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Evaluation of non-invasive transcranial brain stimulation protocols for future clinical use: aspects of interindividual variability and safety in healthy subjects and schizophrenia patients.

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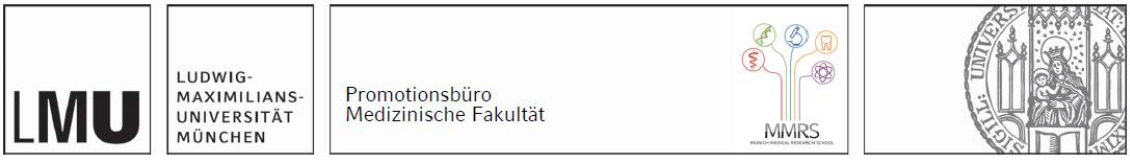

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List of Abbreviations

ANS: autonomic nervous system

DLPFC: Dorsolateral Prefrontal Cortex

HRV: heart rate variability

iPAS: individualized paired-associative stimulation

ISI: Inter-stimulus interval

LTD: long-term depression

LTP: long-term potentiation

M1: Primary motor cortex

MEP: motor-evoked potential

NTBS: Non-invasive transcranial brain stimulation

PAS: Paired-associative stimulation

rTMS: repetitive transcranial magnetic stimulation

TMS: Transcranial magnetic stimulation

List of Publications

1. Campana M, Papazova I, Pross B, Hasan A, Strube W. Motor-cortex excitability and response variability following paired-associative stimulation: a proof-of-concept study comparing individualized and fixed inter-stimulus intervals. Exp Brain Res. 2019 Jul; 237(7):1727-1734. doi: 10.1007/s00221-019-05542-x. Epub 2019 Apr 25. PMID: 31025050.

2. Campana M, Wagner E, Wobrock T, Langguth B, Landgrebe M, Eichhammer P, Frank E, Cordes J, Wölwer W, Winterer G, Gaebel W, Hajak G, Ohmann C, Verde PE, Rietschel M, Malchow B, Ahmed R, Strube W, Häckert J, Schneider-Axmann T, Falkai P, Hasan A. Effects of high-frequency prefrontal rTMS on heart frequency rates and blood pressure in schizophrenia. J Psychiatr Res. 2021 Jun 7;140:243-249. doi: 10.1016/j.jpsychires.2021.06.010. Epub ahead of print. PMID: 34119909.

1. Contribution to the publications

1.1 Contribution to Paper I

This publication concerns the development of a novel non-invasive transcranial brain stimulation (NTBS) protocol to investigate in-vivo motor cortex excitability and plasticity in healthy human subjects. I contributed to this work throughout multiple stages of its realization. I first laid out the conceptualization of the project in collaboration with two senior authors (W.S., A.H.). More specifically, I outlined the physiological idea behind it, which was to individualize the aforementioned NTBS protocol to take into account between-subjects anatomical and neurophysiological differences. Together with W.S. and A.H., we translated this idea into a specific stimulation protocol. I then screened and recruited 21 healthy volunteers. I personally run all experimental stimulation sessions, which involved around 6 hours of non-invasive brain stimulation per volunteer over 3 weeks. Under W.S. and A.H.´s supervision, I undertook all statistical data analysis using the software "IBM SPSS" as described in more detail in the paper. The original article´s draft was written by me in its entirety (Introduction, Methods, Statistics, Results, and Discussions). Together with the other co-authors, we refined the original draft and sent the article to the journal: "Experimental Brain Research" to be considered for publication. Under W.S. and A.H.´s supervision, I reviewed and modified the article according to each revision suggested by the journal´s peer reviewers until its final publication. Selected preliminary data from this research project have been included in my graduation thesis at the Universita´ Cattolica del Sacro Cuore in Rome.

1.2 Contribution to Paper II

This publication is a secondary analysis of the first large-scale randomized controlled trial investigating the impact of prefrontal repetitive transcranial magnetic stimulation (rTMS) on autonomic function assessed with systolic and diastolic blood pressure and heart rate in two different positions in patients with schizophrenia. The data used in this publication stem from the "rTMS for the Treatment of Negative Symptoms in Schizophrenia" (RESIS) trial (2007-2011), where, after a 2-week pretreatment phase, 76 patients were treated with 10-Hz rTMS to the left dorsolateral prefrontal cortex (DLPFC) added to the ongoing treatment, and 81 patients were subjected to sham rTMS applied similarly.

The realization of this work entailed multiple steps in many of which I could contribute. Most of the co-authors of this article were involved in the original clinical trial and data collection. The research question behind this secondary analysis was first outlined by A.H. and later discussed and refined with me. The statistical analysis was designed and performed by me and A.H. in close collaboration with a professional experienced statistician (T.S.A). Results of the analysis were interpreted and reported by me under A.H. supervision. I finally wrote, under E.W. supervision, the original article´s draft in its entirety (Introduction, Methods, Results, Discussions, and Conclusions) as well as designing Tables and Figures. Together with the other co-authors, we finalized the original draft and sent the article to the journal: "Journal of Psychiatric Research" to be considered for publication. Under E.W. and A.H.´s supervision, I reviewed and modified the article according to each revision during the peer-review process until its final publication.

2. Introduction

2.1 Theoretical background

2.1.1 NTBS and PAS

Non-invasive transcranial brain stimulation (NTBS) is a term that entails various kinds of stimulation protocols, which have diverse technical and neurophysiological backgrounds as well as a rich spectrum of applications. Undeniably, NTBS protocols have contributed to the study of in vivo brain plasticity at a systems level in humans. Their non-invasive nature has opened the possibility for clinicians and neuroscientists to investigate the functions and characteristics of different brain areas in patients as they are awake resting or even completing tasks. The applications of NTBS protocols can range from neurophysiological investigations on the excitability and plasticity of different brain cortical areas to clinical therapeutic applications like in Major Depressive Disorder or in post-stroke rehabilitation[1, 2]. In the clinical field, the rationale behind some NTBS applications is to enhance traditional neurorehabilitation practices by exploiting the physiological changes induced through NTBS. One of the two papers presented in this dissertation (Paper I) revolves around a specific NTBS protocol called Paired-associative Stimulation (PAS). PAS has been used both as a therapeutic intervention[1, 3] and as a neurophysiological investigation tool to better characterize Hebbian principles of synaptic plasticity. At the core of PAS lies the idea of simultaneously delivering two electrical stimuli on a specific cortical spot. The first stimulus is delivered to a peripheral nerve (usually at the wrist) a few milliseconds in advance of the second stimulus. The second stimulus is a magnetic stimulus directly administered on the scalp, which induces an electrical field that has a precise area of action on the underlying neurons. This form of magnetic stimulation is referred to as transcranial magnetic stimulation (TMS). The time difference between the two stimuli is called inter-stimulus interval (ISI). In the original PAS protocol described in a 2000 paper by Stefan and colleagues[4], a train

of 90 single electrical stimuli was delivered to the median nerve at the level of the wrist and paired with 90 magnetic stimuli delivered by TMS to the contralateral primary motor cortex (M1). The ISI was set at 25 milliseconds to account for the time it takes for the first electrical stimulus to be transmitted from the periphery to the somatosensory cortex and from there to the primary motor cortex. The coupling of the aforementioned stimuli over an extended period resulted in an augmentation of the excitability of corticospinal neuronal projection from the motor cortex. The change in excitability of a neuronal population regardless of the quality of that change (increase or decrease of excitability) is referred to as plastic adaptation. The ability of a neuronal population (i.e. an entire cortical area) to change its excitability in response to afferent stimuli reflects the plastic adaptation or plasticity of that area.

2.1.2 Plasticity in healthy population and schizophrenia

There is substantial evidence that cortical neuronal circuits in humans exhibit plasticity and have the ability to adapt to new stimuli throughout life. Different brain regions may express different degrees of plasticity[5]; moreover, cortical plasticity can be induced by certain stimuli but not by others[6]. Plasticity can occur as a result of physiological stimuli (i.e. learning to ride a bike) as well as pathological stimuli (i.e. warfare trauma) and its results can be functional (riding a bike) or dysfunctional (excessive autonomic arousals after hearing a loud noise). In general, neural plasticity can occur through the formation of new synapses or alterations of synaptic efficacy. Changes in synaptic efficacy have been linked to physiological phenomena referred to as long-term potentiation (LTP) and long-term depression (LTD). Arguably, the area of the brain where these two mechanisms have been studied most extensively is the primary motor cortex. In this area plasticity is likely driven by learning or by practicing newly acquired movement patterns but not by repetitive simple motor activity[6], implying, as mentioned above, that only certain kinds of stimuli are capable of inducing plastic adaptations in the nervous system. Another factor that greatly influences brain plasticity is the

presence of a neurological or psychiatric disease, although its causal role has not yet been fully understood.

Protocols like PAS or repetitive TMS (rTMS) have been used to investigate as well as to induce changes in plasticity in various cortical areas including M1 both in healthy subjects and in patients affected by psychiatric disorders[4, 7]. Several neurobiological as well as neurophysiological mechanisms were discussed to be implicated in neural plasticity, the most studied mechanisms to date are the removal of cortical inhibitory neurotransmission[8] as well as the activation of NMDA receptors that facilitate LTP[9]. There is evidence that the neurotransmitter mechanisms mediating neural plasticity are altered in schizophrenia[10]. Dysfunctional GABA and NMDA receptor-mediated neurotransmission have been described and linked to changes in neuronal connectivity. Finally -when investigated through PAS- people affected by schizophrenia showed disrupted LTP, demonstrating significant plasticity deficits following PAS and impaired motor learning[11], indicating that the enhancement of plasticity could become a potential target for future treatments.

2.1.3 NTBS safety and autonomic dysfunction

NTBS techniques such as PAS or rTMS are considered to be safe and hence are now being used for the treatment of a wide range of neuropsychiatric disorders. Data regarding rTMS-safety are substantial since the technique has been used in several clinical trials. Seizures are undoubtedly the most severe adverse reactions described in the literature, although estimations rate the risk for seizure between 1 and 67/100,000 sessions[12] with no relevant impact on clinical practice. More frequent adverse reactions include headache, dizziness, and autonomic dysfunction[13]. The autonomic nervous system (ANS) is a branch of the peripheral nervous system that regulates bodily functions, such as heart rate, digestion, respiratory rate, pupillary response, urination, and sexual arousal[14]. The Heart rate variability (HRV) is a reliable index of cardiac autonomic function, as it assesses the beat-to-beat variation in the heart over time[15]. An increasing amount of evidence found a decreased HRV in individuals with psychiatric disorders including

schizophrenia[16]. According to a recent meta-analysis[17], prospective evidence indicates that reductions in HRV are strongly associated with a higher incidence of first cardiac events in patients without known cardiovascular disease[18]. Reductions in HRV have therefore been considered a marker of greater risk for cardiovascular disease and all-cause mortality[19].

This is of great importance since cardiovascular disorders are among the main causes of premature and sudden death in schizophrenia patients[20]. Moreover, the use of a first- or second-generation antipsychotic appears to double the incidence-rate ratio of sudden cardiac death[21]. To date, potential effects of rTMS on autonomic functions have been mainly investigated in healthy subjects and little is known about the potential beneficial or harmful effects of rTMS on autonomic functions in such a vulnerable population like schizophrenia patients.

2.2 Research Projects

2.2.1 Research question and goals

The articles presented here cover two critical aspects of non-invasive transcranial brain stimulation. On one hand, one of the limitations of NTBS protocols is the high interindividual variability reported in the literature, which often hinders studies´ replicability and results´ applicability. On the other hand, safety aspects of NTBS protocols are often researched on healthy volunteers, possibly neglecting more vulnerable populations such as schizophrenia patients when it comes to autonomic dysfunction. The idea behind both articles was to address these critical concerns around NTBS as well as prospectively plan a translational clinical trial where iPAS could be used to potentially improve brain cortical plasticity in schizophrenia patients. Thus, both articles deal with specific effects of NTBS from a point of significant relevance for clinical applications, namely variability and safety.

With this goal in mind, the first research project aimed at establishing a novel NTBS protocol, which took form from the original PAS protocol (see paragraph

2.1.1) and was then individualized to better account for anatomical and functional interindividual differences. This new individualized Paired-associative Stimulation (iPAS) protocol was tested among healthy subjects to assess its safety and feasibility while being compared with two other standard PAS protocols.

The second research project aimed at investigating the safety of a widely used NTBS protocol such as rTMS with focus on potential effects on the autonomic nervous system in a clinical population. Since patients living with schizophrenia often display a compromised autonomic regulation and an increased risk for cardiovascular diseases, the evaluation of potential effects of rTMS on autonomic function remains a central topic in the discourse around TMS safety. Specifically, for the second project my colleagues and I underwent a secondary analysis of a large multicentric randomized controlled trial investigating the impact of prefrontal repetitive transcranial magnetic stimulation (rTMS) on autonomic function in patients with schizophrenia. The data used in this publication stem from the "rTMS for the Treatment of Negative Symptoms in Schizophrenia" (RESIS) trial (2007-2011), where 76 patients were treated with 10-Hz rTMS to the left dorsolateral prefrontal cortex and 81 patients were subjected to sham rTMS. Together, the results from the aforementioned research projects lay the basis for a safe future clinical investigation, where cortical motor cortex plasticity will be assessed using the iPAS protocol in schizophrenia patients in order to potentially improve treatment outcomes in this severely affected population.

2.2.2 Research Project N. 1

Paired-associative stimulation represents an established NTBS technique, which has been shown to produce long-lasting LTP- and LTD-like plasticity in the human motor cortex[22]. Since the original report of the PAS technique in 2000[4], substantial interindividual variability in the elicited cortical effects of the stimulation has been described in the literature. A possible contributor to this ample variability could reside in functional as well as anatomical differences between subjects, which are not taken into consideration when using a standardized ISI of

25ms (see paragraph 2.1.1). We therefore hypothesized that an individualized ISI might result in a more stable after-effect when compared to a fixed ISI.

To prove this hypothesis, a new individualized PAS protocol (iPAS) was developed and compared to two established fixed-ISI variants of the PAS protocol (for more detailed information see the Method section of the first paper presented in this dissertation). To test the feasibility and efficacy of the iPAS protocol, my colleagues and I run a first preliminary pilot-study with 21 healthy volunteers. The results showed a significant increase in average post-stimulation motor-evoked potential (MEP) magnitudes (a measure of increased cortical excitability) only in volunteers receiving the iPAS protocol, while the same analysis obtained no significant differences in the two other established protocols. When corrected for multiple comparisons, the increase in average post-iPAS MEP magnitudes reached trend level. Nevertheless, given the relatively small sample size for this research project, we could establish a new safe PAS protocol that showed a trend level increase in M1 excitability, while the two well-established protocols failed at achieving any statistical significance in their post-stimulation effects.

2.2.3 Research Project N. 2

Repetitive transcranial magnetic stimulation (rTMS) is one of the most widely used NTBS techniques both in clinical settings and in clinical trials targeting numerous diseases such as dementia, schizophrenia or depression[23]. rTMS is characterized by a favorable side-effects profile, with the most frequent adverse reactions being headaches or slight discomfort on the scalp at the site of stimulation. Nevertheless, in clinical practice, side-effects like vertigo or dizziness are often reported by patients undergoing such a treatment[13]. Noteworthy, autonomic dysfunction as a side effect has not been included in a recent international evaluation of rTMS safety[24]. The aim of this research project was to better investigate the impact of rTMS on autonomic function in a cohort of patients affected by schizophrenia. Those patients are particularly vulnerable to autonomic disorders partly because of the underlying condition and partly because of the use of drugs such as antipsychotics which might pronounce autonomic dysfunctions[17].

In order to contribute to the discussion around rTMS safety in patients with schizophrenia, we underwent a secondary analysis of the largest to date randomized multicenter controlled trial investigating the effect of rTMS delivered to the DLPFC on autonomic function assessed through measurements of systolic and diastolic blood pressure as well as heart rate measurements. Our group took data from the 'rTMS for the Treatment of Negative Symptoms in Schizophrenia' (RESIS) trial[25] and evaluated changes in blood pressure and heart rate from screening up to 105 days after the intervention among 157 schizophrenia patients suffering from negative symptoms that received either treatment (rTMS, n=76) or a sham stimulation (n=81). Using Linear Mixed Model (LMM) analyses we were not able to identify time x group interactions nor time effects for the considered variables. Overall rTMS on DLPFC could not show a significant effect compared to sham stimulation when considering heart rate or blood pressure changes over time. This lack of effect was observed both during the intervention and during the follow-up period. These results greatly contribute to the understanding of rTMS safety in schizophrenia by adding high-quality evidence from a large sham-controlled trial.

2.3 Conclusion and future outlooks

Schizophrenia is a brain disorder characterized by various degrees of disability as well as higher rates of comorbidity and mortality resulting in substantial individual and societal costs[26]. Although the advent of antipsychotic drugs revolutionized schizophrenia's treatment, their efficacy remains limited especially when considering the negative and cognitive symptoms of the disorder[27]. Non-invasive transcranial brain stimulation protocols like rTMS are a promising strategy both in the neurophysiological investigation of brain functions and in treating psychiatric symptoms in a more specific and safer way.

As medicine and psychiatry are moving towards a more individualized approach to diagnosis and treatment[28] it appears relevant to pursue this approach also in the field of brain stimulation. Interindividual variability remains one obstacle in achieving high rates of treatment response when applying NTBS protocols[29].

For this reason we established a individualized PAS protocol that took into account anatomical and physiological differences between individuals. This approach appeared to be superior to standard, already established, PAS protocols.

As mentioned above, one of the strengths of NTBS compared to standard pharmacological treatment, is the extremely favorable side-effects profile. As an increasing number of clinical trials have been completed or are being run, more data around safety is being collected. Since psychiatric patients appear to be clinically more vulnerable both because of the underlying disease and because of the often lifelong pharmacological treatment they are receiving, it is of high importance that safety is assessed not only in healthy volunteers but also in patients. We demonstrate that in regard to autonomic (dys-)function rTMS appears to be a safe intervention both during treatment and in the weeks afterward. Moreover, we highlighted the importance of assessing safety-related parameters such as heart rate and heart rate variability also in real-time during the stimulation to achieve a better assessment of rTMS'autonomic effects.

With these two works as background, we will be performing a comparison between the iPAS protocol and a standard one (with an ISI of 25ms) in people affected by schizophrenia. Patients that do not present any contraindication will be recruited and undergo both protocols in a randomized order with a 5 to 10 days break between protocols. This study has been approved with the number 19-0907 by the Ethical Committee of the LMU Munich. The aim of this project would be to assess changes in neuroplasticity in patients already being pharmacologically treated, as well as investigating differences in PAS efficacy when comparing an individualized and a standard stimulation protocol. Patients in the early stage of the disease as well as chronically ill patients will be recruited, thus enabling the investigation of the relationship between disease course and impairment of cortical plasticity. Finally, we hope to further contribute to the discussion around NTBS safety by proving iPAS to be a safe and effective stimulation technique also in schizophrenia patients.

3. Zusammenfassung:

Die In-vivo-Neurophysiologie am Menschen hat dank der Einführung von NTBS-Protokollen (englisch „non-invasive transcranial brain stimulation“) eine Zunahme innovativer Studiendesigns zu verzeichnen gehabt. Ein Nachteil solcher NTBS-Protokolle ist die hohe interindividuelle Variabilität, die sich negativ auf die Reproduzierbarkeit im Kontext von klinischen Studien auswirken kann. Mit Hinblick auf eine prospektive klinische Anwendung haben meine Kollegen und ich zunächst ein gut tolerierbares und sicheres NTBS-Protokoll entwickelt, das inter-individuelle anatomische und funktionelle Unterschiede berücksichtigt. Die Anwendung dieses innovativen Protokolls, genannt „individualisierte paarweise assoziative Stimulation (iPAS) am primären motorischen Kortex bei gesunden Probanden“ führte zu einer Erhöhung der neuronalen Erregbarkeit nach der Stimulation. Generell zeichnen sich NTBS-Protokolle durch ein günstiges Nebenwirkungsprofil aus, in der klinischen Praxis werden jedoch häufig Nebenwirkungen wie Schwindel oder Übelkeit von Patienten berichtet. Insbesondere Patienten, die an einer Schizophrenie erkrankt sind, zeigen im Allgemeinen eine höhere Vulnerabilität für autonome Dysfunktionen verglichen mit der Allgemeinbevölkerung. Das Ziel des zweiten hier vorgestellten Forschungsprojekts war es, die Effekte eines NTBS-Protokolls im Forschungsfeld der repetitiven transkraniellen Magnetstimulation (rTMS) auf die autonome Funktion von Patienten mit einer Schizophrenie besser zu untersuchen und zu verstehen. Zusammenfassend haben wir eine Sekundäranalyse der bisher größten randomisierten, multizentrischen, kontrollierten rTMS-Studie an Schizophrenie-Patienten mit prädominanter Negativsymptomatik durchgeführt. In dieser Sekundäranalyse wurden die Effekte von rTMS durch Stimulation des linken dorsolateralen präfrontalen Kortex (DLPFC) auf das autonome Nervensystemuntersucht. Es konnten durch rTMS im Vergleich zu Sham-Stimulation keine signifikanten Veränderungen der Herzfrequenz oder des Blutdrucks beobachtet werden. Somit erweist sich die rTMS am linken DLPFC als ein sicheres Verfahren, auch bei Menschen mit einer Schizophrenie, die als eine Risikopopulation für dysautonome Prozesse

betrachtet werden können. Indem sie sich mit zwei zentralen Aspekten von NTBS befassen, nämlich der interindividuellen Variabilität und der Sicherheit, legen beide Arbeiten den Grundstein für die Untersuchung potenzieller plastizitätssteigernder Effekte von iPAS bei Schizophrenie-Patienten, bei denen effektive Therapiemöglichkeiten aktuell eingeschränkt verfügbar und die Erforschung und Etablierung innovativer Therapieoptionen in der Zukunft dringend notwendig sind.

4. Abstract:

In vivo neurophysiology in humans has experienced a surge in novel experimental designs thanks to the introduction of non-invasive transcranial brain stimulation (NTBS) protocols. A drawback of such protocols is represented by the high interindividual variability that negatively impacts the replicability of both scientific and clinical trials. With a prospective clinical application in mind, my colleagues and I first developed a safe NTBS protocol that took interindividual anatomical and functional differences into account. The application of this novel protocol called individualized paired-associative stimulation (iPAS) on the primary motor cortex in healthy volunteers resulted in a trend level increase in post-stimulation neuronal excitability. NTBS are generally characterized by a favorable side-effects profile, however, in clinical practice, side-effects like vertigo or dizziness are often reported by patients. In particular, patients affected by schizophrenia are generally more vulnerable to autonomic dysfunction than the general population. The aim of the second research project presented here was to better investigate the impact of a NTBS protocol called repetitive transcranial magnetic stimulation (rTMS) on autonomic function in a cohort of patients affected by schizophrenia. In summary, we underwent a secondary analysis of the largest to date randomized multicenter controlled trial involving schizophrenia patients investigating the effect on autonomic function of rTMS delivered to the dorsolateral prefrontal cortex. We were able to report a lack of significant changes in heart rate and blood pressure in individuals receiving rTMS compared to individuals receiving a sham stimulation. Thus, rTMS appears to be a safe technique even in more vulnerable populations.

By addressing two crucial aspects of NTBS, namely interindividual variability and safety both works lay the foundation for a further investigation of potential plasticity-augmenting effects of iPAS in schizophrenia patients, where currently effective therapeutic options are limited and more research is urgently needed.

5. Paper I

Campana M, Papazova I, Pross B, Hasan A, Strube W. Motor-cortex excitability and response variability following paired-associative stimulation: a proof-of-concept study comparing individualized and fixed inter-stimulus intervals. Exp Brain Res. 2019 Jul; 237(7):1727-1734. doi: 10.1007/s00221-019-05542-x. Epub 2019 Apr 25. PMID: 31025050.

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6. Paper II

Campana M, Wagner E, Wobrock T, Langguth B, Landgrebe M, Eichhammer P, Frank E, Cordes J, Wölwer W, Winterer G, Gaebel W, Hajak G, Ohmann C, Verde PE, Rietschel M, Malchow B, Ahmed R, Strube W, Häckert J, Schneider-Axmann T, Falkai P, Hasan A. Effects of high-frequency prefrontal rTMS on heart frequency rates and blood pressure in schizophrenia. J Psychiatr Res. 2021 Jun 7;140:243-249. doi: 10.1016/j.jpsychires.2021.06.010. Epub ahead of print. PMID: 34119909.

See: https://doi.org/10.1016/j.jpsychires.2021.06.010

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