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SHORT COMMUNICATION

Effects of cholinergic receptor activation and magnetic fields on motor behavior in ischemic gerbils

Efectos de la activación de receptores colinérgicos y campos magnéticos en el comportamiento motor de jerbos isquémicos

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ABSTRACT

Introduction: ischemic stroke stands as a leading global cause of death and disability, prompting the need for animal model experiments in stroke research and the protection of motor function. Recently, magnetic fields have gained significant interest in various biological contexts, showing promise in preserving neurons and reversing behavioral and morphological changes in stroke models. This study explores the potential synergy between static magnetic field and nAChR agonist administration in safeguarding motor behavior in ischemic gerbils.
Objective: to determine whether the combined use of a static magnetic field and an agonist for nicotinic acetylcholine receptors (nAChR) can preserve motor behavior in ischemic gerbils.
Methods: in this experimental study, 72 Mongolian gerbils were randomly allocated into nine groups (n=8): S, SISM, SINSM, ISM, INP, ISP, INSM, INNP, INSP, distributed according to surgical procedure and treatment. The animals were trained and evaluated on the Rotarod (RR) to assess motor performance.
Results: the main finding was the preservation of motor behavior in the Sham Ischemia and Nicotine and Sham Magnetic Stimulation (SINSM) and Ischemia and Nicotine and South Pole Magnetic Field (INSP) groups, as evidenced by the results of the RR test.
Conclusions: the findings are consistent with previous literature and provide insight into the mechanism of potentiation, as results showed that adding a nAChR agonist to the magnetic field preserved motor performance in the RR test of ischemic animals.

Keywords: Ischemia; Cholinergic Agonists; Magnetic Field Therapy; Motor Activity; Gerbillinae.

RESUMEN

Introducción: el accidente cerebrovascular isquémico es una de las principales causas globales de muerte y discapacidad, lo que hace necesarios experimentos en modelos animales para la investigación del accidente cerebrovascular y la protección de la función motora. Recientemente, los campos magnéticos han despertado un interés significativo en diversos contextos biológicos, mostrando promesas en la preservación de neuronas y la reversión de cambios conductuales y morfológicos en modelos de accidente cerebrovascular. Este estudio explora la posible sinergia entre los campos magnéticos estáticos y la administración de un agonista de los receptores de acetilcolina nicotínica (nAChR) para salvaguardar el comportamiento motor en jerbos isquémicos.
Objetivo: determine si el uso combinado de un campo magnético estático y un agonista de los receptores de acetilcolina nicotínica (nAChR) puede preservar el comportamiento motor en jerbos isquémicos.
Métodos: en este estudio experimental, 72 jerbos mongoles a nueve grupos (n=8):S, SISM, SINSM, ISM, INP,

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ISP, INSM, INNP, INSP, S, SISM, SINSM, ISM, INP, ISP, INSM, INNP, INSP, distribuidos según el procedimiento quirúrgico y el tratamiento. Los animales fueron entrenados y evaluados en el Rotarod (RR) para evaluar el rendimiento motor.
Resultados: el hallazgo principal fue la preservación del comportamiento motor en los grupos de Isquemia Simulada y Nicotina y Estimulación Magnética Simulada (ISNEMS) e Isquemia y Nicotina y Campo Magnético del Polo Sur (INPS), según lo evidenciado por los resultados de la prueba de RR.
Conclusiones: los hallazgos son consistentes con la literatura previa y proporcionan información sobre el mecanismo de potenciación, ya que los resultados mostraron que agregar un agonista de nAChR a los campos magnéticos preservó el rendimiento motor en la prueba de RR de animales isquémicos.

Palabras clave: Isquemia; Agonista Colinérgico; Terapia de Campo Magnético; Actividad Motora; Jerbo.

INTRODUCTION

Ischemic stroke is one of the leading causes of death and functional disability worldwide.[1] Therefore, animal models are essential to study stroke and potential treatments for motor behavior protection.[2,3,4]

In recent years, there has been a high interest in magnetic fields in various biological areas. For example, there is evidence of neuronal preservation and behavioral and morphological reversal changes in Gerbils subjected to a brain ischemia model of stroke.[4] Among the several mechanisms of action, magnetic field could interfere with cholinesterase. Cholinesterase is an enzyme that plays an essential role in cholinergic neurotransmission, related to motor control, as its inhibition increases the extracellular concentration of acetylcholine, which binds to the nicotinic acetylcholine receptor (nAChR).[5]

Given that magnetic field and cholinergic receptor activation have a synergistic mechanism in the cholinergic system, this work aims to determine whether the association of a static magnetic field and the administration of a nAChR agonist can preserve animal behavior in ischemic gerbils. The hypothesis is that magnetic field and cholinergic receptor activation have a synergistic mechanism in the cholinergic system that can preserve motor behavior.

MATERIALS AND METHODS

Animals and Groups

This experimental study took place at the Laboratory of Neuropsychobiology and Motor Behavior of the Ribeirão Preto Medical School of the University of São Paulo (FMRP-USP). The animals were divided into nine groups, totaling 72 gerbils. Animals received intraperitoneal injection of nAChR or saline at 2 mg/kg, depending on their group. Magnetic stimulation, either sham or verum, was applied using a helmet during surgery.

I - Saline (S): Naive Animals;
II - Sham Ischemia and Sham Magnetic Stimulation (SISM);
III - Sham Ischemia, Nicotine, and Sham Magnetic Stimulation (SINSM);
IV - Ischemia and Sham Magnetic Stimulation (ISM);
V - Ischemia and North Pole Magnetic Stimulation (INP);
VI - Ischemia and South Pole Magnetic Stimulation (ISP);
VII - Ischemia, Nicotine, and Sham Magnetic Stimulation (INSM);
VIII - Ischemia, Nicotine, and North Pole Magnetic Field (INNP);
IX - Ischemia, Nicotine, and South Pole Magnetic Field (INSP).

Drug Administration

A solution of nicotine sulfate nAChR agonist [pyrrolidine, 1 methyl-2-(3-pyridyl)-, sulfate; grade II; PM 422-6; Sigma®, Brazil] dissolved in saline at a dose of 2 mg/kg were used. The volume was recalculated to 1 mg of nicotine for every 2 mL saline. The drug was given intraperitoneally for eight days, starting three days before the experimental surgery, on the day of surgery, and for four days after surgery (figure 1). All evaluation procedures were conducted 30 minutes after the injections to prevent interference with the results.[6]

Surgery Procedure

On the fourth day of administering the nAChR agonist or saline, the animals were anesthetized intramuscularly with Zoletil® (60 mg/kg) and positioned on the surgical table. After anesthesia was established, trichotomy followed by application of iodized alcohol for sterilization and an incision was made. The subcutaneous and muscular tissues were then separated to visualize the carotid arteries, which were bilaterally occluded for five minutes using a silk thread in the ischemic groups. After five minutes, blood flow was restored by releasing the occlusion. A 3-0 silk thread suture was made in the ventral region of the neck using Shalon® to complete the procedure. The same procedure was performed in the Sham group but without occlusion of the arteries.

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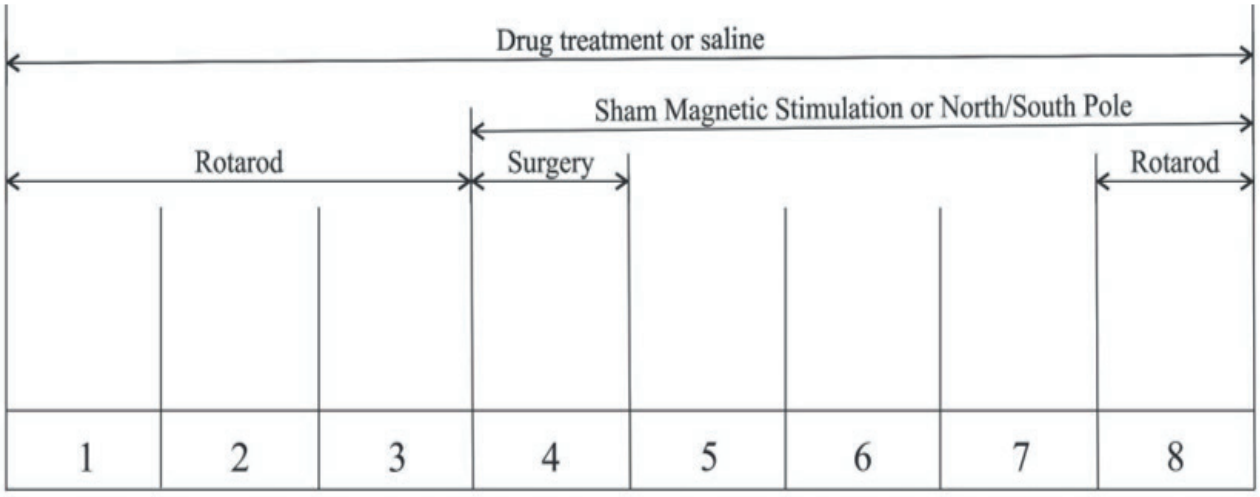

Figure 1. Experimental Design

Magnetic Field

A bipolar (North Pole and South Pole), circular magnets composed of neodymium, boron, and iron (Nd2Fe14B) were used. These magnets had dimensions of 3x8mm and a magnetic strength of 3 200 Gauss (0,32 Tesla). The magnets used in the SMS groups had the same composition and dimensions but were not magnetized.

After suturing, the animals were placed on the surgical board. Trichotomy was performed on the skull, and asepsis with iodized alcohol. A 2 % lidocaine solution [Cristália, Brazil] was injected subcutaneously to form a papule to guide the scalp removal. The skull cap was exposed, allowing the cranial sutures to be visualized. The position of the skull allowed the lambda and bregma to be in the same horizontal plane. In this area, a circular magnet was fixed and covered with self-curing acrylic, which occluded the incision and contained the magnet.

After the process, the animals were kept warm until they regained consciousness, with controlled temperature.

Motor behavior test

The Rotarod (RR) is a cylinder that rotates at a constant speed of 10 rotations per minute. Baseline recordings were taken three days before surgery, with each animal completing three consecutive daily trials over 3 seconds. The same baseline protocol was used to re-record the test on the eighth day. The test ended when the animal fell, and the average of the three recorded times was used as the permanence time data.[4]

Statistical analysis

The behavioral data were initially analyzed using a Shapiro-Wilk test for normality assessment, followed by a one-way analysis of variance (ANOVA) and identified differences between groups with Tukey's post hoc test. The statistical tests were carried out using the SigmaPlot®13 program and significance and defined at p-values <0,05.

Ethical aspects

The study was performed following the Committee on Ethics in Animal Experimentation of the Ribeirão Preto Medical School, University of São Paulo, and based on the Brazilian College of Animal Ethics in Research (Proc. 023/2020). All experimental design recommendations by ARRIVE guidelines for animals were adopted to reduce animal suffering.[7] This manuscript follows the 3Rs principles (Replacement, Reduction, and Refinement) in animals research, prioritizing ethical practices by exploring alternatives where possible (Replacement), minimizing the use of animals (Reduction) and optimizing experimental procedures to enhance animal welfare (Refinement).[8]

RESULTS

The data from the RR test revealed that the SINSM and INSP groups spent significantly more time in the test than the other groups (F=7,48, p<0,05) (figure 2).

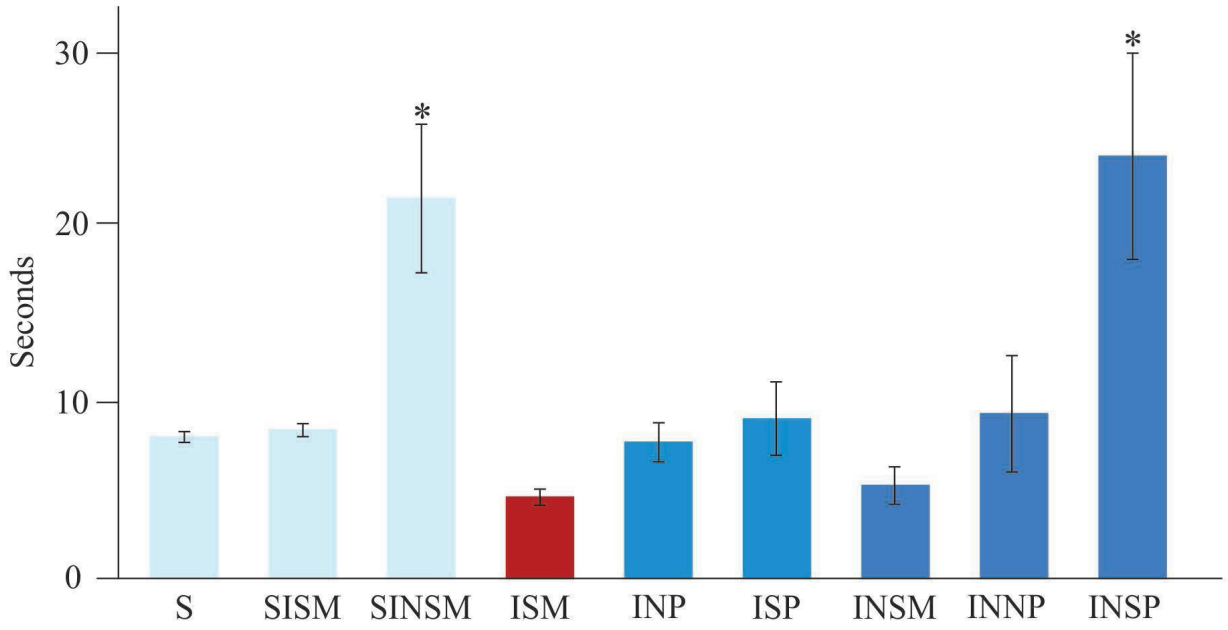

Figure 2. Time spent in the Rotarod (RR) test in seconds

The different treatment groups are shown on the x-axis: S (Naive Animals), SISM (Sham Ischemia and Sham Magnetic Stimulation), SINSM (Sham Ischemia, Nicotine, and Sham Magnetic Stimulation), ISM (Ischemia and Sham Magnetic Stimulation), INP (Ischemia and North Pole Magnetic Stimulation), ISP (Ischemia and South Pole Magnetic Stimulation), INSM (Ischemia, Nicotine, and Sham Magnetic Stimulation), INNP (Ischemia, Nicotine, and North Pole Magnetic Field) and, INSP (Ischemia, Nicotine, and South Pole Magnetic Field). All groups were injected with saline or nicotine. The y-axis shows the time spent in the RR test in seconds. The asterisk (*) indicates a statistically significant difference ($p<0,05$) compared to the other groups. The statistical analysis was performed using ANOVA followed by Tukey's post hoc test.

DISCUSSION

This study investigated the effects of a static magnetic field with a nAChR agonist on behavioral and motor responses in ischemic gerbils. Our study found that the SINSM and INSP showed preserved motor behavior, as indicated by the widely used RR test results, which evaluate motor performance, coordination, and balance in a forced motor evaluation.[9]

Improved motor behavior in animals that received the nAChR agonist, as evidenced by longer permanence time in the RR test compared to groups without cholinergic activation. This effect may be attributed to the cholinergic system activation, closely linked to neural structures modulating learning. Research has established that motor and behavioral modulation is associated with learning, and following an ischemic event, this can have negative consequences in areas such as CA1 of the hippocampus, M1 of the motor cortex, and striatum.[2,3]

The findings corroborate the beneficial effects of nAChR agonists on motor performance in ischemic gerbils.

Bertolino et al. demonstrated that magnetic field could reverse the damage and reduce neuronal death through modulation in the cell membrane, leading to changes in cell permeability and intracellular calcium metabolism, using the same animal model of ischemia as in our study.[4,10] The study demonstrates that the ischemic groups, when stimulated with the South Pole side of the magnet exhibited a behavioral response similar to that of non-ischemic animals. This corroborates the hypothesis that the magnetic field preserves motor behavior after ischemia.

In recent studies conducted by Giorgetto et al. and Brito et al., it was demonstrated that magnetic field could interact with and potentiate the effects of drugs that act on the central nervous system.[11,12] The study supports this finding by demonstrating the potentiation of the behavioral effects in the RR test of the animals treated with the magnetic field and nAChR agonists.[5] This interaction may modulate calcium in cholinergic receptors, affecting memory and learning. An alternative explanation for our observations with the naive animals treated with the south pole of the magnetic field and nicotine is that administering nAChR agonists can stimulate dopaminergic neurons, which may improve associative memory and mechanisms involved in executing previously learned motor tasks through positive reinforcement.[13]

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One potential limitation of this study is the lack of a morphological analysis, which may have provided additional insights into the underlying mechanisms driving the observed behaviors. However, this limitation is mitigated by the robust behavioral data collected, which provides strong evidence for the conclusions drawn in the study. Considering the known effects of morphological preservation and the widely accepted use of the RR test as a standard for assessing motor performance, the focus was on examining the impact of treatment on the animals' motor behavior.[14,15]

Overall, the study provides evidence for the potential of combining magnetic field with nAChR agonists to preserve motor function after ischemic events. Further studies investigating the underlying mechanisms of this potentiation effect could pave the way for new therapeutic interventions in stroke rehabilitation.

CONCLUSION

The findings demonstrate that the adding a nAChR agonist to the magnetic field can preserve motor performance in the RR test, suggesting a potential potentiation mechanism in the context of ischemia. These results contribute to the understanding of the behavioral mechanisms underlying the effects of nAChR agonists and highlight the potential of this approach as a therapeutic strategy.

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CONFLICT OF INTERESTS

The authors have no conflicts of interest to declare.

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