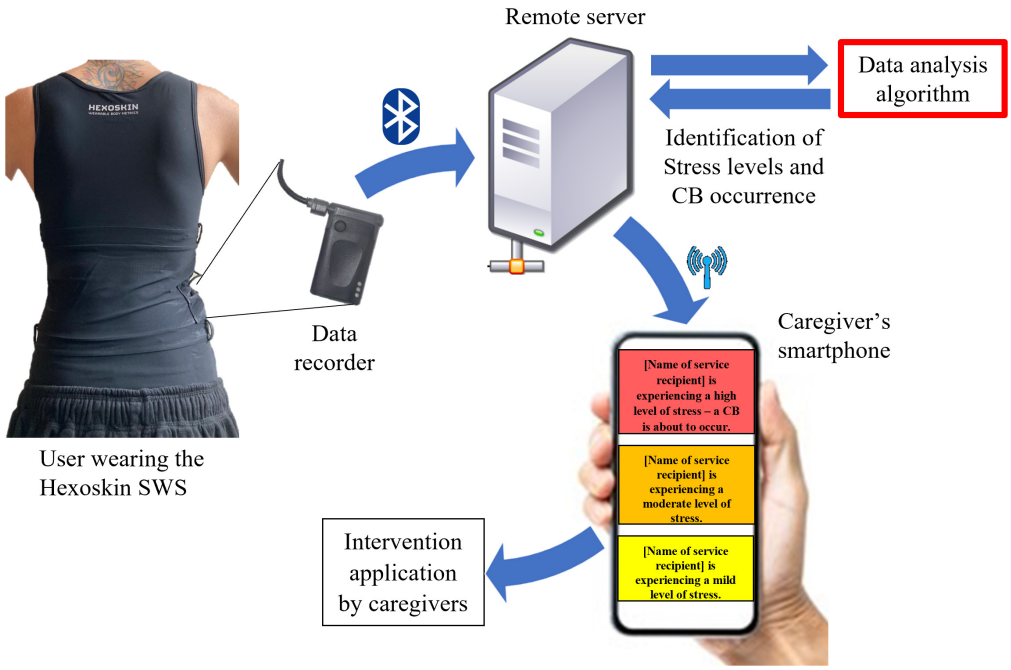


FIGURE 2
Brief system architecture description. Physiological signals are captured by the Hexoskin smart wearable shirt (SWS) and recorded by the provided data recorder. Recorded data are transferred in real time via Bluetooth technology to a remote server where they are analyzed. If the *ad hoc* developed algorithm detects the incoming data suggesting the occurrence of challenging behavior (CB), the remote server immediately sends a notification alert to the caregiver's smartphone.

have been collected, a deep learning long short-term memory algorithm (LSTM), which is perceived as a neural network algorithm composed of many layers that the neural network accumulates over time, will be developed in order to understand the variations in the individual' physiological parameters that occur before a CB and predict the eruption of future CBs. The SWS data will be sent in real-time via Bluetooth technology to a remote server, where it will be classified and analyzed through the developed algorithm, thus yielding a CB behavior alert. In case of "normal" behavior, the algorithm will not classify the existing behavior of the participant as CB. Based on the algorithm mentioned above, a smartphone application will be developed to receive the data. If the algorithm detects the possibility of an incoming CB, a notification will be sent to the care provider's smartphone to inform on the possible oncoming CB, thereby enabling the implementation of the selected intervention strategy. The system architecture is explained in **Figure 2**.

Phase three – System proof of concept

The developed system prototype and its efficacy will be tested on one participant with ASD for 7 days at the participant's residence. The participant will wear the Hexoskin SWS during waking hours. Before the beginning of the POC phase, the teachers and caregivers who will interact with the system will be trained on its use, and the authors will be available to clarify any doubts and provide technical assistance during the POC week. At the end of those 7 days, the QUEST 2.0 will be administered to each professional interacting with the system. In addition, a focus group will be carried out with the same care providers, addressing the research questions mentioned above. The focus group will discuss on the information obtained from the QUEST 2.0 administration. In the last part of the focus group, a summation of solutions to each research question will be proposed to the group, and the number of participants who agree or disagree with the proposed summation solutions will be collected.

Data analyses

Section 1

Data collected by the Hexoskin SWS from participants in the OG and CG will be analyzed and compared. From electrocardiogram data, HR will be calculated between two consecutive QRS complexes. Considering the time interval between two QRS complexes as "t," the corresponding temporal HR will be 60/t (Becker, 2006). In order to remove unwanted artifacts from the HR, a percentage threshold value will be set using the sliding window method, and a minimum allowed peak width will be identified. The removal process will be performed for positive and negative peaks in two rounds. A window

will be slid over the HR signal, and its median value will be calculated. The maximum (positive and negative) allowed peak amplitude will be determined for every window by multiplying the window's median value by a threshold value. The threshold value for positive peaks was set at 30% (for the first removal round) and 25% (for the second removal round) of the window's mean value. For negative peaks, the threshold value was set at 50% (for the first removal round) and 30% (for the second removal round) of the window's mean value. Then, all peaks with amplitude larger than the allowed value will be identified from every window. If one of these peaks is found as narrower than the minimum allowed peak width, it will be replaced with the reference window median value. Otherwise, if an identified peak width is larger than the pre-set peak width, its value will be replaced with the maximal allowed HR (for positive peaks) or the minimal allowed HR (for negative peaks). The maximal allowed HR will be calculated with the following formula:"209 − (0.7 × (Participant age))" (Tanaka et al., 2001). The minimal allowed HR will be 60 beats per minute (Sidhu and Marine, 2020). After removing abnormal peaks, the signal will be filtered with a Gaussian filter with a sigma equal to one. After the HR signal filtering process, the obtained cleaned HR signal will be used to classify the participant's CBs (herein "stress") within the following levels: "no stress," "mild stress," "moderate stress," and "high stress." Each stress level will refer to an HR signal positioned within a specific range of values. The "no stress" level will include the HR values below 90% of the cleaned HR signal's lowest peak. The "high stress" level will comprise values above 90% of the cleaned HR signal's highest peak. If this value exceed the maximal allowed HR, it will be substituted with 90% of the maximal allowed HR value. The range left between these two thresholds will be divided into two equal parts (lower and upper half). The HR data positioned in the lower half of this range will be classified as "mild stress" and those positioned in the upper half as "moderate stress." Each HR value will be classified and assigned with a numerical value corresponding to a stress level ("no stress" = 0, "mild stress" = 1, "moderate stress" = 2, and "high stress" = 3). After acquiring the sequences of the stress levels of all participants of section two, the sequences of the subjects in the OG and CG will be compared using a version of the Smith-Waterman algorithm adapted for the analysis of the obtained data.

Section 2

The data gathered by the Hexoskin SWS from participants enrolled in section two will be analyzed as described above. A deep learning algorithm will be developed to predict the incoming participants' stress levels. To find CB patterns among subjects, the authors intend to construct a classifier based on supervised learning to find anomalies in the subject's data that might indicate an upcoming CB. Therefore, an LSTM algorithm along with other ML strategies (e.g., RF algorithm) will be guided through pre-defined rule sets to recognize data patterns corresponding to CB occurrence using the data collected by the participants' caregivers via the behavioral diary and information collected by the SWS.

Long short-term memory algorithm is an extension of the recurrent neural network (RNN). In contrast to the application of machine learning and deep learning, in the process of analyzing and predicting time series information, each data point is based on previous information, which must be examined as well. RNN is the most used network for time series applications since it can form the target vector observing the current input data history, using shared weights among the hiding units of the network across each time step of the data. The authors chose the usage of LSTM, and not RNN, since RNN has one significant problem (the vanishing gradient), where the gradient of the output error is based on previous inputs vanishes when time lags between inputs and errors increases. To overcome this problem, the LSTM is introduced. LSTM is designed as having a memory, which comes into practice by replacing the nonlinear units of RNN in the hidden layers with memory blocks. The network propagates errors throughout the entire network, and as a result, it can learn long-term dependencies and forget unnecessary information based on the data at hand (El Boujnouni and Tali, 2019).

The best classification algorithm will be selected based on the obtained prediction accuracy. The accuracy of the prediction model will be calculated according to common estimation methods such as the confusion matrix, and the area under the curve (AUC) values corresponding to the receiver operator characteristic curve (Ozdenizci et al., 2018; Goodwin et al., 2019; Nuske et al., 2019). These values range from 0.5 to 1 and will be designated as follow: 0.90–1 = excellent, 0.80–0.90 = good, 0.70–0.80 = fair, 0.60–0.70 = poor, and 0.50–0.60 = fail.

Section 3

In section three, the themes that will emerge from the focus group will be extracted from the discussion transcription. An axial coding strategy will be applied to calculate the extensiveness of each theme. This qualitative data analysis consists of assigning a reference number to each theme and marking any sentence related to that theme with that number. A reliability check for the code-to-sentence matches will be applied by giving the list of codes to an independent researcher experienced in qualitative analysis and asking him or her to identify the sentence that matches each code (Breen, 2006). The level of agreement with each summation answer to the research questions will be obtained by calculating the percentage of participants that agree with the proposed statement. The authors will discuss the developed answers to the research questions in light of the relevant themes that will have emerged, along with the level of agreement of the discussion group. The participants' responses to the focus group will be used to improve the usage of the SWS within the context of CB and ASD, as well as further develop the mobile application.

Results

The expected results for each part of the current investigation are summarized below.

Phase one

From the data gathered in the study phase one, the authors expect to recognize a higher stress level within the sequences obtained from participants in the OG compared to those from the CG. Although the literature reported a similar HR variation in adults with and without ASD exposed to stressful situations (Bishop-Fitzpatrick et al., 2017; Dijkhuis et al., 2019), adults with ASD are overall experiencing higher stress levels than typically developed peers when exposed to stressors (Gillott and Standen, 2007; Hirvikoski and Blomqvist, 2015; Bishop-Fitzpatrick et al., 2017). Therefore, the authors expect to be able to detect a difference within the stress sequences obtained from the proposed HR classification system during the disturbing video watching using the adapted Smith-Waterman algorithm. On the other hand, the authors expect no difference between the stress sequences obtained from individuals in the OG and CG when the participants watched at the relaxing video.

Phase two

Although, to the authors' knowledge, there is no literature reporting the capability of HR analysis in predicting the occurrence of CB in adults with ASD, the reports related to children and youth with ASD are encouraging. Relying on previous findings, the authors expect that the developed LSTM algorithm will be able to predict CBs that are about to occur at least in the upcoming 1 min with an AUC value at least above 0.70 (representing a fair prediction sensitivity) by analyzing the data gathered in the previous 60 s (Ozdenizci et al., 2018; Goodwin et al., 2019; Nuske et al., 2019).

Phase three

Quest 2.0

At the end of the protocol's POC phase, the QUEST 2.0 questionnaire will be administered to each teacher and caregiver who use the system with the participant with ASD. Within the area related to user satisfaction with the assistive technologies, the authors expect to achieve a high satisfaction value (mean score above 4.0) as the Hexoskin SWS only has to be worn by the participant, and the caregiver side of the system will be integrated into his smartphone (smartphone application). Once the SWS and the smartphone are paired with the remote server, no other actions are required from the caregiver. Therefore, no safety problems are anticipated, and easy use of the system is expected. Moreover, the smartphone application will provide visual and auditory stimuli related to the participant's stress level leading to comfort use and the possibility of intervening when a high-stress level is identified.

A high satisfaction score is anticipated concerning the area of provided service (mean score above 4.0) as there will be a training period for all who will interact with the system and the availability to provide technical assistance during the POC week. Moreover, the focus group that will be conducted represents a reasonable opportunity to verify the system's functioning.

Focus group

As to the authors' knowledge, the current protocol represents the first attempt to conduct a focus group evaluating the experience of caregivers with the use of a smart wearable device to predict the CBs of adults with ASD. It is challenging to rely on previous reports that mainly focus on design suggestions for wearable devices for people with ASD. In the discussion of the first research question ("Did wearing the SWS upset the participants?"), the authors expect that no difficulties will be reported on the selected SWS wearing during the POC week as it is a soft undershirt out of the participant's direct field of vision. Moreover, garments, such as t-shirts, have been found to be a highly preferred device to be used by individuals with ASD (Koo et al., 2018). However, reflections are anticipated about the individual sensory preferences of each person that may compromise the use of SWS in some people with ASD. Themes similar to this one emerged from a previous focus group related to the design of wearable technologies for people with ASD (Cantin-Garside et al., 2021). Furthermore, concerns can arise related to the hottest times of the year, when wearing a tank top under the shirt may be inappropriate.

Concerning the second, third, and fourth research questions ("Was the system able to detect all relevant CBs?"; "Was the system's operational speed sufficient to allow the in-time application of appropriate prevention strategies?"; "Has the use of the system reduced the amount of CBs?"), the authors expect that the emerging themes will crosscut them. Anticipated themes relate to the different CBs that can occur and the system's ability to predict all of them. Moreover, considerations are expected about the usefulness of the classification system's ability to reflect the current participant status and how the real-time knowledge of his arousal level changes the caregivers' carrying strategies. Finally, anticipated themes comprise the discussion of the usefulness of the time with which the CB is predicted. Reflections may emerge about whether the prediction time is sufficient or not to implement the appropriate CB prevention strategies.

During the fifth research question discussion, one anticipated theme relates to the possibility of using the proposed system in several environments, as the current architecture requires a Bluetooth connection with a server

nearby. Moreover, reflections may occur about the benefit of a smaller wearable device, which is less recognizable by the participant, and the possibility of having a wider prediction window. The system's availability in all the participant's daily living environments can help the prevention of during the whole day. A smaller device can improve the wearability of the system, increasing the acceptability of the device. Finally, a wider prediction window may be required in some cases for appropriate prevention strategies application.

Discussion

Many individuals with ASD present aggressive or disruptive CB, negatively affecting the quality of life of the person presenting the CBs. CBs can also reduce the possibility of receiving a proper education, social participation, and job opportunities for the person with ASD. Although numerous interventions have been proposed in the literature regarding how to cope with such behaviors, to date, most of them have not been found to affect CBs in a significant manner positively. Therefore, there is a need for effective strategies to support such interventions that can anticipate oncoming CBs. The results obtained from the analysis described in "Section 1" will deepen the knowledge related to the relationship between HR and stress levels in adults with ASD. Moreover, to the authors' best knowledge, this study represents the first attempt of using SWSs and physiological parameters to predict CBs of adults with ASD. The results obtained from the prediction algorithm development will lay the foundation for expanding the field of study of CBs prediction through ML techniques to the adult population with ASD. The availability of an effective strategy to anticipate the CBs occurrence will allow the caregivers to intervene, applying the adequate procedure to reduce the person's stress level and avoid the behavioral meltdown. Such technology can potentially improve the quality of life of people with ASD presenting aggressive and disruptive CBs and their peers, care providers, and healthcare professionals. Moreover, the proposed system will be cost-effective, easy to use even for non-experts, and widely accessible. Furthermore, results that will be obtained at the end of this project can assist in the further development of wearable devices to predict CB, a field of growing interest among health and medical researchers with the potential to help other populations presenting CBs, such as those with intellectual and developmental disabilities, dementia, and other mental challenges. Finally, the mixed-method design proposed for phase three of the protocol will involve care providers and healthcare professionals who handle CB daily. Their participation will allow for the integration of their clinical experience and perceived needs and will provide valuable information to be considered for developing the current application and future similar devices. The current method presents some limitations. First, one participant only will be included in the POC phase. However, although this choice can limit the external validity of the results obtained in the POC phase, it will provide initial data on the usability of the developed system. Moreover, the videos that were chosen for the study phase one were not previously validated to elicit the desired stress increase (or reduction) in the population enrolled in the study (typically developed adults and adults with ASD).

Data availability statement

The original contributions presented in this study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ariel University Institutional Review Board (AU-HEA-ML-20201203), Asaf Harofe Institutional Review Board (0136-21-ASF), and the Israeli Ministry of Health (MOH_2022-01-25_010570). The patients/participants provided their written informed consent to participate in this study.

Author contributions

MZ and ML obtained the funds for the current research project. MZ, RT, and ML designed the protocol and selected the SWS to be used. MZ, RT, and HH identified the adequate data analysis for the algorithm development and wrote the software requirements specification for the application interfaces. MZ and HH defined the design requirements for the application implementation phase. ML and AR defined the methodology for the clinical part of the data collection (behavioral diary and focus group) and were involved in the data collection section of the proposed research. AR and HH wrote the protocol text. All authors read the protocol draft and suggested improvement until consensus was reached.

Funding

This work was supported by the Israeli Innovation Authority under the grant number: 72359.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders*. 5th Edn. Washington, DC: American Psychiatric Association, doi: 10.1176/appi.books.9780890425596

Arnold, S., and Reed, P. (2016). Reading assessments for students with ASD: a survey of summative reading assessments used in special educational schools in the UK. *Br. J. Special Educ.* 43, 122–141. doi: 10.1111/1467-8578.12127

Baker, A. E. Z., Lane, A., Angley, M. T., and Young, R. L. (2008). The relationship between sensory processing patterns and behavioural responsiveness in autistic disorder: A pilot study. *J. Autism. Dev. Disord.* 38, 867–875.

Banaee, H., Ahmed, M. U., and Loutfi, A. (2013). Data mining for wearable sensors in health monitoring systems: A review of recent trends and challenges. *Sensors* 13, 17472–17500. doi: 10.3390/s131217472

Baron-Cohen, S., Scott, F. J., Allison, C., Williams, J., Bolton, P., Matthews, F. E., et al. (2009). Prevalence of autism-spectrum conditions: UK school-based population study. *Br. J. Psychiatry* 194, 500–509. doi: 10.1192/BJP.BP.108.059345

Barton, E. E., Reichow, B., Schnitz, A., Smith, I. C., and Sherlock, D. (2015). A systematic review of sensory-based treatments for children with disabilities. *Res. Dev. Disabil.* 37, 64–80. doi: 10.1016/J.RIDD.2014.11.006

Becker, D. E. (2006). Fundamentals of electrocardiography interpretation. *Anesth. Prog.* 53, 53–64. doi: 10.2344/0003-3006200653[53:FOEI]2.0.CO;2

Berg, W. K., Peck, S., Wacker, D. P., Harding, J., McComas, J., Richman, D., et al. (2000). The effects of presession exposure to attention on the results of assessments of attention as a reinforcer. *J. Appl. Behav. Anal.* 33, 463–477. doi: 10.1901/jaba.2000.33-463

Bishop-Fitzpatrick, L., Minshew, N. J., and Eack, S. M. (2014). "A systematic review of psychosocial interventions for adults with autism spectrum disorders," in *Adolescents and Adults with Autism Spectrum Disorders*, F. R. Volkmar, B. Reichow and J. C. McPartland, (Berlin: Springer), 315–327. doi: 10.1007/978-1-4939-0506-5_16

Bishop-Fitzpatrick, L., Minshew, N. J., Mazefsky, C. A., and Eack, S. M. (2017). Perception of life as stressful, not biological response to stress, is associated with greater social disability in adults with autism spectrum disorder. *J. Autism Dev. Disord.* 47, 1–16. doi: 10.1007/s10803-016-2910-6

Blacher, J., and McIntyre, L. L. (2006). Syndrome specificity and behavioural disorders in young adults with intellectual disability: cultural differences in family impact. *J. Intel. Disabil. Res.* 50, 184–198. doi: 10.1111/J.1365-2788.2005.00768.X

Black, M. H., Milbourn, B., Chen, N. T. M., McGarry, S., Wali, F., Ho, A. S. V., et al. (2020). The use of wearable technology to measure and support abilities, disabilities and functional skills in autistic youth: a scoping review. *Scand. J. Child Adolesc. Psychiatr. Psychol.* 8, 48–69. doi: 10.21307/sjcapp-2020-006

Blankenship, K., Erickson, C. A., and McDougle, C. J. (2010). Pharmacotherapy of Autism and related disorders. *Psychiatr. Ann.* 40, 203–209. doi: 10.3928/00485713-20100330-06

Breen, R. L. (2006). A practical guide to focus-group research. *J. Geogr. High. Educ.* 30, 463–475. doi: 10.1080/03098260600927575

Bremer, E., Crozier, M., and Lloyd, M. (2016). A systematic review of the behavioural outcomes following exercise interventions for children and youth with autism spectrum disorder. *Autism* 20, 899–915. doi: 10.1177/1362361315616002

Cantin-Garside, K. D., Nussbaum, M. A., White, S. W., Kim, S., Kim, C. do, Fortes, D. M. G., et al. (2021). Understanding the experiences of self-injurious behavior in autism spectrum disorder: Implications for monitoring technology design. *J. Am. Med. Inform. Assoc.* 28, 303–310. doi: 10.1093/jamia/ocaa169

Case-Smith, J., Weaver, L. L., and Fristad, M. A. (2015). A systematic review of sensory processing interventions for children with autism spectrum disorders. *Autism* 19, 133–148. doi: 10.1177/1362361313517762

Chiang, H. M. (2008). Expressive communication of children with autism: the use of challenging behaviour. *J. Intel. Disabil. Res.* 52, 966–972. doi: 10.1111/J.1365-2788.2008.01042.X

Cohen, I. L., Yoo, J. H., Goodwin, M. S., and Moskowitz, L. (2011). "Assessing challenging behaviors in autism spectrum disorders: prevalence, rating scales, and autonomic indicators," in *International Handbook of Autism and Pervasive Developmental Disorders*, eds J. Matson and P. Sturmey (New York, NY: Springer New York), 247–270. doi: 10.1007/978-1-4419-8065-6_15

Cooper, J. O., Heron, T. E., and Heward, W. L. (2020). *Applied Behavior Analysis*. 3rd Edn. Harlow: Pearson.

Cotelli, M., Manenti, R., and Zanetti, O. (2012). Reminiscence therapy in dementia: A review. *Maturitas* 72, 203–205. doi: 10.1016/j.maturitas.2012.04.008

Demers, L., Weiss-Lambrou, R., and Ska, B. (2002). The Quebec User Evaluation of Satisfaction with Assistive Technology (QUEST 2.0): An overview and recent progress. *Technol. Disabil.* 14, 101–105. doi: 10.3233/TAD-2002-14304

Demers, L., Weiss-Lambrou, R., Ska, B., and Demers, L. (2000). Item analysis of the quebec user evaluation of satisfaction with assistive technology (QUEST). *Assist. Technol.* 12, 96–105. doi: 10.1080/10400435.2000.10132015

Didden, R., Korzilius, H., Van Oorsouw, W., and Sturmey, P. (2006). Behavioral treatment of challenging behaviors in individuals with mild mental retardation: Meta-analysis of single-subject research. *Am. J. Mental Retard.* 111, 290–298. doi: 10.1352/0895-80172006111[290:BTOCBI]2.0.CO;2

Dijkhuis, R. R., Ziermans, T., van Rijn, S., Staal, W., and Swaab, H. (2019). Emotional arousal during social stress in young adults with autism: insights from heart rate, heart rate variability and self-report. *J. Autism Dev. Disord.* 49, 2524–2535. doi: 10.1007/s10803-019-04000-5

Doyle, K. L., Morgan, E. E., Morris, S., Smith, D. M., Little, S., Iudicello, J. E., et al. (2013). Real-world impact of neurocognitive deficits in acute and early HIV infection. *J. Neurovirol.* 19, 565–573. doi: 10.1007/s13365-013-0218-2

Eggermont, L. H. P., and Scherder, E. J. A. (2006). Physical activity and behaviour in dementia: A review of the literature and implications for psychosocial intervention in primary care. *Dementia* 5, 411–428. doi: 10.1177/1471301206067115

El Boujnouni, I., and Tali, A. (2019). Heart rate variability prediction based on the combination of wavelet decomposition and LSTM networks. *Int. J. Sci. Eng. Res.* 10, 61–65.

Emerson, E. (2001). *Challenging Behaviour: Analysis and Intervention in People with Severe Intellectual Disabilities*. 2nd Edn. Cambridge: Cambridge University Press.

Erturk, B., Machalicek, W., and Drew, C. (2018). Self-injurious behavior in children with developmental disabilities: a systematic review of behavioral intervention literature. *Behav. Modif.* 42, 498–542. doi: 10.1177/0145445517741474

Fakhoury, N., Wilhelm, N., Sobota, K. F., and Kroustos, K. R. (2017). Impact of music therapy on dementia behaviors: A literature review. *Consult. Pharm.* 32, 623–628. doi: 10.4140/TCP.N.2017.623

Felce, D., Perry, J., Lowe, K., and Jones, E. (2011). The impact of autism or severe challenging behaviour on lifestyle outcome in community housing. *J. Appl. Res. Intel. Disabil.* 24, 95–104. doi: 10.1111/J.1468-3148.2010.00571.X

Ferguson, J., Craig, E. A., and Dounavi, K. (2019). Telehealth as a model for providing behaviour analytic interventions to individuals with autism spectrum disorder: a systematic review. *J. Autism. Dev. Disord.* 49, 582–616. doi: 10.1007/S10803-018-3724-5/FIGURES/5

Fettig, A., and Barton, E. E. (2014). Parent implementation of function-based intervention to reduce children's challenging behavior: a literature review. *Topics Early Child. Spec. Educ.* 34, 49–61. doi: 10.1177/0271121413513037

Flores, J. G., and Alonso, C. G. (1995). Using focus groups in educational research: exploring teachers' perspectives on educational change. *Eval. Rev.* 19, 84–101. doi: 10.1177/0193841X9501900104

Forbes, D., Forbes, S. C., Blake, C. M., Thiessen, E. J., and Forbes, S. (2015). Exercise programs for people with dementia. *Cochrane Database Syst. Rev.* 2015, 195–196. doi: 10.1002/14651858.CD006489.pub4

Francese, R., and Yang, X. (2021). Supporting autism spectrum disorder screening and intervention with machine learning and wearables: a systematic literature review. *Complex Intel. Syst.* 8, 3659–3674. doi: 10.1007/S40747-021-00447-1

Gerow, S., Hagan-Burke, S., Rispoli, M., Gregori, E., Mason, R., and Ninci, J. (2018). A Systematic Review of Parent-Implemented Functional Communication Training for Children With ASD. *Behav. Modif.* 42, 335–363. doi: 10.1177/0145445517740872

Gillott, A., and Standen, P. J. (2007). Levels of anxiety and sources of stress in adults with autism. *J. Intel. Disabil.* 11, 359–370. doi: 10.1177/1744629507083585

Gold, C., Wigram, T., and Elefant, C. (2006). Music therapy for autistic spectrum disorder. *Cochrane Database Syst. Rev.* 2006:CD004381. doi: 10.1002/14651858.CD004381.PUB2/INFORMATION/EN

Goldstein, H. (2002). Communication intervention for children with autism: a review of treatment efficacy. *J. Autism Dev. Disord.* 32, 373–396. doi: 10.1023/A:1020589821992

Goodwin, M. S., Mazefsky, C. A., Ioannidis, S., Erdogmus, D., and Siegel, M. (2019). Predicting aggression to others in youth with autism using a wearable biosensor. *Autism Res.* 12, 1286–1296. doi: 10.1002/aur.2151

Goodwin, M. S., Özdenizci, O., Cumpanasoiu, C., Tian, P., Guo, Y., Stedman, A., et al. (2018). "Predicting imminent aggression onset in minimally-verbal youth with autism spectrum disorder using preceding physiological signals," in *Proceedings of the 12th EAI International Conference on Pervasive Computing Technologies for Healthcare* (New York, NY: ACM), 201–207. doi: 10.1145/3240925.3240980

Gregori, E., Wendt, O., Gerow, S., Peltier, C., Genc-Tosun, D., Lory, C., et al. (2020). Functional communication training for adults with autism spectrum disorder: a systematic review and quality appraisal. *J. Behav. Educ.* 29, 42–63. doi: 10.1007/s10864-019-09339-4

Gulshan, V., Peng, L., Coram, M., Stumpe, M. C., Wu, D., Narayanaswamy, A., et al. (2016). Development and validation of a deep learning algorithm for detection of diabetic retinopathy in retinal fundus photographs. *JAMA* 316, 2402–2410. doi: 10.1001/jama.2016.17216

Hirvikoski, T., and Blomqvist, M. (2015). High self-perceived stress and poor coping in intellectually able adults with autism spectrum disorder. *Autism* 19, 752–757. doi: 10.1177/1362361314543530

Holden, B., and Gitlesen, J. P. (2006). A total population study of challenging behaviour in the county of Hedmark, Norway: Prevalence, and risk markers. *Res. Dev. Disabil.* 27, 456–465. doi: 10.1016/J.RIDD.2005.06.001

Hutt, C., and Hutt, S. J. (1965). Effects of environmental complexity on stereotyped behaviours of children. *Anim. Behav.* 13, 1–4. doi: 10.1016/0003-3472(65)90064-3

Imbiriba, T., Cumpanasoiu, D. C., Heathers, J., Ioannidis, S., Erdomuş, D., and Goodwin, M. S. (2020). Biosensor prediction of aggression in youth with autism using kernel-based methods. *ACM Int. Conf. Proceeding Ser.* 88–93. doi: 10.1145/3389189.3389199

Inoue, M. (2019). Assessments and interventions to address challenging behavior in individuals with intellectual disability and autism spectrum disorder in Japan: a consolidated review. *Yonago Acta Med.* 62, 169–181. doi: 10.33160/YAM.2019.06.001

James, R., Sigafoos, J., Green, V. A., Lancioni, G. E., O'Reilly, M. F., Lang, R., et al. (2015). Music therapy for individuals with autism spectrum disorder: a systematic review. *Rev. J. Autism Dev. Disord.* 2, 39–54. doi: 10.1007/s40489-014-0035-4

Jang, J., Dixon, D. R., Tarbox, J., and Granpeesheh, D. (2011). Symptom severity and challenging behavior in children with ASD. *Res. Autism Spectr. Disord.* 5, 1028–1032. doi: 10.1016/j.rasd.2010.11.008

Kinsbourne, M. (1980). Do repetitive movement patterns in children and animals serve a dearousing function? *J. Dev. Behav. Pediatr.* 1, 39–42. doi: 10.1097/00004703-198003000-00009

Kitzinger, J. (1994). The methodology of Focus Groups: the importance of interaction between research participants. *Sociol. Health Illn.* 16, 103–121. doi: 10.1111/1467-9566.ep11347023

Klusek, J., Roberts, J. E., and Losh, M. (2015). Cardiac autonomic regulation in autism and Fragile X syndrome: A review. *Psychol. Bull.* 141, 141–175. doi: 10.1037/a0038237

Knapp, M., Romeo, R., and Beecham, J. (2009). Economic cost of autism in the UK. *Autism* 13, 317–336. doi: 10.1177/1362361309104246

Koo, S. H., Gaul, K., Rivera, S., Pan, T., and Fong, D. (2018). Wearable technology design for autism spectrum disorders. *Arch. Design Res.* 31, 37–55. doi: 10.15187/adr.2018.02.31.1.37

Lang, R., O'Reilly, M., Healy, O., Rispoli, M., Lydon, H., Streusand, W., et al. (2012). Sensory integration therapy for autism spectrum disorders: A systematic review. *Res. Autism Spectr. Disord.* 6, 1004–1018. doi: 10.1016/j.rasd.2012.01.006

Leong, H. M., Carter, M., and Stephenson, J. (2015). Systematic review of sensory integration therapy for individuals with disabilities: Single case design studies. *Res. Dev. Disabil.* 47, 334–351. doi: 10.1016/J.RIDD.2015.09.022

Lequia, J., MacHalicek, W., and Rispoli, M. J. (2012). Effects of activity schedules on challenging behavior exhibited in children with autism spectrum disorders: A systematic review. *Res. Autism Spectr. Disord.* 6, 480–492. doi: 10.1016/j.rasd.2011.07.008

Levine, T. P., Conradt, E., Goodwin, M. S., Sheinkopf, S. J., and Lester, B. (2014). "Psychophysiological arousal to social stress in autism spectrum disorders," in *Comprehensive Guide to Autism*, eds V. B. Patel, V. R. Preedy and C. R. Martin (New York, NY: Springer New York), 1177–1193. doi: 10.1007/978-1-4614-4788-7_66

Lim, J. M. (2019). Emotion regulation and intervention in adults with autism spectrum disorder: a synthesis of the literature. *Adv. Autism* 6, 48–62. doi: 10.1108/AIA-12-2018-0050

Lindgren, S., Wacker, D., Suess, A., Schieltz, K., Pelzel, K., Kopelman, T., et al. (2016). Telehealth and autism: treating challenging behavior at lower cost. *Pediatrics* 137, S167–S175. doi: 10.1542/PEDS.2015-2851O

Lloyd, B. P., and Kennedy, C. H. (2014). Assessment and treatment of challenging behaviour for individuals with intellectual disability: a research review. *J. Appl. Res. Intel. Disabil.* 27, 187–199. doi: 10.1111/JAR.12089

Lydon, S., Healy, O., O'Reilly, M., and McCoy, A. (2013). A systematic review and evaluation of response redirection as a treatment for challenging behavior in individuals with developmental disabilities. *Res. Dev. Disabil.* 34, 3148–3158. doi: 10.1016/J.RIDD.2013.06.010

Lydon, S., Healy, O., Reed, P., Mulhern, T., Hughes, B. M., and Goodwin, M. S. (2016). A systematic review of physiological reactivity to stimuli in autism. *Dev. Neurorehabil.* 19, 335–355. doi: 10.3109/17518423.2014.971975

Machalicek, W., O'Reilly, M. F., Beretvas, N., Sigafoos, J., and Lancioni, G. E. (2007). A review of interventions to reduce challenging behavior in school settings for students with autism spectrum disorders. *Res. Autism Spectr. Disord.* 1, 229–246. doi: 10.1016/J.RASD.2006.10.005

Machalicek, W., Raulston, T., Knowles, C., Ruppert, T., Carnett, A., and Alresheed, F. (2016). "Challenging behavior," in *Comorbid Conditions Among Children with Autism Spectrum Disorders*, Ed. J. L. Matson (Cham: Springer), 137–170. doi: 10.1007/978-3-319-19183-6_6

MacNaul, H. L., and Neely, L. C. (2018). Systematic review of differential reinforcement of alternative behavior without extinction for individuals with autism. *Behav. Modif.* 42, 398–421. doi: 10.1177/0145445517740321

Malone, R. P., Gratz, S. S., Delaney, M. A., and Hyman, S. B. (2005). Advances in drug treatments for children and adolescents with autism and other pervasive developmental disorders. *CNS Drugs* 19, 923–934. doi: 10.2165/00023210-200519110-00003/FIGURES/TAB1

Masino, A. J., Forsyth, D., Nuske, H., Herrington, J., Pennington, J., Kushleyeva, Y., et al. (2019). "M-Health and autism: Recognizing stress and anxiety with machine learning and wearables data," in *Proceedings of the IEEE Symposium on Computer-Based Medical Systems 2019-June*, Shenzhen, 714–719. doi: 10.1109/CBMS.2019.00144

Matson, J. L., Wilkins, J., and Macken, J. (2008). The relationship of challenging behaviors to severity and symptoms of autism spectrum disorders. *J. Ment. Health Res. Intellect. Disabil.* 2, 29–44. doi: 10.1080/19315860802611415

McDonnell, A., Sturmey, P., Oliver, C., Cunningham, J., Hayes, S., Galvin, M., et al. (2008). The effects of staff training on staff confidence and challenging behavior in services for people with autism spectrum disorders. *Res. Autism. Spectr. Disord.* 2, 311–319. doi: 10.1016/J.RASD.2007.08.001

McPheeters, M. L., Warren, Z., Sathe, N., Bruzek, J. L., Krishnaswami, S., Jerome, R. N., et al. (2011). A systematic review of medical treatments for children with autism spectrum disorders. *Pediatrics* 127, e1312–e1321. doi: 10.1542/peds.2011-0427

Mirenda, P. (1997). Supporting individuals with challenging behavior through functional communication training and AAC: research review. *Augment. Alter. Commun.* 13, 207–225. doi: 10.1080/07434619712331278048

Mukaddes, N. M., and Topcu, Z. (2006). Case report: homicide by a 10-year-old girl with autistic disorder. *J. Autism Dev. Disord.* 36, 471–474. doi: 10.1007/S10803-006-0087-0

Murphy, G. H., Beadle-Brown, J., Wing, L., Gould, J., Shah, A., and Holmes, N. (2005). Chronicity of challenging behaviours in people with severe intellectual disabilities and/or autism: a total population sample. *J. Autism Dev. Disord.* 35, 405–418. doi: 10.1007/S10803-005-5030-2

Murphy, K. P. (2012). *Machine Learning: A Probabilistic Perspective*. Cambridge, MA: MIT press.

Murphy, O., Healy, O., and Leader, G. (2009). Risk factors for challenging behaviors among 157 children with autism spectrum disorder in Ireland. *Res. Autism. Spectr. Disord.* 3, 474–482. doi: 10.1016/j.rasd.2008.09.008

Neal, M., and Barton Wright, P. (2003). Validation therapy for dementia. *Cochrane Database Syst. Rev.* 2003:CD001394 doi: 10.1002/14651858.CD001394

Nissen, J. M. J. F., and Haveman, M. J. (1997). Mortality and avoidable death in people with severe self-injurious behaviour: results of a Dutch study. *J. Intel. Disabil. Res.* 41, 252–257. doi: 10.1046/J.1365-2788.1997.04545.X

Nuske, H. J., Finkel, E., Hedley, D., Parma, V., Tomczuk, L., Pellecchia, M., et al. (2019). Heart rate increase predicts challenging behavior episodes in preschoolers with autism. *Stress* 22, 303–311. doi: 10.1080/10253890.2019.1572744

O'Donnell, S., Deitz, J., Kartin, D., Nalty, T., and Dawson, G. (2012). Sensory processing, problem behavior, adaptive behavior, and cognition in preschool children with autism spectrum disorders. *Am. J. Occupat. Ther.* 66, 586–594. doi: 10.5014/ajot.2012.004168

O'Neil, M. E., Freeman, M., Christensen, V., Telerant, R., Addleman, A., and Kansagara, D. (2011). *A Systematic Evidence Review of Non-Pharmacological Interventions for Behavioral Symptoms of Dementia*. Washington, DC: Department of Veterans Affairs.

Ogg-Groenendaal, M., Hermans, H., and Claessens, B. (2014). A systematic review on the effect of exercise interventions on challenging behavior for people with intellectual disabilities. *Res. Dev. Disabil.* 35, 1507–1517. doi: 10.1016/J.RIDD.2014.04.003

Ornitz, E. M., and Ritvo, E. R. (1968). Perceptual inconstancy in early infantile autism: the syndrome of early infant autism and its variants including certain cases of childhood schizophrenia. *Arch. Gen. Psychiatry* 18, 76–98. doi: 10.1001/ARCHPSYC.1968.01740010078010

Ozdenizci, O., Cumpanasoiu, C., Mazefsky, C., Siegel, M., Erdogmus, D., Ioannidis, S., et al. (2018). "Time-series prediction of proximal aggression onset in minimally-verbal youth with autism spectrum disorder using physiological biosignals," in *Proceedings of the Annual International Conference of the IEEE Engineering in Medicine and Biology Society (IEEE)*, Sydney, 5745–5748. doi: 10.1109/EMBC.2018.8513524

Prizant, B. M., and Wetherby, A. M. (2005). "Critical issues in enhancing communication abilities for persons with autism spectrum disorders," in *Handbook of Autism and Pervasive Developmental Disorders: Assessment, Interventions, and Policy*, eds F. R. Volkmar, R. Paul, A. Klin, and D. Cohen (Hoboken, NJ: John Wiley & Sons, Inc.), 925–945. doi: 10.1002/9780470939352.ch10

Reese, R. M., Richman, D. M., Belmont, J. M., and Morse, P. (2005). Functional characteristics of disruptive behavior in developmentally disabled children with and without autism. *J. Autism Dev. Disord.* 35, 419–428. doi: 10.1007/S10803-005-5032-0

Reichow, B., Servili, C., Yasamy, M. T., Barbui, C., and Saxena, S. (2013). Non-specialist psychosocial interventions for children and adolescents with intellectual disability or lower-functioning autism spectrum disorders: a systematic review. *PLoS Med.* 10:e1001572. doi: 10.1371/JOURNAL.PMED.1001572

Rose, D., Horne, S., Rose, J. L., and Hastings, R. P. (2004). Negative emotional reactions to challenging behaviour and staff burnout: Two replication studies. *J. Appl. Res. Intel. Disabil.* 17, 219–223. doi: 10.1111/j.1468-3148.2004.00194.x

Sawyer, A., Lake, J. K., Lunsky, Y., Liu, S. K., and Desarkar, P. (2014). Psychopharmacological treatment of challenging behaviours in adults with autism and intellectual disabilities: A systematic review. *Res. Autism. Spectr. Disord.* 8, 803–813. doi: 10.1016/J.RASD.2014.03.021

Scherer, M. J. (2005). *Living in the State of Stuck: How Technology Impacts the Lives of Persons with Disabilities*. 4th Edn. Brookline, MA: Brookline Books.

Scotti, J. R., Ujcich, K. J., Weigle, K. L., Holland, C. M., and Kirk, K. S. (1996). Interventions with challenging behavior of persons with developmental disabilities: a review of current research practices. *J. Assoc. Persons Severe Handic.* 21, 123–134. doi: 10.1177/154079699602100303

Seida, J. K., Ospina, M. B., Karkhaneh, M., Hartling, L., Smith, V., and Clark, B. (2009). Systematic reviews of psychosocial interventions for autism: An umbrella review. *Dev. Med. Child. Neurol.* 51, 95–104. doi: 10.1111/j.1469-8749.2008.03211.x

Sidhu, S., and Marine, J. E. (2020). Evaluating and managing bradycardia. *Trends Cardiovasc. Med.* 30, 265–272. doi: 10.1016/j.tcm.2019.07.001

Simpson, K., and Keen, D. (2011). Music interventions for children with autism: Narrative review of the literature. *J. Autism Dev. Disord.* 41, 1507–1514. doi: 10.1007/s10803-010-1172-y

Sorensen, C., and Zarrett, N. (2014). Benefits of physical activity for adolescents with autism spectrum disorders: a comprehensive review. *Rev. J. Autism Dev. Disord.* 1, 344–353. doi: 10.1007/S40489-014-0027-4/TABLES/4

Stephenson, J. (2006). Music therapy and the education of students with severe disabilities. *Educ. Train Dev. Disabil.* 41, 290–299.

Subramaniam, P., and Woods, B. (2012). The impact of individual reminiscence therapy for people with dementia: Systematic review. *Expert Rev. Neurother.* 12, 545–555. doi: 10.1586/ern.12.35

Taj-Eldin, M., Ryan, C., O'Flynn, B., and Galvin, P. (2018). A review of wearable solutions for physiological and emotional monitoring for use by people with autism spectrum disorder and their caregivers. *Sensors* 18:4271. doi: 10.3390/s18124271

Tanaka, H., Monahan, K. D., and Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *J. Am. Coll. Cardiol.* 37, 153–156. doi: 10.1016/S0735-1097(00)01054-8

Tanner, K., Hand, B. N., O'Toole, G., and Lane, A. E. (2015). Effectiveness of interventions to improve social participation, play, leisure, and restricted and repetitive behaviors in people with autism spectrum disorder: A systematic review. *Am. J. Occupat. Ther.* 69, 6905180010p1–6905180010p12. doi: 10.5014/ajot.2015.017806

Vanderkerken, L., Heyvaert, M., Maes, B., and Onghena, P. (2013). Psychosocial interventions for reducing vocal challenging behavior in persons with autistic disorder: A multilevel meta-analysis of single-case experiments. *Res. Dev. Disabil.* 34, 4515–4533. doi: 10.1016/J.RIDD.2013.09.030

Wahman, C. L., Pustejovsky, J. E., Ostrosky, M. M., and Santos, R. M. (2022). Examining the effects of social storiestm on challenging behavior and prosocial skills in young children: a systematic review and meta-analysis. *Topics Early Child Spec. Educ.* 41, 267–279. doi: 10.1177/0271121419855692

Walker, V. L., and Snell, M. E. (2013). Effects of augmentative and alternative communication on challenging behavior: A meta-analysis. *Augment. Alter. Commun.* 29, 117–131.

Walker, V. L., Carpenter, M. E., Lyon, K. J., and Button, L. (2021). A meta-analysis of paraprofessional-delivered interventions to address challenging behavior among students with disabilities. *J. Posit Behav. Interv.* 23, 68–80. doi: 10.1177/1098300720911147

Wan Yunus, F., Liu, K. P. Y., Bissett, M., and Penkala, S. (2015). Sensory-based intervention for children with behavioral problems: a systematic review. *J. Autism Dev. Disord.* 45, 3565–3579. doi: 10.1007/S10803-015-2503-9/TABLES/4

Watling, R., and Hauer, S. (2015). Effectiveness of Ayres sensory integration® and sensory-based interventions for people with autism spectrum disorder: a systematic review. *Am. J. Occupat. Ther.* 69, 6905180030p1–6905180030p12. doi: 10.5014/ajot.2015.018051

Weston, R., Hodges, A., and Davis, T. N. (2018). Differential reinforcement of other behaviors to treat challenging behaviors among children with autism: a systematic and quality review. *Behav. Modif.* 42, 584–609. doi: 10.1177/0145445517743487

Woodbury-Smith, M. R., Clare, I. C. H., Holland, A. J., and Kearns, A. (2006). High functioning autistic spectrum disorders, offending and other law-breaking: Findings from a community sample. *J. Forensic Psychiatry Psychol.* 17, 108–120. doi: 10.1080/14789940600589464

Zetteler, J. (2008). Effectiveness of simulated presence therapy for individuals with dementia: A systematic review and meta-analysis. *Aging Ment. Health* 12, 779–785. doi: 10.1080/13607860802380631

Zheng, Z. K., Staubitz, J. E., Weitlauf, A. S., Staubitz, J., Pollack, M., Shibley, L., et al. (2021). A predictive multimodal framework to alert caregivers of problem behaviors for children with ASD (PreMAC). *Sensors* 21:370. doi: 10.3390/S21020370

frontiers | Frontiers in Behavioral Neuroscience

TYPE Review
PUBLISHED 23 January 2023
DOI 10.3389/fnbeh.2022.992223

OPEN ACCESS

EDITED BY
Patricio Huerta,
Feinstein Institute for Medical Research,
United States

REVIEWED BY
Chen Liu,
Beijing United Family Hospital, China
Attila Szabo,
University of Oslo, Norway

*CORRESPONDENCE
Zhaofu Li
lzf0871@126.com
Dongdong Qin
qindong108@163.com
Jiangyun Peng
pengjiangyuntx@163.com

†These authors have contributed equally to this work and share first authorship

SPECIALTY SECTION
This article was submitted to Pathological Conditions, a section of the journal Frontiers in Behavioral Neuroscience

RECEIVED 12 July 2022
ACCEPTED 30 December 2022
PUBLISHED 23 January 2023

CITATION
Liu N, Yan W, Su R, Zhang L, Wang X, Li Z, Qin D and Peng J (2023) Research progress on rheumatoid arthritis-associated depression. Front. Behav. Neurosci. 16:992223. doi: 10.3389/fnbeh.2022.992223

Research progress on rheumatoid arthritis-associated depression

Nian Liu[1†], Weitian Yan[1†], Rong Su[2], Lin Zhang[1], Xingqiang Wang[2], Zhaofu Li[3]*, Dongdong Qin[3]* and Jiangyun Peng[2]*

[1]First Clinical Medical School, Yunnan University of Chinese Medicine, Kunming, China, [2]Rheumatism Center, Yunnan Provincial Hospital of Traditional Chinese Medicine, Kunming, China, [3]Basic Medical School, Yunnan University of Chinese Medicine, Kunming, China

Depression is an independent mood disorder and one of the most common comorbidities of rheumatoid arthritis (RA). Growing evidence suggests that there is two-way regulation between RA and depression, resulting in a vicious cycle of RA, depression, poor outcomes, and disease burden. The rising prevalence of RA-associated depression warrants a re-examination of the relationships between them. Here we provide an overview of the etiology and pathological mechanisms of RA-associated depression, and recent advances in treatment with biologics, which will facilitate the development of new and effective prevention and treatment strategies.

KEYWORDS

rheumatoid arthritis, depression, etiology, pathology, biological therapies, research progress

1. Introduction

Rheumatoid arthritis (RA) is a systemic autoimmune disorder characterized by synovitis, joint erosion, and cartilage damage. In the global burden of disease study in 2010, the disability imposed by RA ranked 42 among the 291 diseases included (Cross et al., 2014). In addition to the disability caused by joint pain, swelling, and deformation, the extra-articular symptoms of RA also require attention. Depression is a mood disorder affecting 322 million people worldwide, and one of the most common comorbidities in RA (World Health Organization, 2017; Baerwald et al., 2019). Depression can be triggered by multiple stimuli such as repeated physical pain, fatigue, gradual loss of function, lack of social role, and financial burden. In a cross-sectional study conducted in 17 countries, depression was the most frequent complication in RA, with prevalence ranging between 14% and 48% (Nerurkar et al., 2019). In China, the proportion was as high as 48%, and the respective prevalence of mild, moderate, and severe depression was 30%, 18%, and 18% (Fu et al., 2017). Although the prevalence of depression in RA patient groups varies in different countries and regions due to measurement methods as well as the non-uniform threshold of diagnostic criteria for major depressive disorder (MDD; Sturgeon et al., 2016), it also reminds us that depression is a link that cannot be ignored in RA treatment. This article reviews the research progress on RA-associated depression from: (1) etiology; (2) pathology; and (3) biological therapies, hoping to provide a reference for future basic and clinical research on RA-associated depression.

2. What causes depression in RA patients?

In 1977, Professor Engel of the Medical School of the University of Rochester put forward a new medical model, the bio-psycho-social medical model.

This new model emphasized the combination of biology, psychology, and sociology to search for the causes, diagnosis, and treatment methods of diseases, instead of investigating diseases from a single biomedical perspective. Given the debate as to why RA is often associated with depression, more complex and comprehensive factors covering biological, psychological, and sociological needed to be considered rather than a simple causal model of psychological impairment due to chronic pain and long-term disability associated with RA.

There is a bidirectional association between RA and depression. On the one hand, under the influence of pain, fatigue, drugs, diet, micronutrient, gender, lack of exercise, aberrant testosterone levels, and social support (including social tool support, emotional support, and financial assistance), RA patients often face poor health-related quality of life, reduced chance of joint symptom relief, and a higher risk of death (Marrie et al., 2018; Shadick et al., 2019; Vallerand et al., 2019; Lwin et al., 2020; **Figure 1**). They have to overcome more severe obstacles in maintaining biological function, mental health, as well as social participation. As a result, the risk of depression in RA patients is significantly higher than in non-RA groups (Lin et al., 2015; Lu et al., 2016; Marrie et al., 2018). On the other hand, RA patients associated with depression will bear "overload hospitalization costs" due to more physician visits, increased emergency care utilization, and the use of more drug types to treat depression (Hitchon et al., 2021). Thus, some patients have to reduce the cost of RA treatment, which aggravates RA. In a word, RA is a risk factor for depression, and depression can exacerbate the severity of RA. The two diseases fed on each other, pushing the patients into a vicious cycle of "RA-depression-adverse outcomes-social and economic burden".

2.1. Physical and mental symptoms

Pain is the most typical symptom of RA. Even if the inflammation has been controlled, patients often experience chronic pain. A German cross-sectional study based on data from nationwide statutory health insurance fund (BARMER GEK) reported that depressive symptoms were far more likely to develop in RA patients with severe pain (75.3%) than in those with moderate pain (53.1%) or mild/no pain (21.0%; Jobski et al., 2017).

Fatigue is a common mental symptom in RA patients. Approximately one-sixth of RA patients experience severe fatigue, which is related to pain, personality characteristics, gender, sleep, social support, and comorbidities (Nikolaus et al., 2013). In addition, it is affected by drugs such as methotrexate (Pope, 2020). A study investigating risk factors for depression and deterioration of depressive symptoms in 2018 indicated that depression and depressive symptom deterioration in RA positively correlated with the degree of fatigue [odds ratio (OR) 1.26] (Cheon et al., 2018). These results suggest that doctors need to pay more attention to the possibility of depression for RA patients who are prone to fatigue symptoms.

2.2. Drugs

The finding that recurrent depressive disorder without antidepressant treatment is a significant predictor of the progression of joint destruction in RA suggests both RA and depression have to be taken into account in the treatment for RA-associated depression (Abramkin et al., 2020). Similarly, it is necessary to be aware of the possibility of depression caused by the drugs for RA treatment. Among the drugs currently used to treat RA, glucocorticoids (GCs) and infliximab need attention. GCs have a series of biological effects, including anti-inflammation, immunosuppression, regulation of metabolism, and cognitive signal transduction (Scherholz et al., 2019). Exogenous GCs supplementation is a conventional treatment for RA. Long-term exposure to exogenous GCs can also cause some severe adverse effects however, such as infection, osteoporosis, cushing syndrome, and some emotional disorder symptoms including depression (Pamukcu et al., 2021). A German study compared the physical condition of RA patients whose daily dose of prednisone exceeded 0.5 mg in the past 6 months with others that had not received any GCs therapy in the past 12 months. The results showed that a daily dose of prednisone exceeding 7.5 mg was a threshold for a significant increase in the frequency of depression (Huscher et al., 2009). Through the study of the macaque rhesus model of depression, the mechanism of depression induced by chronic GCs exposure was found to be related to the decrease of hypothalamic-pituitary-adrenal (HPA) axis cortisol level in blood, the increase of hair cortisol concentration, and the decrease of dopamine level in cerebrospinal fluid (Qin et al., 2019).

Another drug with a high possibility of causing depression is infliximab, the first tumor necrosis factor α (TNF-α) antagonist used to treat chronic inflammatory diseases, and characterized by rapid therapeutic effect and high bioavailability. The up-regulation of TNF levels in depressed patients has been demonstrated, therefore, infliximab is used to treat depression also (Rani et al., 2022). In 2014, a clinical study involving 34 RA-associated depression patients showed that infliximab could reduce RA disease activity and improve symptoms of depression (Miwa et al., 2014). However, randomized controlled trials conducted in Canada and the United States showed that infliximab did not significantly reduce depression in adults with bipolar depression compared with a placebo (McIntyre et al., 2019). Infliximab is ineffective in reducing depressive symptoms when used for treatment-resistant depression, which is significantly related to TNF levels. Furthermore, according to the research of the Thillard team, which enrolled 118,528 RA patients, the hazard ratio of developing depression associated with infliximab exposure is 3.49 (Thillard et al., 2020). Compared with infliximab, RA patients who received etanercept had a lower risk of depression, and it is suggested that etanercept may be the more appropriate biologic drug for RA-associated depression (Ng et al., 2020). Although there is still a lack of high-quality research evidence on the risk of depression caused by RA treatment drugs, it is undeniable that the existing evidence may still help clinicians adjust the choice of drugs and improve the benefit-hazard ratio.

2.3. Vitamin D and magnesium deficiency

Vitamins and minerals in a healthy body are maintained at a relatively constant concentration, involved energy metabolism, DNA synthesis, oxidative stress, and neuronal function to support the normal function of bone, muscle, and brain. Once the stability of this concentration is broken, it means the possibility of disease (Tardy et al., 2020). Recent studies have shown that vitamins, magnesium (Mg), zinc, selenium, copper, and other trace elements with antioxidant effects are involved in RA inflammation, whereas

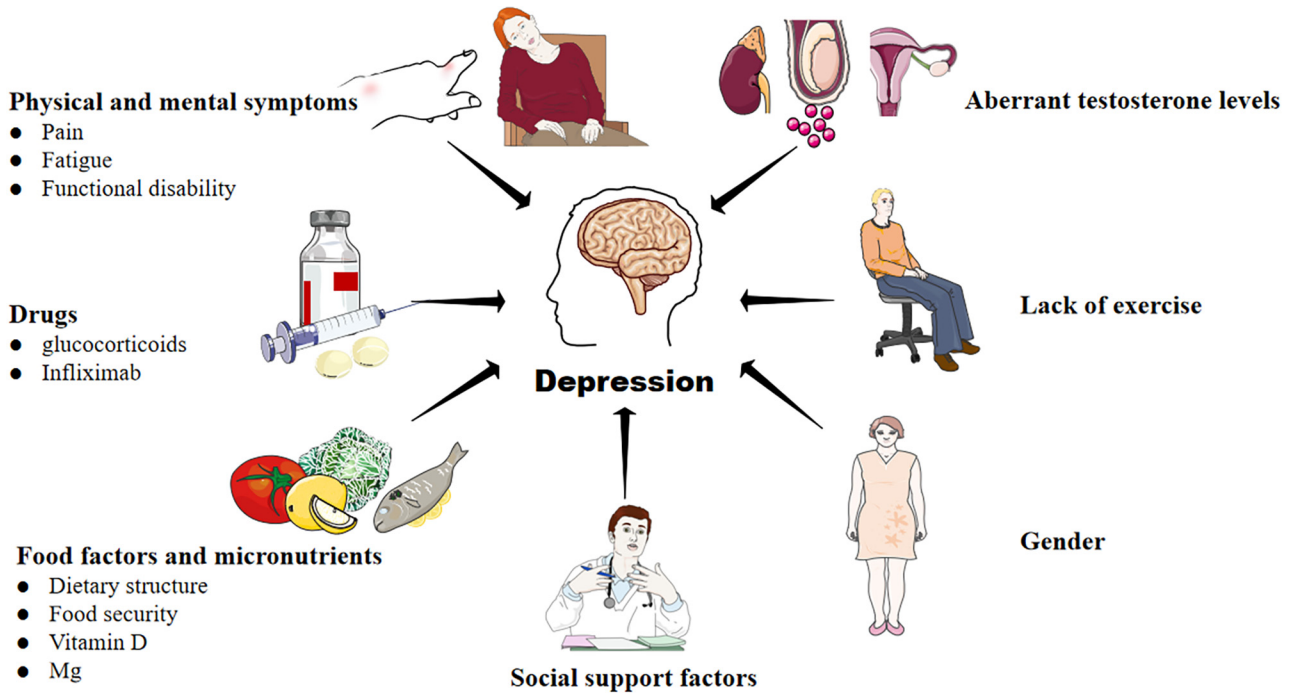


FIGURE 1
Factors that contribute to the development of depression in RA. A variety of complex and comprehensive factors, including physical and mental symptoms, drugs, food and micronutrients, lack of exercise, aberrant testosterone levels, and social support may contribute to depression in RA.

research on RA-associated depression has mainly focused on vitamin D and magnesium.

Vitamin D is a fat-soluble vitamin that can bind to vitamin D receptors in different tissues and cells, and plays an essential role in calcium homeostasis and bone metabolism (Sizar et al., 2022). Evidence suggests that vitamin D can also affect mental health (Föcker et al., 2017). In a 2017 study of 161 RA patients, serum vitamin D levels in those with depression were significantly lower than those without depression, and vitamin D levels were negatively correlated with Hamilton Depression Scale scores and Hamilton Anxiety Scale scores (Pu et al., 2017). The results suggested that vitamin D deficiency may be a risk factor for depression in RA patients.

Mg is an antioxidant micronutrient to improve the function of antioxidant enzymes and reduces inflammatory conditions. 50%–60% of Mg is stored in bone tissue to maintain bone health, and relieve chronic musculoskeletal pain in RA (Arablou et al., 2019; Capozzi et al., 2020; Elma et al., 2020). In terms of brain biochemistry, patients with depression show abnormal glutamate and gamma-aminobutyric acid (GABA) neurotransmission. Mg can increase the expression of GluN2B, a subunit of the glutamatergic n-methyl-D-Aspartate receptor (NMDAR), and inhibit the phosphorylation of eukaryotic elongation factor 2 (eEF2) in cells, antagonize the NMDAR to affect the transmission of glutamate and other neurotransmitters, resulting in antidepressant effects (Górska et al., 2019). Although Mg plays a vital role in the regulation of both inflammation and brain biochemistry, its efficacy in the treatment of RA-associated depression remains controversial. Cross-sectional studies indicated that the dosage of Mg on diet was inversely associated with the risk of RA and depression (Sun et al., 2018; Hu et al., 2020). RA prevalence was kept to a minimum when Mg intake was between 181 and 446 mg/day, and the risk of depression was reduced at 320 mg/day (Li et al., 2017; Hu et al., 2020). However, a prospective study in the SUN Mediterranean cohort with an expanded sample size of 15,836, and an extended follow-up (median = 10.2 years) study confirmed that no significant association between Mg intake and low risk of depression (OR = 0.85, 95% CI: 0.60–1.22; Martínez-González and Sánchez-Villegas, 2016). In addition, low Mg intake has been identified as a protective factor in reducing the risk of depression in older adults (Tarleton and Littenberg, 2015). These results may be influenced by the uncertainty and complexity of causality in cross-sectional studies. More prospective studies are needed to evaluate the effect of magnesium on RA-associated depression in the future.

2.4. Exercise

Sedentary behavior is prevalent in RA patients due to impaired physical function and persistent fatigue (Fenton et al., 2018). Overwhelming data indicate that exercise treatment has a therapeutic effect on various chronic diseases involved in many systems, including the neuropsychiatric system, endocrine system, cardiovascular system, and musculoskeletal system, among others. Reductions in daily physical activity can lead to impaired functionality and premature damage to health (Booth et al., 2012; Pedersen and Saltin, 2015). For RA patients, any exercise will get more clinical benefits than no exercise, whether it is hand exercise, rejoice exercise, Taichi, strength training, aquatic exercise, resistance exercise, or cryotherapy (Hu et al., 2021).

Exercise also has a therapeutic effect on depression. Studies have found that exercise and antidepressant drugs can increase the secretion of brain-derived neurotrophic factor (BDNF), serotonin,

and norepinephrine, enhance the activity of the HPA axis, reduce systemic inflammatory signals to promote the development of new neurons, and strengthen synaptic connections between neurons to alleviate depression. In addition, exercise changed the structure of the hippocampus, anterior cingulate, orbitofrontal cortex, and enriched the blood vessels of the brain (Gujral et al., 2017). Aerobic exercise and/or strength training can significantly relieve depressive symptoms in adults with arthritis and other rheumatic diseases (Kelley et al., 2015). Tai Chi (Waite et al., 2013), Pilates (Yentür et al., 2021), yoga (Bosch et al., 2009), and medium-high intensity exercise (Kucharski et al., 2019) were also influential. What calls for special attention is that some patients may have limited exercise patterns due to arthritis or functional impairment. To maximize the benefit, the intensity, frequency, and cycle of exercise should be formulated according to individual symptoms and wishes. It poses a greater challenge to the professionalism of healthcare personnel and the improvement of social movement facilities.

2.5. Diet

The involvement of dietary structure in the pathogenesis of depression in general population has long been confirmed by clinical studies. In 2021, scholars introduced this concept into the field of RA, and results showed that the eating habits of RA patients were also associated the occurrence of depression. It has been demonstrated that among 20 foods, including vegetables, grains, meat, fish, and fruits, intake of fish, vegetables, and fruits were inversely related to depression scores in RA patients with frequent intake of fish ($\geq$3 times per week). Improving eating habits, especially increasing intake of fish may contribute to alleviating depression in RA patients (Minamino et al., 2021). In addition to dietary structure, food source security is highly correlated with the development of depression in RA, and their correlation becomes much more conspicuous as soon as food safety declines. Compared to RA patients with complete food security, food-insecure patients had a significantly higher risk of depression (OR = 2.96, 95% CI: 1.48–5.90; Cai et al., 2022). Once food insecurity is improved, the statistical significance of the correlation gradually declines.

2.6. Gender

Patients with RA are at greater risk for severe depression than gender-matched healthy individuals (Khan et al., 2021), and the risk within the RA group is reflected in the higher risk of depression in females compared to males (Albrecht, 2014; Kim et al., 2020). In addition, there are also gender-related differences in the causes of severity of depression among RA patients. According to McQuillan et al. (2022), RA functional disability is more strongly associated with depression in males than in females. Depressive symptoms in female patients appear to be more closely related to poor sleep quality or family pressure (Hughes et al., 2021; Hamasaki et al., 2022).

2.7. Aberrant testosterone levels

Testosterone is a sex hormone synthesized in the gonads and the adrenal gland. In genera, testosterone levels are significantly higher in men than in women. Previous studies have shown that testosterone has an immunosuppressive effect, which can inhibit the onset of RA to an extent. A decline in testosterone level is related to RF-negative RA, and may also induce depression (Pikwer et al., 2014; Gubbels Bupp and Jorgensen, 2018; Walther et al., 2019; Maharjan et al., 2021). However, there is also evidence that excessive testosterone can have adverse effects on mental and physical health. In a two-sample Mendelian randomization study conducted in 2021 abnormally high testosterone level is associated with a risk of RA and depression (Syed et al., 2020). Thus, aberrant fluctuation of testosterone may contribute to RA and depression.

2.8. Social support

Social tools and social emotional support are independent factors affecting the severity of depression in RA. Based on DAS28 score, an analysis of psychosocial characteristics in RA patients with and without remission showed that emotional support had a significantly beneficial effect on the severity of depressive symptoms in RA in remission, whereas instrumental support had an extremely limited effect. In the non-remission group, the positive regulatory effect of instrumental support was relatively significant, and emotional support was also helpful for depression (Yasuoka et al., 2021). The results indicated that the treatment of RA-associated depression should not focus solely on the medical control of disease activity by doctors, but should also recognize the need for social support to cover instrumental and emotional to improve overall physical and mental wellbeing (Khan et al., 2021).

3. Pathology of interaction between RA and depression

Although the mechanism of interaction between RA and depression is still unclear, some previous findings have provided insight into directions for further investigation. As an immune-mediated inflammatory disease, RA has associated the abnormal expression of pro-inflammatory and anti-inflammatory mediators induced by an imbalance in immune tolerance. Similarly, depression is associated with abnormal activation of the immune system and inflammatory responses (Beurel et al., 2020). Depressed patients are likely to exhibit increases in neutrophil/lymphocyte, platelet/lymphocyte, monocyte/lymphocyte ratios (Marazziti et al., 2021), and a shift from classical monocytes toward non-classical monocytes (Hasselmann et al., 2018). Serum interleukin 6 (IL-6), TNF, and C-reactive protein (CRP) were also higher in depressed patients than in a healthy control group (Beurel et al., 2020). The levels of IL-6 and TNF in cerebrospinal fluid, and translocator protein (PET marker of central inflammation) in the anterior cingulate cortex and temporal cortex are higher in MDD patients when compared to controls, suggesting that central inflammation may be involved in MDD (Enache et al., 2019). Notably, there is significant heterogeneity in the levels of circulating inflammatory factors in patients with depression, and this heterogeneity is also reflected in responses to antidepressants (Liu J. J. et al., 2020). For example, increased CRP is seen in resistant MDD, rather than in depressed patients generally (Chamberlain et al., 2019). It seems that as well as immune

disorder and inflammation, other mechanisms are also involved in the interaction between RA and depression.

3.1. Immune inflammatory stimulation

The stimulating effects of peripheral inflammation in RA on the central nervous system (CNS) are regarded as the main triggering mechanism of depression. The inflammatory bias is one of the most important mechanisms that connect the two diseases. A genetically-based inflammatory bias that arose during early human evolution is critical for humans to fight infection, heal wounds, and maintain vigilance to attack. This inflammatory bias is suppressed by regulatory T (Treg) cells, regulatory B (Breg) cells, and immunoregulatory M2 macrophages, as well as the anti-inflammatory cytokines interleukin-10 (IL-10) and transforming growth factor β (TGF-β) in the rural environment. In modern society, psychological challenges have been increasing along with the decline in infectious challenges that have left the former immune checks and balances lacking. These psychological challenges stimulate the overproduction of inflammasome in myeloid cells, which mediates responses to non-pathogenic or "sterile" stressors and leads to the development of a variety of disorders, including depression (Miller and Raison, 2016). In previous studies, the activation of inflammasome also plays a crucial role in immune dysregulation and joint inflammation (Jiang et al., 2022). NLRP3 inflammasome expression in the synovium is increased in collagen-induced arthritis (CIA) model, and targeted inhibition of NLRP3 activation, contributes to inhibiting the progression of RA (Zhang et al., 2016; Liu P. et al., 2020).

Peripheral inflammatory signals can reach the brain through humoral and neural pathways. There are three types of humoral pathways. First, pro-inflammatory cytokines can cross the blood-brain barrier (BBB), and contact the brain *via* periventricular organs and the choroid plexus. Second, TNF and other inflammatory mediators bind to cytokine receptors on the membranes of cerebrovascular endothelial cells directly, activate a second messenger, are transported into the CNS, which leads to the activation of microglia and the subsequent secretion of pro-inflammatory factors in the brain. Third, blood-derived immune cells and pro-inflammatory cytokines can access the brain through a damaged BBB (Süß et al., 2020). A recent study found that microglia in the area postrema (a brain region lacking a BBB) significantly increased in density and kept highly activated during persistent autoimmune arthritis, which demonstrates that chronic inflammation in RA may affect microglia in brain regions lacking a BBB and result in CNS-mediated symptoms, such as depression (Matsushita et al., 2021).

In the neural pathways, pro-inflammatory mediators stimulate active primary afferent nerves to transmit peripheral inflammation to the CNS (Fakra and Marotte, 2021). The immune system and nervous system can communicate with each other mainly depending on the activation of the HPA axis by pro-inflammatory cytokines and afferent vagal fibers. Cytokines also directly impact the cerebral cortex and nuclei in the brain stem (Ingegnoli et al., 2020). How the vagus transduces inflammatory signals to the CNS and causes depression is still not fully understood. Vagus nerve stimulation is routinely used in the clinic to treat depression. It has been shown that severing the connections between the nucleus of the solitary tract (NTS) and the higher brain regions can reduce stimulation-induced activation for NTS neurons receiving myelinated vagal input, suggesting that higher brain regions play a significant role in maintaining both regular activity in NTS and indirect mechanisms of enhancing NTS neuronal activity during vagus nerve stimulation (Cooper et al., 2021). The study indirectly explains the vagal pathway that transmits inflammatory signals to the CNS. Once the level of inflammatory cytokines in the CNS increases, activated indoleamine 2,3-dioxygenase may enhance tryptophan catabolism, inducing serotonin depletion and kynurenine production. Kynurenine is then further transformed to 3-hydroxykynurenine and quinolinic acid, which can lead to an elevated glutamate level and oxidative stress response, reduce GABAergic inhibitory control, and cause apoptosis in the hippocampal and medial prefrontal cortex (Belleau et al., 2019). Previous studies have also shown that IL-1β and TNF reduced serotonin levels by activating serotonin transporters, ultimately causing a depressive state (Zhu et al., 2006; Malynn et al., 2013). Furthermore, pro-inflammatory cytokines can affect synaptic plasticity and neurogenesis by reducing the expression of brain-derived neurotrophic factors, resulting in structural and functional alteration of the brain (Calabrese et al., 2014). Cortical neural circuits involved in emotion and stress regulation trigger depression under the above triple stimuli (Fakra and Marotte, 2021). The over-active HPA axis, which is wildly associated with depression, can also be induced by pro-inflammatory cytokines. Adzic et al. (2015) have already shown that depressive-like behavior caused by lipopolysaccharide-inducing peripheral inflammation in rats emerges from HPA axis activation and sex-specific alterations of hypothalamic molecular signaling. Interestingly, it is found that MDD can in turn trigger pro-inflammatory shifts in monocyte subsets and decrease the expression of steroid signaling-related genes (Hasselmann et al., 2018). Under stress, upregulated calcium/calmodulin-dependent protein kinase II in the hippocampus will promote the transcription and expression of cyclooxygenase-2, enhance the level of the pro-inflammatory factor prostaglandin E2, and aggravate RA joint synovial inflammation (Vallerand et al., 2019). Furthermore, depression is associated with the overactivation of the sympathetic nervous system (SNS; Bucciarelli et al., 2020). It has been demonstrated that RA patients frequently have an unbalanced autonomic nervous system, with decreased parasympathetic and increased sympathetic tone (Koopman et al., 2011). In a murine model of lymphoproliferative disease, the SNS induces apoptosis in immunosuppressive CD4(+) Foxp3(+) regulatory T cells, which suggests overactive SNS driven by depression can lead to RA *via* peripheral immune activation (Wirth et al., 2014).

3.2. Signal pathways

In the bi-directional feedback between RA and depression, the transduction of immune and inflammatory signals inside and outside cells is mainly completed by JAK/STAT and MAPK signal pathways. JAK/STAT is a rapid membrane nuclear signal module composed of transcription factors of the Janus kinase family and the STAT family, which regulate the pathological and physiological processes of RA by mediating interferon (Villarino et al., 2017). The JAK/STAT pathway is driven by pro-inflammatory cytokines, leading to elevated expression of the matrix metalloproteinase gene, accelerated chondrocyte apoptosis, and decreased apoptosis resistance in inflamed synovial tissue, which plays a critical role in the development of RA (Malemud, 2018). Cytokines can also activate

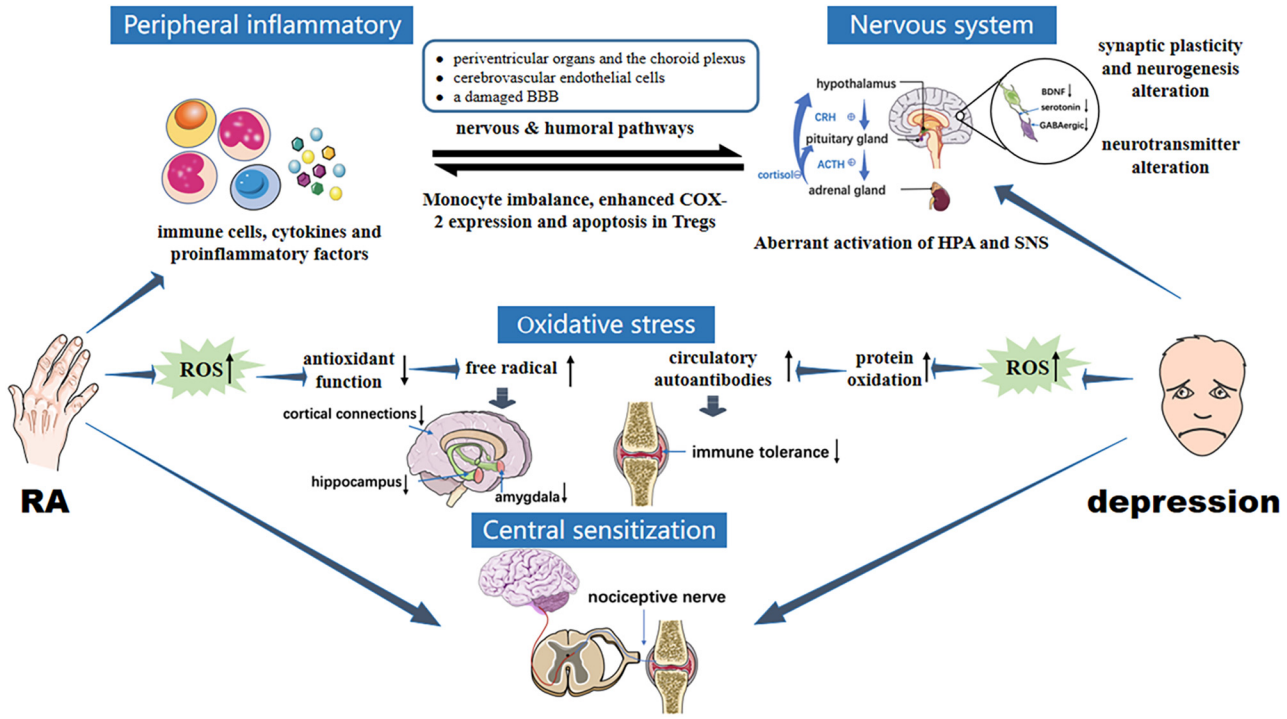


FIGURE 2
Pathology of interaction between RA and depression. RA immune tolerance imbalance induces abnormal expression of inflammatory mediators, activated peripheral inflammatory signals enter the brain through humoral and neural pathways. CNS inflammation is induced, as are overactivation of HPA, changes in brain structure and function, upregulation of glutamate levels, reduced GABA expression and brain-derived neurotrophic factors, enhanced oxidative stress, and increased ROS levels, leading to depression. In the two-way feedback between RA and depression, the transduction of immune and inflammatory signals in and out of cells mainly involves JAK/STAT and MAPK signaling cascades. In addition, central sensitization enhances pain perception and aggravates depression under the stimulus of chronic inflammation in RA. Oversensitivity caused by depression in turn exacerbates pain, creating a vicious circle between RA and depression.

indoleamine 2,3-dioxygenase in glial cells by stimulating STAT1, leading to a reduced source of serotonin production and subsequent depression (Yan et al., 2018). MAPK is a group of threonine/serine protein kinases that transduce extracellular stimuli to the nucleus. In RA, inflammatory factors activate the MAPK signaling pathway, causing synovial tissue proliferation and joint destruction. It can also accelerate the clearance of serotonin in synapses *via* the p38 MAPK signaling pathway, enhance glucocorticoid resistance, cause synaptic plasticity imbalance, and ultimately lead to depression (Malemud and Miller, 2008).

3.3. Oxidative stress

Oxidative stress is a pathological state of redox imbalance caused by increased production of reactive oxygen species (ROS) and/or decreased antioxidant capacity (Salim, 2017), which produces free radicals that act as oxidants and inflammatory mediators involved in RA pathology. Excessive ROS in RA patients can reduce the function of free radical enzyme defense systems, lead to a rapid increase in free radical levels, aggravate weakening effects on the hippocampus, amygdala, and cortex connection, and eventually accelerate the occurrence of depression (Bala et al., 2017; Salim, 2017). Alouffi et al. (2018) found that compared to patients with RA alone, levels of carbonyl (a protein oxidation marker mediated by ROS) were higher in patients with RA and depression. It is speculated that inhibiting the process of oxidative stress in RA will help to reduce the probability of RA-associated depression, or alleviate the degree of depression.

3.4. Central sensitization and pain

Pain is not only the leading cause of the medical behavior of RA, it is also strongly associated with the occurrence of depression (Lwin et al., 2020). Recent studies have shown that the pain symptoms in RA are co-regulated by both the peripheral nervous system and the CNS (Harth and Nielson, 2019). In the pathological process of RA, adaptive and innate immune systems are activated, producing a series of inflammatory mediators. Then, neutrophils, T lymphocytes, and B lymphocytes are driven into the synovium, leading to local synovial inflammation. In the inflammatory environment, fibroblast-like synovial cells secrete nerve growth factors and upregulate the release of substance P, neuropeptide, kinin, IL-6, TNF, and other molecules, sensitizing the nociceptor terminals of inflammatory periarticular tissues and primary afferent neurons, resulting in the production of pain (Walsh and McWilliams, 2014). Central sensitization in the spinal dorsal horn of the cerebrospinal fluid expands and enhances pain perception in the sensory area. Remodeling of inflammatory joint nerve fibers may also contribute to the generation and maintenance of arthritis pain (Gonçalves Dos Santos et al., 2020). Inflammation, the central source of pain in RA, is also closely related to non-inflammatory factors. Researchers have revealed

that cytokines can directly cause central sensitization through the nociceptive nervous system, and reduce the pain threshold, resulting in persistent pain (Schaible, 2014; Sebba, 2021). Although there is no direct correlation between depression and central sensitization, patients with depression are more sensitive to psychological and physical pain than patients without depression (Conejero et al., 2018; **Figure 2**). In RA patients, chronic inflammation impairs physiological stress resistance and effective coping behavior, leading to depression. Hypersensitivity caused by depression will also undoubtedly aggravate pain, and indirectly promote the deterioration of RA. Clinicians should be mindful that anti-depressants is considered if pain symptoms persist after early, standardized, combined DMARDs, NSAIDs, and GC treatment, if patients have achieved remission but still experience joint pain based on the DAS28 score (Zhang and Lee, 2018).

4. Biological therapies for RA-associated depression

The treatment of depression mainly includes drug therapies, psychological intervention, and comprehensive nursing. In a study conducted by Yasuoka et al. (2021), the depressive symptoms of RA patients in remission (DAS28 score < 2.6) could be significantly improved by emotional support, but whether this applies to non-remission patients remains uncertain. From this study, there is general uncertainty about the efficacy of non-pharmacological therapies for depression with RA. With the role of cytokines in the pathological mechanism of RA-associated depression gradually being discovered, the value of biological agents in the treatment of RA and depression has become a hot research topic (**Table 1**).

4.1. bDMARDs

Variation in responses to conventional antidepressants is a recognized limitation of evidence-based pharmacotherapy for MDD. Shariq et al. (2018) reported that cytokine blockade effectively improved the therapeutic efficacy of MDD patients with immune dysfunction, which cannot be achieved by conventional antidepressants alone. Another large-scale, self-controlled study including 18,241 RA-associated depressed patients showed that 20% to 40% of patients who received biologics improved their depressive symptoms after 1 year. Patients with pre-existing depressive symptoms who received biologics for the first time had a lower rate of response to treatment (Matcham et al., 2018). In addition, females, younger ages, and lower baseline HAMD scores were positive factors for improving the response rate to biological disease modifying anti-rheumatic drugs (bDMARDs; Miwa et al., 2018).

It should be noted that bDMARDs are not an absolute advantage in the effect of RA-associated depression, some studies have reached the opposite conclusion. A randomized controlled trial involving 90 patients (Yayikci and Karadag, 2019) indicated that bDMARDs were not better than conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs) for the treatment of RA-associated depression. Compared with methotrexate, leflunomide, and hydroxychloroquine, bDMARDs are associated with high rates of depression, anxiety, and suicide in RA patients (Pinho de Oliveira Ribeiro et al., 2013). These results showed that the efficacy of biological agents for RA-associated depression is controversial, but the specified types of biological agents are not clear in these studies, which may be one of the reasons for the controversial conclusions. Of course, there are also some studies on specific biological agents that may provide more valuable evidence.

4.2. Anti-TNF

The levels of TNF in RA patients with depression generally rise (Köhler et al., 2017; Enache et al., 2019). Compared with those who do not respond to anti-TNF treatment, those who do respond to it take a lower risk of depression (Deb et al., 2019). Accordingly, depression may be a predictor of no response or a poor response after 3–6 months of anti-TNF or methotrexate treatment of RA. Infliximab was the first anti-TNF drug used in RA. Early studies found that infliximab could reduce disease activity and improve depression in RA patients (Michelsen et al., 2017). In a 2020 systematic retrospective meta analysis of four randomized controlled studies, infliximab did not have any therapeutic effects on depressive symptoms in RA patients (Bavaresco et al., 2020), and it even seemed to induce suicidal tendencies in a subset of RA patients according to a French retrospective cohort study (Thillard et al., 2020).

4.3. IL-6 antibody

IL-6 antibody may have a positive effect on mental health in RA patients. Tocilizumab was the first humanized anti IL-6 receptor monoclonal antibody approved for treating RA refractory to methotrexate or TNF inhibitors. The weekly use of tocilizumab *via* subcutaneous injection has been widely claimed to improve depression in RA patients (Figueiredo-Braga et al., 2018; Tiosano et al., 2020). In contrast to tocilizumab, which targets the IL-6 receptor, sirukumab, and siltuximab directly antagonize IL-6 and block its function. Two-phase double-blind placebo-controlled trials to evaluate the efficacy of sirukumab and siltuximab in RA patients with depression showed that both drugs could improve depressive symptoms, even in patients who did not respond to RA treatment (Sun et al., 2017). However, safety may be a considerably important issue. In the phase 3 double-blind sirukumab study, the respective incidences of adverse events and serious adverse events were 93.4% and 7.4% (Takeuchi et al., 2018).

In summary, compared with non-biological therapies, the efficacy of biological agents in RA patients with depression is still controversial. They may even be associated with more severe depression, anxiety, and suicidal tendencies. Moreover, biotherapies may also lead to adverse effects such as tumors, abnormal blood parameters, infection, and allergy. Hence, the value of biological agents for the treatment of RA-associated depression requires further research.

5. Conclusion and prospects

RA can be associated with various comorbidities, among which depression has attracted much attention due to its high incidence

TABLE 1 Biotherapies for RA-associated depression.

Research type	Intervention	Number of subjects	Time	Results	Reference
Randomized control trial	bDMARDS vs. csDMARDS	90	24 w	bDMARDs were not superior to csDMARDs with regard to their effects on anxiety and depression in patients with RA.	Yayikci and Karadag (2019)
Randomized control trial	bDMARDs vs. MTX vs. LEF vs. HCQ	105	unclear	RA with bDMARDs had the high rate of depression compared with MTX, LEF, and HCQ.	Pinho de Oliveira Ribeiro et al. (2013)
Self-control trial	bDMARDS	18,241	48 w	Depressive symptoms improved by 20%–40% after 1 year of biologic therapy, but preexisting depressive symptoms at the time of receiving the first biologic may reduce the chance of treatment response.	Matcham et al. (2018)
Retrospective study	bDMARDS	152	24 w	Younger patients with lower depression scores at baseline can achieve depressive remission with bDMARDs.	Miwa et al. (2018)
Retrospective observational cohort study	Anti-TNF	4,222	48 w	RA patients who responded to anti-TNF had significantly lower risk of depression	Deb et al. (2019)
Prospective cohort study	Anti-TNF vs. MTX	1,326	48 w	Depression was a strong negative predictor of disease remission in patients with RA after 3 and 6 months of anti-TNF or MTX treatment.	Michelsen et al. (2017)
Systematic review and meta-analysis	Infliximab vs. placebo	152	12 w	There was no statistically significant effect of infliximab as an adjuvant treatment for treatment-resistant depression.	Bavaresco et al. (2020)
Retrospective cohort study	Infliximab	7,600	unclear	Infliximab treatment increased the risk of adverse events of mental illness, and may increase suicidal tendencies in RA patients.	Thillard et al. (2020)
Case control study	Tocilizumab vs. non-biological systemic treatments	82	4 w	High IL-10 in RA is associated with an increased risk of depression, tocilizumab can reduce depressive symptoms.	Figueiredo-Braga et al. (2018)
Multi-center, single-arm study	Tocilizumab	91	24 w	Tocilizumab treatment may be significantly associated with improvement RA-associated depression.	Tiosano et al. (2020)

Note: This table summarizes research of bDMARDs for the treatment of RA-associated depression. HCQ, hydroxychloroquin; LEF, leflunomide; MTX, methotrexate; w, weeks.

10.3389/fnbeh.2022.992223

and seriousness. The etiology and pathological mechanism of RA- associated depression are complex, in addition to somatic symptoms, drugs, diet, and exercise habits, vitamin D deficiency, Mg deficiency, abnormal testosterone levels, social support, and RA disease activity itself may induce or aggravate the depression, resulting in a vicious circle of "RA-depression-adverse outcomes-social and economic burden". Immune imbalance and inflammatory stimulation are important pathological mechanisms leading to the bidirectional association between RA and depression. Taking these factors into consideration when choosing a treatment regimen will help with disease remission. At present, some studies have attempted to use biological agents for the efficacy of RA-associated depression, but there is no consensus. Doctors should be alert to the possible risks of biological agents. In the future, larger sample, multi-center, higher-level evidence-based studies related to biologics are needed to provide high-quality evidence for clinical decision-making pertaining to biologics for treating of RA-associated depression.

Author contributions

NL and WY wrote the manuscript. RS and LZ identified and retrieved the original documents. XW drew the figure. DQ and ZL revised the manuscript. JP raised the idea for the article. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by the National Natural Science Foundation of China (No. 81960863, No. 82160901) and Yunnan Clinical Research Center for Rheumatism of Traditional Chinese Medicine (No. 202002AA310005).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abramkin, A. A., Lisitsyna, T. A., Veltishchev, D. Y., Seravina, O. F., Kovalevskaya, O. B., Glukhova, S. I., et al. (2020). Depression and severity of articular destruction in patients with rheumatoidarthritis. *Terapevticheskii arkhiv* 92, 22–32. doi: 10.26442/00403660.2020.05.000624

Adzic, M., Djordjevic, J., Mitic, M., Brkic, Z., Lukic, I., and Radojcic, M. (2015). The contribution of hypothalamic neuroendocrine, neuroplastic and neuroinflammatory processes to lipopolysaccharide-induced depressive-like behaviour in female and male rats: involvement of glucocorticoid receptor and C/EBP-β. *Behav. Brain Res.* 291, 130–139. doi: 10.1016/j.bbr.2015.05.029

Albrecht, K. (2014). Gender-specific differences in comorbidities of rheumatoid arthritis. *Z Rheumatol.* 73, 607–614. doi: 10.1007/s00393-014-1410-3

Alouffi, S., Sherwani, S., Al-Mogbel, M. S., Sherwani, M. K. A., and Ali Khan, M. W. (2018). Depression and smoking augment the production of circulating autoantibodies against glycated HSA in rheumatoid arthritis patients. *Int. Arch. Allergy Immunol.* 177, 170–180. doi: 10.1159/000489896

Arablou, T., Aryaeian, N., Djalali, M., Shahram, F., and Rasouli, L. (2019). Association between dietary intake of some antioxidant micronutrients with some inflammatory and antioxidant markers in active rheumatoid arthritis patients. *Int. J. Vitam. Nutr. Res.* 89, 238–245. doi: 10.1024/0300-9831/a000255

Baerwald, C., Manger, B., and Hueber, A. (2019). Depression as comorbidity of rheumatoid arthritis. *Z Rheumatol.* 78, 243–248. doi: 10.1007/s00393-018-0568-5

Bala, A., Mondal, C., Haldar, P. K., and Khandelwal, B. (2017). Oxidative stress in inflammatory cells of patient with rheumatoid arthritis: clinical efficacy of dietary antioxidants. *Inflammopharmacology* 25, 595–607. doi: 10.1007/s10787-017-0397-1

Bavaresco, D. V., Uggioni, M. L. R., Ferraz, S. D., Marques, R. M. M., Simon, C. S., Dagostin, V. S., et al. (2020). Efficacy of infliximab in treatment-resistant depression: a systematic review and meta-analysis. *Pharmacol. Biochem. Behav.* 188:172838. doi: 10.1016/j.pbb.2019.172838

Belleau, E. L., Treadway, M. T., and Pizzagalli, D. A. (2019). The impact of stress and major depressive disorder on hippocampal and medial prefrontal cortex morphology. *Biol. Psychiatry* 85, 443–453. doi: 10.1016/j.biopsych.2018.09.031

Beurel, E., Toups, M., and Nemeroff, C. B. (2020). The bidirectional relationship of depression and inflammation: double trouble. *Neuron* 107, 234–256. doi: 10.1016/j.neuron.2020.06.002

Booth, F. W., Roberts, C. K., and Laye, M. J. (2012). Lack of exercise is a major cause of chronic diseases. *Compr. Physiol.* 2, 1143–1211. doi: 10.1002/cphy.c110025

Bosch, P. R., Traustadóttir, T., Howard, P., and Matt, K. S. (2009). Functional and physiological effects of yoga in women with rheumatoid arthritis: a pilot study. *Altern. Ther. Health Med.* 15, 24–31. doi: 10.1016/j.ctim.2020.102639

Bucciarelli, V., Caterino, A. L., Bianco, F., Caputi, C. G., Salerni, S., Sciomer, S., et al. (2020). Depression and cardiovascular disease: the deep blue sea of women's heart. *Trends Cardiovasc. Med.* 30, 170–176. doi: 10.1016/j.tcm.2019.05.001

Cai, Q., Pesa, J., Wang, R., and Fu, A. Z. (2022). Depression and food insecurity among patients with rheumatoid arthritis in NHANES. *BMC Rheumatol.* 6:6. doi: 10.1186/s41927-021-00236-w

Calabrese, F., Rossetti, A. C., Racagni, G., Gass, P., Riva, M. A., and Molteni, R. (2014). Brain-derived neurotrophic factor: a bridge between inflammation and neuroplasticity. *Front. Cell Neurosci.* 8:430. doi: 10.3389/fncel.2014.00430

Capozzi, A., Scambia, G., and Lello, S. (2020). Calcium, vitamin D, vitamin K2 and magnesium supplementation and skeletal health. *Maturitas* 140, 55–63. doi: 10.1016/j.maturitas.2020.05.020

Chamberlain, S. R., Cavanagh, J., de Boer, P., Mondelli, V., Jones, D. N. C., Drevets, W. C., et al. (2019). Treatment-resistant depression and peripheral C-reactive protein. *Br. J. Psychiatry* 214, 11–19. doi: 10.1192/bjp.2018.66

Cheon, Y. H., Lee, S. G., Kim, M., Kim, H. O., Sun, S. Y., Park, K. S., et al. (2018). The association of disease activity, pro-inflammatory cytokines and neurotrophic factors with depression in patients with rheumatoid arthritis. *Brain Behav. Immun.* 73, 274–281. doi: 10.1016/j.bbi.2018.05.012

Conejero, I., Olié, E., Calati, R., Ducasse, D., and Courtet, P. (2018). Psychological pain, depression and suicide: recent evidences and future directions. *Curr. Psychiatry Rep.* 20:33. doi: 10.1007/s11920-018-0893-z

Cooper, C. M., Farrand, A. Q., Andresen, M. C., and Beaumont, E. (2021). Vagus nerve stimulation activates nucleus of solitary tract neurons *via* supramedullary pathways. *J. Physiol.* 599, 5261–5279. doi: 10.1113/JP282064

Cross, M., Smith, E., Hoy, D., Carmona, L., Wolfe, F., Vos, T., et al. (2014). The global burden of rheumatoid arthritis: estimates from the global burden of disease 2010 study. *Ann. Rheum. Dis.* 73, 1316–1322. doi: 10.1136/annrheumdis-2013-204627

Deb, A., Dwibedi, N., LeMasters, T., Hornsby, J. A., Wei, W., and Sambamoorthi, U. (2019). Tumor necrosis factor inhibitor therapy and the risk for depression among working-age adults with rheumatoid arthritis. *Am. Health Drug Benefits* 12, 30–38.

Elma, Ö., Yilmaz, S. T., Deliens, T., Coppieters, I., Clarys, P., Nijs, J., et al. (2020). Do nutritional factors interact with chronic musculoskeletal pain? a systematic review. *J. Clin. Med.* 9:702. doi: 10.3390/jcm9030702

Enache, D., Pariante, C. M., and Mondelli, V. (2019). Markers of central inflammation in major depressive disorder: a systematic review and meta-analysis of studies examining cerebrospinal fluid, positron emission tomography and post-mortem brain tissue. *Brain Behav. Immun.* 81, 24–40. doi: 10.1016/j.bbi.2019.06.015

Fakra, E., and Marotte, H. (2021). Rheumatoid arthritis and depression. *Joint Bone Spine* 88:105200. doi: 10.1016/j.jbspin.2021.105200

Fenton, S. A. M., Veldhuijzen van Zanten, J. J. C. S., Duda, J. L., Metsios, G. S., and Kitas, G. D. (2018). Sedentary behaviour in rheumatoid arthritis: definition, measurement and implications for health. *Rheumatology (Oxford)* 57, 213–226. doi: 10.1093/rheumatology/kex053

Figueiredo-Braga, M., Cornaby, C., Cortez, A., Bernardes, M., Terroso, G., Figueiredo, M., et al. (2018). Influence of biological therapeutics, cytokines and disease activity on depression in rheumatoid arthritis. *J. Immunol. Res.* 2018:5954897. doi: 10.1155/2018/5954897

Föcker, M., Antel, J., Ring, S., Hahn, D., Kanal, Ö., Öztürk, D., et al. (2017). Vitamin D and mental health in children and adolescents. *Eur. Child Adolesc Psychiatry* 26, 1043–1066. doi: 10.1186/s12981-022-00499-4

Fu, X., Li, Z. J., Yang, C. J., Feng, L., Sun, L., Yao, Y., et al. (2017). The prevalence of depression in rheumatoid arthritis in china: a systematic review. *Oncotarget* 8, 53623–53630. doi: 10.18632/oncotarget.17323

Gonçalves Dos Santos, G., Jimenéz-Andrade, J. M., Woller, S. A., Muñoz-Islas, E., Ramírez-Rosas, M. B., Ohashi, N., et al. (2020). The neuropathic phenotype of the K/BxN transgenic mouse with spontaneous arthritis: pain, nerve sprouting and joint remodeling. *Sci. Rep.* 10:15596. doi: 10.1038/s41598-020-72441-5

Górska, N., Słupski, J., Szałach, Ł. P., Włodarczyk, A., Szarmach, J., Jakuszkowiak-Wojten, K., et al. (2019). Magnesium and ketamine in the treatment of depression. *Psychiatr. Danub.* 31, 549–553. doi: 10.1186/s13063-022-07010-4

Gubbels Bupp, M. R., and Jorgensen, T. N. (2018). Androgen-induced immunosuppression. *Front. Immunol.* 9:794. doi: 10.3389/fimmu.2018.00794

Gujral, S., Aizenstein, H., Reynolds, C. F., 3rd, Butters, M. A., and Erickson, K. I. (2017). Exercise effects on depression: possible neural mechanisms. *Gen. Hosp. Psychiatry* 49, 2–10. doi: 10.1016/j.genhosppsych.2017.04.012

Hamasaki, M., Origuchi, T., and Matsuura, E. (2022). Factors associated with depressive symptoms in Japanese women with rheumatoid arthritis. *Rheumatol. Adv. Pract.* 6:rkac006. doi: 10.1093/rap/rkac006

Harth, M., and Nielson, W. R. (2019). Pain and affective distress in arthritis: relationship to immunity and inflammation. *Exp. Rev. Clin. Immunol.* 15, 541–552. doi: 10.1080/1744666X.2019.1573675

Hasselmann, H., Gamradt, S., Taenzer, A., Nowacki, J., Zain, R., Patas, K., et al. (2018). Pro-inflammatory monocyte phenotype and cell-specific steroid signaling alterations in unmedicated patients with major depressive disorder. *Front. Immunol.* 9:2693. doi: 10.3389/fimmu.2018.02693

Hitchon, C. A., Walld, R., Peschken, C. A., Bernstein, C. N., Bolton, J. M., El-Gabalawy, R., et al. (2021). Impact of psychiatric comorbidity on health care use in rheumatoid arthritis: a population-based study. *Arthritis Care Res. (Hoboken)* 73, 90–99. doi: 10.1002/acr.24386

Hu, C., Zhu, F., Liu, L., Zhang, M., and Chen, G. (2020). Relationship between dietary magnesium intake and rheumatoid arthritis in US women: a cross-sectional study. *BMJ Open* 9:e039640. doi: 10.1136/bmjopen-2020-039640

Hu, H., Xu, A., Gao, C., Wang, Z., and Wu, X. (2021). The effect of physical exercise on rheumatoid arthritis: an overview of systematic reviews and meta-analysis. *J. Adv. Nurs.* 77, 506–522. doi: 10.1111/jan.14574

Hughes, M., Chalk, A., Sharma, P., Dahiya, S., and Galloway, J. (2021). A cross-sectional study of sleep and depression in a rheumatoid arthritis population. *Clin. Rheumatol.* 40, 1299–1305. doi: 10.1007/s10067-020-05414-8

Huscher, D., Thiele, K., Gromnica-Ihle, E., Hein, G., Demary, W., Dreher, R., et al. (2009). Dose-related patterns of glucocorticoid-induced side effects. *Ann. Rheum. Dis.* 68, 1119–1124. doi: 10.1136/ard.2008.092163

Ingegnoli, F., Buoli, M., Antonucci, F., Coletto, L. A., Esposito, C. M., and Caporali, R. (2020). The link between autonomic nervous system and rheumatoid arthritis: from bench to bedside. *Front. Med. (Lausanne)* 7:589079. doi: 10.3389/fmed.2020.589079

Jiang, Q., Wang, X., Huang, E., Wang, Q., Wen, C., Yang, G., et al. (2022). Inflammasome and its therapeutic targeting in rheumatoid arthritis. *Front. Immunol.* 12:816839. doi: 10.3389/fimmu.2021.816839

Jobski, K., Luque Ramos, A., Albrecht, K., and Hoffmann, F. (2017). Pain, depressive symptoms and medication in german patients with rheumatoid arthritis-results from the linking patient-reported outcomes with claims data for health services research in rheumatology (PROCLAIR) study. *Pharmacoepidemiol. Drug Saf.* 26, 766–774. doi: 10.1002/pds.4202

Kelley, G. A., Kelley, K. S., and Hootman, J. M. (2015). Effects of exercise on depression in adults with arthritis: a systematic review with meta-analysis of randomized controlled trials. *Arthritis Res. Ther.* 17:21. doi: 10.1186/s13075-015-0533-5

Khan, A., Pooja, V., Chaudhury, S., Bhatt, V., and Saldanha, D. (2021). Assessment of depression, anxiety, stress and quality of life in rheumatoid arthritis patients and comparison with healthy individuals. *Ind. Psychiatry J.* 30, S195–S200. doi: 10.4103/0972-6748.328861

Kim, S. Y., Chanyang, M., Oh, D. J., and Choi, H. G. (2020). Association between depression and rheumatoid arthritis: two longitudinal follow-up studies using a national sample cohort. *Rheumatology (Oxford)* 59, 1889–1897. doi: 10.1093/rheumatology/kez559

Köhler, C. A., Freitas, T. H., Maes, M., de Andrade, N. Q., Liu, C. S., Fernandes, B. S., et al. (2017). Peripheral cytokine and chemokine alterations in depression: a meta-analysis of 82 studies. *Acta Psychiatr. Scand.* 135, 373–387. doi: 10.1111/acps.12698

Koopman, F. A., Stoof, S. P., Straub, R. H., Van Maanen, M. A., Vervoordeldonk, M. J., Tak, P. P., et al. (2011). Restoring the balance of the autonomic nervous system as an innovative approach to the treatment of rheumatoid arthritis. *Mol. Med.* 17, 937–948. doi: 10.2119/molmed.2011.00065

Kucharski, D., Lange, E., Ross, A. B., Svedlund, S., Feldthusen, C., Önnheim, K., et al. (2019). Moderate-to-high intensity exercise with person-centered guidance influences fatigue in older adults with rheumatoid arthritis. *Rheumatol. Int.* 39, 1585–1594. doi: 10.1007/s00296-019-04384-8

Li, B., Lv, J., Wang, W., and Zhang, D. (2017). Dietary magnesium and calcium intake and risk of depression in the general population: a meta-analysis. *Aust. N Z J Psychiatry* 51, 219–229. doi: 10.1177/0004867416676895

Lin, M. C., Guo, H. R., Lu, M. C., Livneh, H., Lai, N. S., and Tsai, T. Y. (2015). Increased risk of depression in patients with rheumatoid arthritis: a seven-year population-based cohort study. *Clinics (Sao Paulo)* 70, 91–96. doi: 10.6061/clinics/2015(02)04

Liu, J. J., Wei, Y. B., Strawbridge, R., Bao, Y., Chang, S., Shi, L., et al. (2020). Peripheral cytokine levels and response to antidepressant treatment in depression: a systematic review and meta-analysis. *Mol. Psychiatry* 25, 339–350. doi: 10.1038/s41380-019-0474-5

Liu, P., Wang, J., Wen, W., Pan, T., Chen, H., Fu, Y., et al. (2020). Cinnamaldehyde suppresses NLRP3 Derived IL-1β *via* activating succinate/HIF-1 in rheumatoid arthritis rats. *Int. Immunopharmacol.* 84:106570. doi: 10.1016/j.intimp.2020.106570

Lu, M. C., Guo, H. R., Lin, M. C., Livneh, H., Lai, N. S., and Tsai, T. Y. (2016). Bidirectional associations between rheumatoid arthritis and depression: a nationwide longitudinal study. *Sci. Rep.* 6:20647. doi: 10.1038/srep20647

Lwin, M. N., Serhal, L., Holroyd, C., and Edwards, C. J. (2020). Rheumatoid arthritis: the impact of mental health on disease: a narrative review. *Rheumatol. Ther.* 7, 457–471. doi: 10.1007/s40744-020-00217-4

Maharjan, D. T., Syed, A. A. S., Lin, G. N., and Ying, W. (2021). Testosterone in female depression: a meta-analysis and mendelian randomization study. *Biomolecules* 11:409. doi: 10.3390/biom11030409

Malemud, C. J. (2018). The role of the JAK/STAT signal pathway in rheumatoid arthritis. *Ther. Adv. Musculoskelet. Dis.* 10, 117–127. doi: 10.1177/1759720X18776224

Malemud, C. J., and Miller, A. H. (2008). Pro-inflammatory cytokine-induced SAPK/MAPK and JAK/STAT in rheumatoid arthritis and the new anti-depression drugs. *Expert. Opin. Ther. Targets* 12, 171–183. doi: 10.1517/14728222.12.2.171

Malynn, S., Campos-Torres, A., Moynagh, P., and Haase, J. (2013). The pro-inflammatory cytokine TNF-α regulates the activity and expression of the serotonin transporter (SERT) in astrocytes. *Neurochem. Res.* 38, 694–704. doi: 10.1007/s11064-012-0967-y

Marazziti, D., Torrigiani, S., Carbone, M. G., Mucci, F., Flamini, W., Ivaldi, T., et al. (2021). Neutrophil/lymphocyte, platelet/lymphocyte and monocyte/lymphocyte ratios in mood disorders. *Curr. Med. Chem.* 29, 5758–5781. doi: 10.2174/0929867328666210922160116

Marrie, R. A., Walld, R., Bolton, J. M., Sareen, J., Patten, S. B., Singer, A., et al. (2018). CIHR team in defining the burden and managing the effects of psychiatric comorbidity in chronic immunoinflammatory disease. psychiatric comorbidity increases mortality in immune-mediated inflammatory diseases. *Gen. Hosp. Psychiatry* 53, 65–72. doi: 10.1016/j.genhosppsych.2018.06.001

Martínez-González, M. Á., and Sánchez-Villegas, A. (2016). Magnesium intake and depression: the SUN cohort. *Magnesium Res.* 29, 102–111. doi: 10.1684/mrh.2016.0409

Matcham, F., Davies, R., Hotopf, M., Hyrich, K. L., Norton, S., Steer, S., et al. (2018). The relationship between depression and biologic treatment response in rheumatoid arthritis: an analysis of the british society for rheumatology biologics register. *Rheumatology (Oxford)* 57, 835–843. doi: 10.1093/rheumatology/kex528

Matsushita, T., Otani, K., Oto, Y., Takahashi, Y., Kurosaka, D., and Kato, F. (2021). Sustained microglial activation in the area postrema of collagen-induced arthritis mice. *Arthritis Res. Ther.* 23:273. doi: 10.1186/s13075-021-02657-x

McIntyre, R. S., Subramaniapillai, M., Lee, Y., Pan, Z., Carmona, N. E., Shekotikhina, M., et al. (2019). Efficacy of adjunctive infliximab vs placebo in the treatment of adults with bipolar I/II depression: a randomized clinical trial. *JAMA Psychiatry* 76, 783–790. doi: 10.1001/jamapsychiatry.2019.0779

McQuillan, J. T., Andersen, J. A., Berdahl, T. A., and Willett, J. (2022). "Associations of rheumatoid arthritis and depressive symptoms over time: are there differences by education, race/ethnicity and gender?". *Arthritis Care Res. (Hoboken)* 74, 2050–2058. doi: 10.1002/acr.24730

Michelsen, B., Kristianslund, E. K., Sexton, J., Hammer, H. B., Fagerli, K. M., Lie, E., et al. (2017). Do depression and anxiety reduce the likelihood of remission in rheumatoid

arthritis and psoriatic arthritis? data from the prospective multicentre NOR-DMARD study. *Ann. Rheum. Dis.* 76, 1906–1910. doi: 10.1136/annrheumdis-2017-211284

Miller, A. H., and Raison, C. L. (2016). The role of inflammation in depression: from evolutionary imperative to modern treatment target. *Nat. Rev. Immunol.* 16, 22–34. doi: 10.1038/nri.2015.5

Minamino, H., Katsushima, M., Hashimoto, M., Fujita, Y., Torii, M., Ikeda, K., et al. (2021). Influence of dietary habits on depression among patients with rheumatoid arthritis: a cross-sectional study using KURAMA cohort database. *PLoS One* 16:e0255526. doi: 10.1371/journal.pone.0255526

Miwa, Y., Ikari, Y., Hosonuma, M., Hatano, M., Hayashi, T., Kasama, T., et al. (2018). A study on characteristics of rheumatoid arthritis patients achieving remission in depression with 6 months of bDMARDs treatment. *Eur. J. Rheumatol.* 5, 111–114. doi: 10.5152/eurjrheum.2018.17147

Miwa, Y., Nishimi, A., Nishimi, S., Saito, M., Tokunaga, T., Yanai, R., et al. (2014). Combined infliximab and methotrexate treatment improves the depressive state in rheumatoid arthritis patients more effectively than methotrexate alone. *Eur. J. Rheumatol.* 1, 147–149. doi: 10.5152/eurjrheumatol.2014.140074

Nerurkar, L., Siebert, S., McInnes, I. B., and Cavanagh, J. (2019). Rheumatoid arthritis and depression: an inflammatory perspective. *Lancet Psychiatry* 6, 164–173. doi: 10.1016/S2215-0366(18)30255-4

Ng, K. J., Huang, K. Y., Tung, C. H., Hsu, B. B., Wu, C. H., Lu, M. C., et al. (2020). Risk factors, including different biologics, associated with depression and anxiety in patients with rheumatoid arthritis: a cross-sectional observational study. *Clin. Rheumatol.* 39, 737–746. doi: 10.1007/s10067-019-04820-x

Nikolaus, S., Bode, C., Taal, E., and van de Laar, M. A. (2013). Fatigue and factors related to fatigue in rheumatoid arthritis: a systematic review. *Arthritis Care Res. (Hoboken)* 65, 1128–1146. doi: 10.1002/acr.21949

Pamukcu, M., İzci Duran, T., Ulusoy, H., and Altinbaş, K. (2021). Investigation of the correlation between mood disorder symptoms and disease activity and functional status in rheumatoid arthritis patients. *Turk. J. Med. Sci.* 51, 3008–3016. doi: 10.3906/sag-2107-283

Pedersen, B. K., and Saltin, B. (2015). Exercise as medicine-evidence for prescribing exercise as therapy in 26 different chronic diseases. *Scand. J. Med. Sci. Sports* 3, 1–72. doi: 10.1111/sms.12581

Pikwer, M., Giwercman, A., Bergström, U., Nilsson, J. Å., Jacobsson, L. T., and Turesson, C. (2014). Association between testosterone levels and risk of future rheumatoid arthritis inmen: a population-based case-control study. *Ann. Rheum. Dis.* 73, 573–579. doi: 10.1136/annrheumdis-2012-202781

Pinho de Oliveira Ribeiro, N., Rafael de Mello Schier, A., Ornelas, A. C., Pinho de Oliveira, C. M., Nardi, A. E., and Silva, A. C. (2013). Anxiety, depression and suicidal ideation in patients with rheumatoid arthritis in use of methotrexate, hydroxychloroquine, leflunomide and biological drugs. *Compr. Psychiatry* 54, 1185–1189. doi: 10.1016/j.comppsych.2013.05.010

Pope, J. E. (2020). Management of fatigue in rheumatoid arthritis. *RMD Open* 6:e001084. doi: 10.1136/rmdopen-2019-001084

Pu, D., Luo, J., Wang, Y., Ju, B., Lv, X., Fan, P., et al. (2017). Prevalence of depression and anxiety in rheumatoid arthritis patients and their associations with serum vitamin D level. *Clin. Rheumatol.* 37, 179–184. doi: 10.1007/s10067-017-3874-4

Qin, D., Li, Z., Li, Z., Wang, L., Hu, Z., Lü, L., et al. (2019). Chronic glucocorticoid exposure induces depression-like phenotype in rhesus macaque (*Macaca mulatta*). *Front. Neurosci.* 13:188. doi: 10.3389/fnins.2019.00188

Rani, T., Behl, T., Sharma, N., Makeen, H. A., Albratty, M., Alhazmi, H. A., et al. (2022). Exploring the role of biologics in depression. *Cell. Signal.* 98:110409. doi: 10.1016/j.cellsig.2022.110409

Salim, S. (2017). Oxidative stress and the central nervous system. *J. Pharmacol. Exp. Ther.* 360, 201–205. doi: 10.1124/jpet.116.237503

Schaible, H. G. (2014). Nociceptive neurons detect cytokines in arthritis. *Arthritis Res. Ther.* 16:470. doi: 10.1186/s13075-014-0470-8

Scherholz, M. L., Schlesinger, N., and Androulakis, I. P. (2019). Chronopharmacology of glucocorticoids. *Adv. Drug. Deliv. Rev.* 151–152, 245–261. doi: 10.1016/j.addr.2019.02.004

Sebba, A. (2021). Pain: a review of interleukin-6 and its roles in the pain of rheumatoid arthritis. *Open Access Rheumatol.* 13, 31–43. doi: 10.2147/OARRR.S291388

Shadick, N. A., Katz, P., Iannaccone, C. I., Maica, G., Coblyn, J., Weinblatt, M. E., et al. (2019). The impact of exercise, lifestyle and clinical factors on perceived cognitive function in patients with rheumatoid arthritis: results from a prospective cohort study. *ACR Open Rheumatol.* 1, 620–626. doi: 10.1002/acr2.11088

Shariq, A. S., Brietzke, E., Rosenblat, J. D., Pan, Z., Rong, C., Ragguett, R. M., et al. (2018). Therapeutic potential of JAK/STAT pathway modulation in mood disorders. *Rev. Neurosci.* 30, 1–7. doi: 10.1515/revneuro-2018-0027

Sizar, O., Khare, S., Goyal, A., and Givler, A. (2022). "Vitamin D deficiency," in *StatPearls [Internet]*. Treasure Island, FL: StatPearls Publishing.

Sturgeon, J. A., Finan, P. H., and Zautra, A. J. (2016). Affective disturbance in rheumatoid arthritis: psychological and disease-related pathways. *Nat. Rev. Rheumatol.* 12, 532–542. doi: 10.1038/nrrheum.2016.112

Sun, C., Wang, R., Li, Z., and Zhang, D. (2018). Dietary magnesium intake and risk of depression. *J. Affect. Disord.* 246, 627–632. doi: 10.1016/j.jad.2018.12.114

Sun, Y., Wang, D., Salvadore, G., Hsu, B., Curran, M., Casper, C., et al. (2017). The effects of interleukin-6 neutralizing antibodies on symptoms of depressed mood and anhedonia in patients with rheumatoid arthritis and multicentric Castleman's disease. *Brain Behav. Immun.* 66, 156–164. doi: 10.1016/j.bbi.2017.06.014

Süß, P., Rothe, T., Hoffmann, A., Schlachetzki, J. C. M., and Winkler, J. (2020). The joint-brain axis: insights from rheumatoid arthritis on the crosstalk between chronic peripheral inflammation and the brain. *Front. Immunol.* 11:612104. doi: 10.3389/fimmu.2020.612104

Syed, A. A. S., He, L., and Shi, Y. (2020). The potential effect of aberrant testosterone levels on common diseases: a mendelian randomization study. *Genes* 11:721. doi: 10.3390/genes11070721

Takeuchi, T., Yamanaka, H., Harigai, M., Tamamura, R., Kato, Y., Ukyo, Y., et al. (2018). Sirukumab in rheumatoid arthritis refractory to sulfasalazine or methotrexate: a randomized phase 3 safety and efficacy study in Japanese patients. *Arthritis Res. Ther.* 20:42. doi: 10.1186/s13075-018-1536-9

Tardy, A. L., Pouteau, E., Marquez, D., Yilmaz, C., and Scholey, A. (2020). Vitamins and minerals for energy, fatigue and cognition: a narrative review of the biochemical and clinical evidence. *Nutrients* 12:228. doi: 10.3390/nu12010228

Tarleton, E. K., and Littenberg, B. (2015). Magnesium intake and depression in adults. *J. Am. Board Fam. Med.* 28, 249–256. doi: 10.3122/jabfm.2015.02.140176

Thillard, E. M., Gautier, S., Babykina, E., Carton, L., Amad, A., Bouzillé, G., et al. (2020). Psychiatric adverse events associated with infliximab: a cohort study from the french nationwide discharge abstract database. *Front. Pharmacol.* 11:513. doi: 10.3389/fphar.2020.00513

Tiosano, S., Yavne, Y., Watad, A., Langevitz, P., Lidar, M., Feld, J., et al. (2020). The impact of tocilizumab on anxiety and depression in patients with rheumatoid arthritis. *Eur. J. Clin. Invest.* 50:e13268. doi: 10.1111/eci.13268

Vallerand, I. A., Patten, S. B., and Barnabe, C. (2019). Depression and the risk of rheumatoid arthritis. *Curr. Opin. Rheumatol.* 31, 279–284. doi: 10.1097/BOR.0000000000000597

Villarino, A. V., Kanno, Y., and O'Shea, J. J. (2017). Mechanisms and consequences of Jak-STAT signaling in the immune system. *Nat. Immunol.* 18, 374–384. doi: 10.1038/ni.3691

Waite, J. M., Hale, C. A., and Lee, H. Y. (2013). Psychosocial effects of tai chi exercise on people with rheumatoid arthritis. *J. Clin. Nurs.* 22, 3053–3061. doi: 10.1111/jocn.12327

Walsh, D. A., and McWilliams, D. F. (2014). Mechanisms, impact and management of pain in rheumatoid arthritis. *Nat. Rev. Rheumatol.* 10, 581–592. doi: 10.1038/nrrheum.2014.64

Walther, A., Breidenstein, J., and Miller, R. (2019). Association of testosterone treatment with alleviation of depressive symptoms in men: a systematic review and meta-analysis. *JAMA Psychiatry* 76, 31–40. doi: 10.1001/jamapsychiatry.2018.2734

Wirth, T., Westendorf, A. M., Bloemker, D., Wildmann, J., Engler, H., Mollerus, S., et al. (2014). The sympathetic nervous system modulates CD4(+)Foxp3(+) regulatory T cells *via* noradrenaline-dependent apoptosis in a murine model of lymphoproliferative disease. *Brain Behav. Immun.* 38, 100–110. doi: 10.1016/j.bbi.2014.01.007

World Health Organization (2017). Depression and other common mental disorders: global health estimates. Available online at: https://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017.2-eng.pdf. Accessed 18 Nov, 2020.

Yan, Z., Gibson, S. A., Buckley, J. A., Qin, H., and Benveniste, E. N. (2018). Role of the JAK/STAT signaling pathway in regulation of innate immunity in neuroinflammatory diseases. *Clin. Immunol.* 189, 4–13. doi: 10.1016/j.clim.2016.09.014

Yasuoka, M., Kojima, T., Waguri-Nagaya, Y., Saito, T., Takahashi, N., Asai, S., et al. (2021). Impact of social support on severity of depressive symptoms by remission status in patients with rheumatoid arthritis. *Mod. Rheumatol.* 12:roab001. doi: 10.1093/mr/roab001

Yayikci, Y. I., and Karadag, A. (2019). Effects of conventional and biological drugs used for the treatment of rheumatoid arthritis on the quality of life and depression. *Eurasian J. Med.* 51, 12–16. doi: 10.5152/eurasianjmed.2018.18018

Yentür, S. B., Ataş, N., Öztürk, M. A., and Oskay, D. (2021). Comparison of the effectiveness of pilates exercises, aerobic exercises and pilates with aerobic exercises in patients with rheumatoid arthritis. *Ir. J. Med. Sci.* 190, 1027–1034. doi: 10.1007/s11845-020-02412-2

Zhang, A., and Lee, Y. C. (2018). Mechanisms for joint pain in rheumatoid arthritis (RA): from cytokines to central sensitization. *Curr. Osteoporos Rep.* 16, 603–610. doi: 10.1007/s11914-018-0473-5

Zhang, Y., Zheng, Y., and Li, H. (2016). NLRP3 inflammasome plays an important role in the pathogenesis of collagen-induced arthritis. *Mediators Inflamm.* 2016:9656270. doi: 10.1155/2016/9656270

Zhu, C. B., Blakely, R. D., and Hewlett, W. A. (2006). The proinflammatory cytokines interleukin-1beta and tumor necrosis factor-alpha activate serotonin transporters. *Neuropsychopharmacology* 31, 2121–2131. doi: 10.1038/sj.npp.1301029

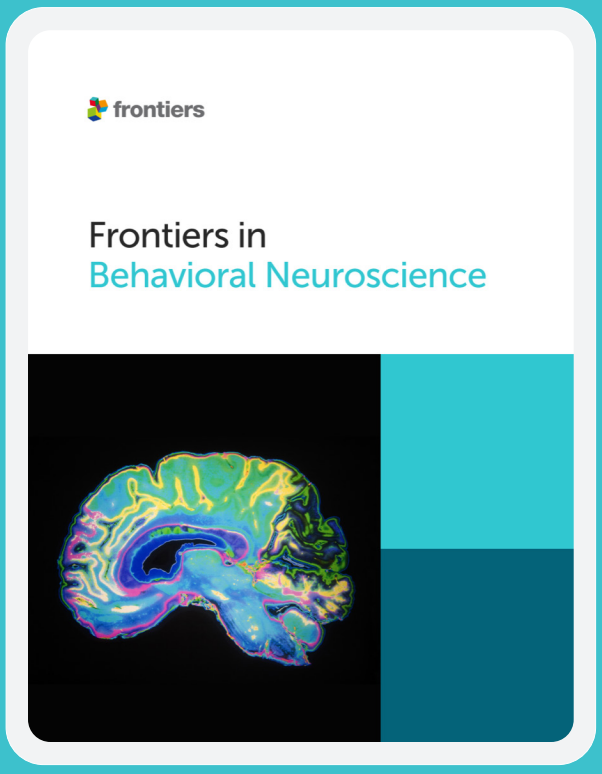
Frontiers in Behavioral Neuroscience

Explores the neural mechanisms underlying animal and human behavior

Part of the world's most cited neuroscience journal series, this journal highlights reseach in all species that advances our understanding of the neural mechanisms underlying behavioral outcomes.

Discover the latest Research Topics

See more →



Frontiers

Avenue du Tribunal-Fédéral 34
1005 Lausanne, Switzerland
frontiersin.org

Contact us

+41 (0)21 510 17 00
frontiersin.org/about/contact