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Research article

Levodopa enhances immobility induced by spinal cord electromagnetic stimulation in rats☆

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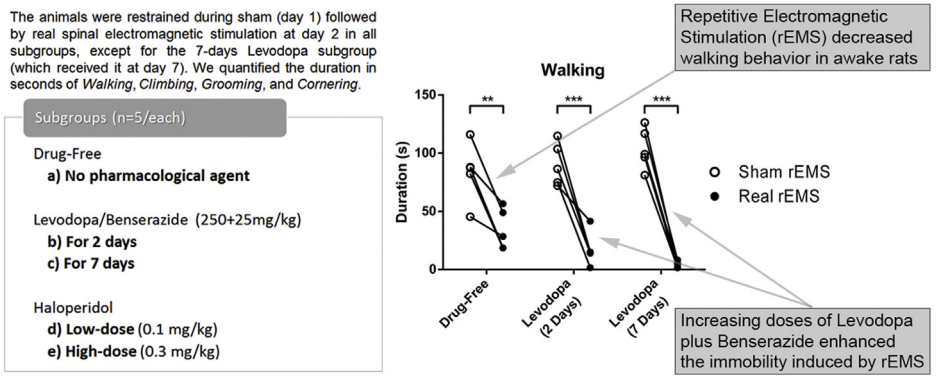
HIGHLIGHTS

- Spinal rEMS reduced the exploratory behavior of awake rats.
- This reduction was enhanced by a pre-treatment with Levodopa plus Benserazide.
- Levodopa or Haloperidol elicited distinct behavioral changes depending on the dose.
- This is the first report of behavioral changes elicited by spinal rEMS.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: The repetitive ElectroMagnetic Stimulation (rEMS) is an innocuous method applied to modulate neurocircuits in real-time to study the physiology of the central nervous system and treat neuropsychiatric conditions. Preliminary data suggest that spinal rEMS induces behavioral changes in awake rats. However, the mechanisms behind this phenomenon remain largely unknown.
Methods: Twenty-five male Wistar rats were divided into five subgroups of five animals each: one subgroup was drug-free, two subgroups received Levodopa + Benserazide 250 + 25 mg/kg for two or seven days, and the remaining two subgroups received Haloperidol 0.1 or 0.3 mg/kg for two days. The animals were restrained during sham rEMS (day 1) followed by real rEMS of the cervicothoracic region at a different day (day 2 or 7, depending on subgroup). Four behavioral parameters were quantified: *Walking*, *Climbing*, *Grooming*, and *Cornering*.
Results: rEMS reduced *Walking* and increased *Cornering* duration when applied over the cervicothoracic region of drug-free animals. A pretreatment with Levodopa + Benserazide for two or seven days induced

Abbreviations: rEMS, repetitive Electro Magnetic Stimulation.

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an additional decrease in *Walking* after rEMS. This reduction was maximum after the treatment for seven days and associated with extinction of *Climbing* and increase in *Cornering*. A pretreatment with Haloperidol 0.1 mg/kg reduced *Grooming* after rEMS, but did not prevent the reduction in *Walking*.
Conclusions: Cervicothoracic rEMS induced complex immobility responses that are in part modulated by dopaminergic pathways in rats. Further studies are necessary to determine the specific mechanisms involved.

1. Introduction

The direct stimulation of the nervous system (faradization) provided important physiological insights in Neurosciences. In the 19th Century, Hitzig and Fritsch localized the motor cortex of dogs for the first time by applying low intensity electrical currents at distinct cortical regions [1]. More recently, after the initial work of Barker et al. in 1985 [2,3], *repetitive ElectroMagnetic Stimulation (rEMS)* emerged as a noninvasive way to treat neuropsychiatric conditions and explore the physiology of the central nervous system.

Several factors influence the responses elicited by rEMS. For instance, frequencies beyond 5 Hz are usually facilitatory, whereas those below 1 Hz are inhibitory [2]. However, the modulation of inhibitory circuits can lead to complex neuronal responses (*e.g.* the inhibition of inhibitory tracts can lead to long-term potentiation) [4], and focal modulation may induce extra-regional "referred changes" in what is known as *diaschisis* [5].

Magnetic fields modulate nociception by direct effects on neuronal discharge, changes in calcium flux, and endorphin levels [6]. Recent evidence from an animal model of spinal injury also supports that rEMS can be used to foster synaptic plasticity and increase connectivity via the activation of NMDA receptors [7].

Despite the potential roles of spinal rEMS in promoting neuroregeneration [7], neuro-rehabilitation [8], autonomic [9] and sensory [10] modulation, a limited number of studies explored the behavioral repercussions of spinal rEMS. In addition, rats pretreated with a D_1 agonist exhibited increased stereotyped *Grooming* behavior, an effect that was abolished by treatment with haloperidol [11]. Therefore, previous studies point to the involvement of the dopaminergic system in the modulation of locomotor activity and stereotyped *Grooming*.

The aim of this study is to evaluate the behavioral changes elicited by rEMS over the cervicothoracic region of awake rats and to evaluate the roles of dopaminergic systems in the associated phenomena. Part of the results were reported in abstract form elsewhere [12].

2. Materials and methods

For the experiments, twenty-five male Wistar rats (Mean Weight ± SEM = 281.7 ± 6.0 g) were housed at the institution's breeding station with free access to water and food. The Institutional Review Board for Animal Research from Universidade Federal do Ceará approved this study (Number 26/09). All experiments and procedures were performed in the morning with similar luminosity and temperature conditions.

The animals were divided into five subgroups of five animals each: one subgroup was drug-free, two subgroups received Levodopa + Benserazide 250 + 25 mg/kg once daily *per os* for two or seven days, and the remaining two subgroups received intraperitoneal Haloperidol 0.1 or 0.3 mg/kg for two days (low and high-dose Haloperidol subgroups, respectively). These animals were restrained during sham rEMS (day 1) followed by real rEMS of the cervicothoracic region at a different day (day 2 in all subgroups, except for the 7-day Levodopa subgroup, which received it after the last administration of the drug at day 7). Each drug was administered 2 h prior to sham or real spinal rEMS.

2.1. Immobilization during experiments

The animals were initially placed on a wooden immobilization apparatus designed by our research team, that restrained the animals in ventral decubitus with both forelimbs and hindlimbs stretched. Hypnotic agents were not necessary, since the immobility was well-tolerated after a brief adaptation period of 3–5 min.

2.2. Electromagnetic stimulation

For the rEMS, a NEURO-MS Stimulator (NEUROSOFT, LTD.; Russia) with a focal 8-shaped, 70-mm coil was used. Firstly, at day 1 (Sham stimulation), a sound device mimicked the rEMS equipment's noise while the probe was in contact with the *vertebra prominens* at C7 level (cervicothoracic region). At day 2, the animals received trains of repetitive stimulation at the same region. The movement threshold was determined as the minimum amount of stimulation capable of eliciting a tail response in 6 of 10 stimuli applied at the C7 level. We employed the following stimulation parameters: Frequency = 20 Hz, Potency = 2x the movement threshold, Burst Duration = 5 s, Pauses = 10 s each, and Duration of Session = 5 min (a total of 2000 pulses/session). These parameters elicited more intense behavioral changes when applied over the cervicothoracic region and were determined after an empirical investigation using low (1 Hz) and high (20 Hz) frequencies in different protocols during open field exploration (data not shown).

2.3. Behavioral analysis

After rEMS or Sham stimulation, we recorded the exploratory behavior for 10 min in an observation cage (100 × 50 × 50 cm) that harbored a small ladder consisting of three steps on the right side (see enclosed video: *Behavioral Analysis*). Three behavioral parameters were quantified in seconds similarly to the protocol from Câmara et al. [13], namely *Walking* (horizontal exploratory behavior), *Climbing* (time spent climbing the set of stairs), and *Grooming*. Additionally, we virtually divided the cage into nine regions and defined *Cornering* as the time spent on the cage's left anterior and posterior regions (corners) (Video 1).

2.4. Statistical analysis

To determine statistically significant differences on the duration of each behavioral parameter in the drug-free subgroup, we conducted a paired Student's *t*-test (sham vs real rEMS). Thereafter, we separated the subgroups in two distinct analyses: a) Pro-Dopaminergic, which consisted of drug-free and Levodopa + Benserazide for 2 or 7 days; and b) Anti-Dopaminergic, which consisted of drug-free and low or high-dose Haloperidol. We evaluated the interactions between rEMS and each subgroup of a) and b) using two distinct repeated measures Two-Way ANOVAs