Elsevier Editorial System(tm) for Neurobiology of Learning and Memory
Manuscript Draft

Manuscript Number:

Title: Sleep deprivation in Octodon Degus: Effects of Transcranial Magnetic Stimulation in spatial learning and memory.

Article Type: Regular Article

Keywords: Sleep deprivation; TMS; Octodon degus; learning; memory.

Corresponding Author: Dr. Maria Trinidad Herrero,

Corresponding Author's Institution: Universitat Jaume I

First Author: Cristina Estrada Esteban

Order of Authors: Cristina Estrada Esteban; Dolores Lopez Lopez; Alvaro Conesa Guillen; Francisco Toledo Romero; Isaac Tunez Fiñana; Francisco Jose Fernandez Gomez; Regis Bordet; Jill C Richardson; Oliver Blin; Emiliano Fernandez Villalba; Maria Trinidad Herrero

Abstract: Sleep is indispensable for maintaining regular daily life activities and a fundamental physiological purpose in cognitive performance. Sleep deprivation (SD) may affect subsequent learning capacity and ability to constitute new memories, particularly in the situation of hippocampus-dependent tasks. Transcranial magnetic stimulation (TMS) is a non-invasive procedure of electromagnetic induction that generates electric currents activating nearby nerve cells in the stimulated cortical area. Several studies have begun to therapeutically use TMS. The present study was designed to evaluate how TMS could improve learning and memory functions after SD in O. degus. Thirty juvenile (aged eighteen months old) females were divided in three groups (control, acute and chronic TMS treatment -with and without SD). To immobilize TMS-treated groups, they were disposed in plastic cylindrical while the O. degus were receiving head magnetic stimulation. SD was achieved by gently handling the animals to keep them awake during the night. Behavioral tests include Radial Arm Maze (RAM), Barnes Maze (BM) and Novel Object Recognition (NOR). When TMS treatment was applied during several days, there was significant improvement of cognitive performance after SD. Noteworthy, only one session of TMS is able to improve some parameters related with spatial memory. No side effects were observed. After SD, chronic TMS treatment provokes significant amelioration in learning and in both spatial and working memory. Nonetheless, an acute treatment of TMS is already sufficient to improve spatial memory.

Suggested Reviewers: Oscar Arias Carrión
arias@ifc.unam.mx

David Bartres Faz
dbartres@ub.edu

Alvaro Pascual-Leone
apleone@bidmc.harvard.edu

David Blum
david.blum@inserm.fr

UNIVERSIDAD DE
MURCIA

Murcia, December 7th, 2015

T. Abel, PhD
Editor-in Chief – Neurobiology of Learning and Memory

Ref.: Sleep deprivation in *Octodon Degus*: Effects of Transcranial Magnetic Stimulation in spatial learning and memory.

Dear Prof. Abel,

It is our pleasure to submit to **Neurobiology of Learning and Memory** the above referenced manuscript by *Estrada C., Lopez D., Conesa A., Toledo F., Tunez I., Fernandez-Gómez F.J., Bordet R., Richardson J.C., Blin O., Fernández-Villalba E. and Herrero MT.,* for your consideration.

To our knowledge, in this manuscript we confirm that sleep deprivation (SD) results in cognitive impairment measured with 3 different psychological tests, and we provide the evidence that transcranial magnetic stimulation (TMS) treatment significantly improve cognitive performance in sleep-deprived animals. Then, after SD, TMS can improve learning, spatial memory and working memory functions.

We feel that these exciting results are novel and important, and can be of interest for scientists, neurologists and physicians working on sleep pathologies.

The experimental work was done in compliance with the National Research Council's Guide for the care and use of laboratory animals, and the Guidelines promulgated by the European Communities Council Directive 2010/63/ECC.

The data presented in the manuscript are original and the manuscript is not under consideration elsewhere. None of the manuscript contents has been previously published. In the present work we do not have any conflict of financial interest.

All authors have seen and approved the manuscript.

Trusting you will consider our manuscript worthy of review by your Journal,

Yours truly,

María-Trinidad Herrero

Prof. María-Trinidad Herrero Ezquerro
Facultad de Medicina
NiCE-CIBERNED. Dept. Anatomía Humana y Psicobiología
Campus Universitario de Espinardo. 30100 Murcia
Tel. 868 88 4683 / Lab. 868 88 8484 8468 / Fax 868 88 4150 / mtherrer@um.es

Sleep deprivation in *Octodon Degus*: Effects of Transcranial Magnetic Stimulation in spatial learning and memory.

Estrada C [a], López D [a], Conesa A [a], Toledo F [a], Tunez I [b], Fernández-Gómez FJ [a], Bordet R[c], Richardson JC[d], Blin O[e], Fernandez-Villalba E [a], Herrero MT [a]

[a] Clinical & Experimental Neuroscience (NiCE-CIBERNED), School of Medicine, *Campus Mare Nostrum*, University of Murcia; Murcia, Spain, [b] Research Network on Ageing and Fragile (RETICEF), Department of Biochemistry and Molecular Biology, Faculty of Medicine, Maimonides Institute of Biomedical Research of Córdoba (IMIBIC), Córdoba University, Córdoba, Spain, [c] Department of Medical Pharmacology, University Lille-North, 1 Place Verdun, 59045 Lille, France, [d] GlaxoSmithKline R&D, Neurosciences Therapeutic Area, Gunnels Wood Road, Stevenage, Herts SG1 2NY, United Kingdom, [e] Department of Pharmacology, Aix-Marseille University, Marseille, France

* Corresponding author:

María Trinidad Herrero Ezquerro, MD, PhD
Clinical and Experimental Neuroscience (NiCE-CIBERNED)
Faculty of Medicine, Campus of Espinardo
University of Murcia, 30100 Murcia. Spain
Tel. +34 868888484 Fax. +34 868884150
E-mail: mtherrer@um.es

Abstract

Sleep is indispensable for maintaining regular daily life activities and a fundamental physiological purpose in cognitive performance. Sleep deprivation (SD) may affect subsequent learning capacity and ability to constitute new memories, particularly in the situation of hippocampus-dependent tasks. Transcranial magnetic stimulation (TMS) is a non-invasive procedure of electromagnetic induction that generates electric currents activating nearby nerve cells in the stimulated cortical area. Several studies have begun to therapeutically use TMS. The present study was designed to evaluate how TMS could improve learning and memory functions after SD in *O. degus.* Thirty juvenile (aged eighteen months old) females were divided in three groups (control, acute and chronic TMS treatment –with and without SD). To immobilize TMS-treated groups, they were disposed in plastic cylindrical while the *O. degus* were receiving head magnetic stimulation. SD was achieved by gently handling the animals to keep them awake during the night. Behavioral tests include Radial Arm Maze (RAM), Barnes Maze (BM) and Novel Object Recognition (NOR). When TMS treatment was applied during several days, there was significant improvement of cognitive performance after SD. Noteworthy, only one session of TMS is able to improve some parameters related with spatial memory. No side effects were observed. After SD, chronic TMS treatment provokes significant amelioration in learning and in both spatial and working memory. Nonetheless, an acute treatment of TMS is already sufficient to improve spatial memory.

Abbrevations

SD: Sleep deprivation

TMS: Transcranial magnetic stimulation

RAM: Radial Arm Maze

BM: Barnes Maze

RME: Reference Memory Errors

WME: Working Memory Errors

NOR: Novel Object Recognition test

NLR: Novel Local Recognition test

RI: Recognition index

ELF MF: Extremely low frequency magnetic fields

1. Introduction

Sleep plays an important role in normal biological functions. It is an essential element of our life and is crucial for maintaining ordinary daily life activities and key physiological objectives such as immune defense (Everson, 1993), thermoregulation (Poirrier et al., 2008), tissue restoration (Adam and Oswald, 1977) energy conservation (Berger and Phillips, 1995) and brain plasticity (Dang-Vu et al., 2006). Sleep is thought to be a procedure that abilities neuronal and synaptic plasticity, which in turns is decisive in cognition, cerebral role and for memory and learning integration (Benington and Frank, 2003) (Meerlo et al., 2009) (Tononi and Cirelli, 2006) (Blissitt, 2001). Many studies suggest the significance of post-training sleep for integration and strengthening of the different types of memories (Graves et al., 2001) (Maquet, 2001) (Walker and Stickgold, 2004). However, sleep prior to learning may influence memory processes as well, by delimiting the ability of neuronal networks to process new knowledge and the capacity to encode novel retention. Insomnia has a deleterious influence in people and sleep deprivation (SD) has been studied as one of the paradigms that most efficiently produces transient cognitive impairment (Walker and Stickgold, 2004) (Jugovac and Cavallero, 2012) (McEwen, 2006) (Huber et al., 2004) in both animals and humans (Alzoubi et al., 2012) (Palchykova et al., 2006) (Alhaider et al., 2011). This produces, in both procedural and declarative memories (Jugovac and Cavallero, 2012) an inadequate integration. Some aspects of sleep function has been studied with SD, as well it has been considered the cognitive levels and brain function in situations of sleep loss (Colavito et al., 2013). There is a large data indicating a robust correlation between SD and memory impairment (Kim et al., 2005). For example, a single night of sleep deprivation impairs working, procedural and implicit memory types in humans (Forest and Godbout, 2000). Furthermore, prior to learning, SD decreases learning ability and damages memory, while the memory formation is impaired by post-learning SD (Harrison and Horne, 2000). Evidence shows that whereas sleep loss reduces hippocampal activity (Yoo et al., 2007), the hippocampus emerges as more energetic and quick when people are allowed to sleep after a learning task (Gais et al., 2007). In addition, it has been seen an increase in the duration of rapid eye movement (REM) sleep

phase after a period of learning (De Koninck et al., 1990). As well, it has been demonstrated the hippocampus-dependent learning and memory in rat impairment after sleep loss (Youngblood et al., 1999).

The significance and consequences of having a bad or damage sleep has been reported on a diversity of cognitive tasks in conventional rodents (McCoy and Strecker, 2011), and SD has also been studied in the *Octodon degus* (*O. degus) (Kas and Edgar, 1999)*. Analyzing memory impairment caused by this challenge in the *O. degus* is particularly important and considerable, since this social rodent has become an increasingly familiar and accepted experimental animal in the latest years. Furthermore, many therapeutic and medical disciplines, particularly those referred to brain functions, has characterized as an important animal model for research (Tarragon et al., 2013). We used SD as a condition which alters the formation and expression of memories and originates a failure in both procedural and declarative memories (Walker and Stickgold, 2004) (Jugovac and Cavallero, 2012) (Huber et al., 2004).

Transcranial magnetic stimulation (TMS) is one of non-invasive ways of brain stimulation procedure which has been in progress over the last few decades (Luber et al., 2013). TMS creates an electric current across the skull without physical contact. The use of the technique has a remarkable tolerability and it is safely. TMS may be a useful way to study neurophysiology and plasticity. Additionally to its employment in research, TMS has been examined in therapeutic trials as a treatment tool (Rajapakse and Kirton, 2013). In order to investigate complicated facets of the human brain as cognition or motor function, TMS has been popularly used (Fregni et al., 2006). In fact, TMS is a manner that is being used to medicate some neuropsychiatric and neurodegenerative disorders, but it is progress the potential therapeutic applications in the therapy of a variety of neurological conditions (Nahas et al., 2004).

Given the insufficient efficacy of pharmacological treatments (Birks, 2006) and the plausible long-term problems in the elderly (Wu et al., 2009), non-pharmacological approaches are of considerable interest and, then, in the present study we tested how TMS could improve learning and memory

functions (spatial learning and memory processing) after sleep deprivation in *O. degus*.

2. Material and methods

2.1. Animals

Thirty healthy juvenile (female, eighteen month-old) weighing between 180-200 g at the beginning study were purchased from our colony. These animals were divided into three groups depending on the TMS treatment: I) control (without TMS treatment), II) 1 only TMS session and III) 2 sessions of TMS per day during several consecutive days depending on the task that we performed. The animals were individually housed in plexiglas cages in an isolated room (Chronolab), with controlled humidity (60%) and temperature (23±1ºC), and under a 12:12 light/dark cycle (light on from 8.00 to 20.00h). Light was provided by fluorescent lamps regulated by an electronic timer (DataMicro, Orbis), and at the cage level there were a light intensity of 350–400 lx. The *O. degus* were fed *ad libitum* throughout the experiment, using a commercial feed (Harlan complete feed for rodents- Maintenance). The experiments were performed during the light period (09:00–15:00 h). Important efforts were made to minimize and refine the number of animals used. The “Three R's principle” was prudently applied in our study, following the most common suggestions from the European Community Council Directive for animal experimentation and care as regards the number of animals to use in preclinical studies. All experimental procedures complied with ethical committee of the University of Murcia and the European Community Council Directive (2010/63/UE).

2.2 Behavioral tests

We use an experimental room with the same noise and temperature conditions to perform all the behavioral tests. It should be mentioned that the animals were allowed to adapt to the experimental room for 24 hours.

2.2.1 Room configuration

The experimental room was configured with visual clues like different colors and shapes (triangle, rectangle, circle or a cross). They were adapted and placed surrounding the maze. Animals had spatial visual signs in the room (for example a chair, a trash can or a computer) and calculated (prepared by the

experimenter). During the experiments, we did not move these clues in order to use it as animal's reference points for locating the target hole or arms.

2.2.2 Radial Arm Maze

To evaluate the learning and memory of the animals, we used the Radial Arm Maze (RAM) (Tarragon et al., 2014). The apparatus consists in a platform with eight equidistantly spaced arms (42×12×12 cm^3). The arms emerge from a central octagonal platform. In this paradigm the *O. degus* were assessed for learning and memory. Before starting the experiment, the animals were kept on a restricted regime, always maintaining their body weight in the 80% of that prior to the training (Dudchenko, 2004) (Srikumar et al., 2006).

Habituation session At the beginning of the training period, our *O. degus* were habituated to the maze. The animals were allowed to examine the eight arms of the maze for 10 minutes.

Learning period The learning period is performed during seven days where the animals were given an acquisition trial per day until they achieved the learning criteria.

At the commencement of each trial, we baited four of the eight arms (1, 4, 5, and 7) with food and cleaned the maze with ethanol (70%). The *O. degus* was placed on the central platform and then, they were allowed to move freely. It was recorded the arm when the animal ate the reward or reached the end of an arm. The correct choise was recorded just the first entrance to the baited arm. When *O. degus* went into the unbaited arms, it was recorded as Reference Memory Errors (RME) and reentrances into the baited arms were recorded as Working Memory Errors (WME). During seven days, all *O. degus* of every group were given one trial and the data acquired were averaged and used in the final analysis. The *O. degus* actions were scored by the latency to the first arm, total time of entrance into all arms, RME and WME (Dudchenko, 2004) (Srikumar et al., 2006) (Karkada et al., 2012).

Retention session (Test). One day after the learning period, the *O. degus* were examined for retention of the task. They were given just one trial, and before start the test we removed all the food that were placed in the maze. Latency time in first arm, total time, WME, and RME was used for analysis.

The 1 TMS group was placed in the cages to receive TMS. After two hours of treatment, they were given the trial. On the other hand, the chronic TMS group received TMS in the retention session, and in addition during the learning period, a total of seven days (Fig. 1 A)

2.2.3 Barnes maze test

The Barnes Maze (BM) is a circular platform with 160 cm of diameter raised 75 cm from the ground and surrounded with 55 cm high plastic wall. The platform was made of white Plexiglas and it was made with eighteen circular holes (8 cm in diameter), with an equidistant distance from each other of 16 cm and 5.5 cm from the outer edge. Just the escape hole has a plastic and transparent escape box, positioned under it, the other holes were blocked with mesh. An open and metallic box (20 x 15 cm) was used as start box. The room where the test was performed, is illuminated by fluorescent lights located on the ceiling (normal room lighting) such that the maze was exposed to an illumination of 210 lx.

Habituation session. During habituation period the animal is placed in the escape cage for 2 minutes. We filled the cage with the bedding from its own home cage. After this period, the animal was placed in the platform near the escape hole and we left free for 1 minute to escape. If the animal did not pass into the escape box, it was kindly picked up and put through the target hole into the escape box. Again, we left the animals in the escape box for 2 minutes. Finally, the animal was put in the centre of the platform, and we left the animals during the following 4 minutes to enter into escape box. If the *O. degus* did not enter in the escape box, it was put into the escape cage as we explain before and we left there for 2 minutes. Every of the parts that we have explained, were separated by a 5 min of resting time, which *O. degus* spent in its home cage. During this period, in order to remove odors we cleaned with ethanol the maze and the start point.

Learning and memory period. One day after the habituation session, the *O. degus* were trained for seven consecutive days. Four trials of 4 minutes were done every day. We left the *O. degus* in their home cage for 5 minutes between each trial. At the initial part of the trial, *O. degus* was kept in the start box for 30 seconds in the centre of the platform. Each *O. degus* was allowed to explore the

maze freely for the 4 minutes of the session. It was picked up and softly placed into the escape box when they did not escape.

Each *O. degus* was left in the escape box for 2 minutes before being returned to its home cage for 5 minutes. The escape hole was kept at the same position throughout all trials and sessions. Between trials, we cleaned perfectly the surfaces of the platform and start box. The following parameters were recorded: i) Latency to the first visit of escape hole; ii) Decision time of entrance into the escape box, time from the initiation of exploration of the escape hole and entrance into the escape hole; iii) Latency to escape; iv) Number of reference memory errors (on each trial, every first visit of a non-escape hole was scored as a RME); and v) Number of working memory errors (repeated visits to the same non-escape hole on the same trial were scored as WME).

Retention session (Test). The next day after the learning period, the *O. degus* were examined for retention of the task. They were given one trial, and the latency time in first arm, total time, WME, and RME was used for analysis. The 1 session TMS group was placed in the cages to receive the treatment. After two hours of TMS, they performed the trial. On the other hand, the chronic TMS group received TMS in the retention session and in addition during the learning period (Fig. 1 B) (Tarragon et al., 2014)

2.2.4 Novel object recognition

The Novel Object Recognition test (NOR) is a moderately simple and direct method to test the working memory in rodents. The essence of NOR is to explore the spontaneous behavior of rodents and it is an absolute test of working memory, free of reference. It has two principal advantages if we compared to other behavioral assays. Firstly, it is a test sociable and pleasant for the animals. As well, NOR test does not demand a positive or negative stimuli or support. The NOR is based on the response of rodents which tender to a novel object to a familiar object and spend more time in exploring the novel object. The experiment was done in their own cages. The procedure for the NOR task consisted of three different retention sessions (Familiar session, Novel Local Recognition, Novel Object Recognition) after a habituation. Each *O. degus* was placed in their cage in the experimental and sound-attenuated room

(day 1). During the familiarization period, two different novel objects were symmetrically fixed to the floor in their own cage, and each *O. degus* was allowed to explore in the box for 10 min (day 2). These objects were different in shape and color but similar in size. We considered that the animal was exploring the object when the head of the *O. degus* was facing the object. The time exploring each object was recorded. One hour after the familiarization, one of the familiar objects that were used during that session was moved to the other side of the cage (NLR). The animals were allowed to examine freely for 5 minutes. One hour after the NLR, the familiar object that was initially changed of site, in this session was replaced by a new object. The animals were allowed to examine openly for 5 min and it was recorded the time that each *O. degus* was exploring both objects. A discrimination index, which is a ratio of the difference in time spent for exploring the novel (place or object) and familiar object to the total time spent for exploring both objects, was used as a variable of cognitive function. They were fixed on the floor of the cage in order to keep them immobile. To avoid the permanence of olfactory cues, both objects were always thoroughly cleaned with ethanol after each trial. Object exploration time was defined when *O. degus* directing its nose within 2 cm distance to the object, or sniffing or pawing the object as we said above, so sitting or standing on the object was not recognized as an examination of the object. The exploration time was calculated manually using 2 stop watches (Fig. 1 C). The Recognition Index (RI) was calculated using the following formula in the testing phase:

$$RI = \frac{\text{Time exploring novel object}}{(\text{Time exploring novel object} + \text{Time exploring familiar object})} \times 100\%$$

2.3 TMS

To immobilize TMS-treated groups, they were disposed in plastic cylindrical while the *O. degus* were receiving head magnetic stimulation. Every coil was formed of 1000 turns of enameled copper wire (7 cm of diameter) hold by plastic receptacles (10,5 x 10,5 x 3,5). A pair of Helmholtz coils generated the magnetic fields (Dhan 1000 (tm); Magnetoterapia S.A. de C.V., Mexico DF, Mexico). The stimulation was formed of an oscillatory magnetic field with the conformation of a sinusoidal wave and we selected a frequency of 60 Hz and

amplitude of 0.7 mT to be applied for 2 hours in the morning in 1 session TMS groups. Chronic TMS groups were treated for a period of four days prior to NOR test and seven days during the training period of RAM and BM, being applied for 2 hours in the morning and 2 hours in the afternoon. Animals showed no signs of discomfort when were exposed to TMS. It were measured that the two hours of TMS induced a highest peak of temperature not superior to 0.5-1ºC inside of the chamber. Dorsally and ventrally to the skull of the animal were placed the two coils. The space between each coil and the midpoint of the cranium was approximately 6 cm. TMS was administered just before performing the tests.

2.4 Sleep Deprivation

SD is simply achieved by gently handling the animals to keep them awake (Webster et al., 2013). Gentle handling is a non-stressful form using to keep the animals awake, so we prevented them from sleeping. After the procedure during 12 hours, the behavioral test takes place (Kas and Edgar, 1999). The SD challenge starts at 7 p.m. in a 12/12-h light and dark cycle.

2.5 Statistical analysis

The data are presented as mean ± standard error of the mean (S.E.M.). The statistical analysis was made using the Statistical 9.0 (StarSoft, Tulsa, OK) software package. It was performed using a factorial ANOVA test following a Fisher´s LSD post hoc analysis, if the repeated measures analysis showed significant differences between groups. Differences were considered statistically significant if $p \leq 0.05$.

3. Results

3.1 Radial Arm Maze

Effect of TMS on the cognitive impairment induced by sleep deprivation evaluated by RAM.

RAM has been used to evaluate the learning and memory in *O. degus*, specific variables (latency, total time, working memory errors and reference memory errors) were used to measure the assessment of learning and memory during the sessions (Fig. 2)

Effect of TMS in 1 session and 7 days

In order to assay the effects of TMS on spatial memory, latency was examined, and there were no differences between control, 1 TMS group and 7 TMS group (Fig. 2 A). In the analysis of total time, two-way (no SD/SD condition and TMS treatment) ANOVA as the between-subject factors was performed. There was a significant effect of TMS treatment ($F(2,29)=20.96$, $p \leq 0.01$). There was no significant effect in no SD/SD condition. In *post hoc* Fisher´s LSD, there were significant differences between control and 1 TMS and 7 TMS in normal sleep and SD ($p \leq 0.01$; ##). TMS-treated animals performed the task significantly better than control groups (Fig. 2 B).

The analysis of working memory errors was done by two-way ANOVA (TMS x no SD/SD condition). It showed a significant effect of no SD/SD condition ($F(1,32)=10.33$, $p \leq 0.01$), TMS treatment ($F(2,32)=13.28$, $p \leq 0.01$) and interaction of TMS and no SD/SD condition ($F(2,32)=14.12$, $p \leq 0.01$). *Post hoc* analysis demonstrated that SD 1 TMS group had significantly less working memory errors than SD control group ($p \leq 0.01$; ##). On the other hand, SD 7 TMS group had significantly less working memory errors than SD control group ($p \leq 0.01$; ##) (Fig. 2 C). Whereas there were no significant differences between normal control and normal 7 TMS groups ($p=0.7984$), indicative that the application of TMS has no negative effect in normal animals.

Reference memory errors analysis showed a significant effect of TMS ($F(2,36)=7.73$, $p \leq 0.01$) and interaction of TMS and no SD/SD condition ($F(2,36)=5.06$, $p \leq 0.05$). *Post hoc* analysis revealed that SD 1 TMS and SD 7 TMS groups had significantly less reference memory errors than SD control group ($p \leq 0.01$; ##) (Fig. 2 D). 1 TMS and 7 TMS SD groups demonstrated improvement in the retention day.

3.2 Barnes Maze (BM)

Effect of TMS in 1 session and 7 days

To further evaluate the effects of TMS on spatial memory, latency to escape hole was examined by the BM test. There was a significant effect in the TMS treatment ($F(2,31)=9.61$, $p \leq 0.01$). *Post hoc* Fisher´s LSD revealed a significant difference between SD 1 TMS and SD 7 TMS groups respect to SD control

groups (p≤0.01; ##) (Fig. 3 A). In the analysis of total time, two-way ANOVA with TMS and no SD/SD condition as the between-subject factors was performed. There was a significant effect both in no SD/SD ($F(1,32)=11.23$, p≤0.01) and in TMS treatment ($F(2,32)=6.97$, p≤0.01). *Post hoc* analysis showed statistic differences between normal and SD of control group (p≤0.01; **). There were no statistic differences between control and 1 TMS groups. In addition, it showed that treated 1 TMS group took a similar total time than control group in performing the task, but the analysis revealed a significant reduction of total time in SD 7 TMS animals compare to SD control group (p≤0.01; ##) (Fig. 3 B).

The analysis of working memory errors was done by two-way ANOVA (TMS x no SD/SD condition). It showed a significant effect of no SD/SD condition ($F(1,32)=57.27$, p≤0.01), TMS treatment ($F(2,36)=33.24$, p≤0.01) and interaction of TMS and no SD/SD condition ($F(2,36)=45.91$, p≤0.01). *Post hoc* analysis demonstrated that SD 1 TMS group had significantly less working memory errors than SD control group (p≤0.01; ##). The analysis revealed that SD 7 TMS group had significantly less working memory errors than SD control group too (p≤0.01; ##), and SD 7 TMS significantly reduce the errors compare to SD 1 TMS groups (p≤0.05; #) (Fig. 3 C). There were no significant differences between normal control and normal 7 TMS groups (without SD) (p=0.536), indicating that the application of TMS alone does not induce side-effects.

Reference memory errors analysis showed a significant effect of no SD/SD condition ($F(1,34)=37.45$, p≤0.01), TMS treatment ($F(2,34)=10.02$, p≤0.01), and between TMS and no SD/SD condition ($F(2,34)=12.94$, p≤0.01). *Post hoc* analysis revealed that SD 1 TMS group had significantly less reference memory errors than SD control group. As well as SD 7 TMS group had significantly less reference memory errors than SD control group (p≤0.01; ##). Whereas there were no significant differences between normal control and normal 7 TMS groups (p=0.82), and normal 1 TMS group (p=0.32) (Fig. 3 D).

3.3 Novel Object Recognition

The NOR results are shown in Figure 4. An analysis of the results was performed to see the effect of TMS and sleep deprivation (two independent variables) on the % recognition index in animals. During the familiarization period, all groups (control, 1 session TMS, 4 days TMS, normal and SD groups) spent a similar amount of time exploring each of the two objects (A and B), indicating that animals had no preference for any specific object (Fig. 4 A) (Effect of condition $F(1,76)=2,1$, $p=0.1514$, Effect of treatment $F(2,76)=0.0578$, $p=0.9439$).

Effect of TMS in 1 session and 4 days

The ANOVA two-way of NLR revealed a significantly effect of the TMS treatment ($F(2,45)=5.364$, $p\leq0.01$) and an effect of interaction between treatment and no SD/SD condition ($F(2,45)=4.863$, $p\leq0.05$) The *post hoc* analysis showed that the SD 1 TMS and SD 4 TMS group explored the novel placed object significantly more than SD control group, suggesting that a session of TMS after a sleep deprivation neutralized the impairment that was seen in the control conditions. There are significant differences between the SD 4 TMS group and SD control group ($p\leq0.01$) and SD 1 TMS group ($p=0.03$) too. SD group with 4 days of TMS treatment increased significantly the recognition index (Fig. 4 B).

The results obtained in the NOR test showed that there are not significant differences between normal sleep and SD 4 TMS groups. The ANOVA showed a significantly effect of no SD/SD condition ($F(1,47)=8.887$, $p\leq0.01$) and TMS treatment ($F(2,47)=12.559$, $p\leq0.01$). There are no significant differences between the normal and SD 1 session of TMS groups, but there are significant differences between SD 4 TMS groups with respect to SD 1 TMS and SD control groups ($p\leq0.01$; ##). Also, it showed a significant difference in the IR% between animals which were treated with only one session of TMS and 4 days of TMS ($p\leq0.05$; #) (Fig. 4 C). All animals showed an increase in the IR% after 4 days of TMS.

4. Discussion

The purpose of the study was to examine the impact of the TMS treatment on spatial memory in *O. degus* in a cognitive alterations model induced by sleep-deprived animals. The experimental methods used (NOR, RAM and BM) are common for assessing deficits in hippocampal-based spatial reference memory (Bryan et al., 2009). RAM and BM are classics methods to examine the spatial learning and memory in rodents, which are widely applied to study the behavioral deficits (Guan et al., 2004). The novel object recognition test is a simple behavioral procedure that is based on the spontaneous answer of rodents to examine novelty and is a pure working memory test as we have said before, being free for reference. Therefore, the use of exploration-based memory tasks let us to remark any especial alteration in spatial reference memory for the different paradigms evaluated.

There are many forms of SD, and it has been shown how it can negatively affect the capacity to retain current information and disturb memory consolidation (Graves et al., 2003). In many studies, it has been corroborated using different models, the detrimental effect of sleep loss on cognition (Hairston et al., 2005). In the current study, we induced sleep-deprivation in *O. degus* in order to evaluate memory impairment caused by this challenge. The connection between sleep and cognition is properly established (Joo et al., 2012) (Sterpenich et al., 2007) (Guzman-Marin et al., 2003) (Yoo et al., 2007), as well it is known that an important sleep loss suppress adult neuronal cell proliferation (Guzman-Marin et al., 2008) (Mirescu et al., 2006) (Mueller et al., 2008) (Aleisa et al., 2011). In this line of observations, our results point out that normal sleep control groups performed properly spatial memory task better than SD control groups, where deficits in spatial reference memory were evident. Many studies support our current findings showing that suppression of some sleep period impacts adversely in memory, independently of any stressful situation of the animal like social anxiety, or humidity (Zagaar et al., 2012). SD provokes an accumulating sleep debt and it has the consequence of an increase of the sacrifice to maintain wakefulness all the time, and this is caused by the homeostatic regulation of sleep. In humans, the procedure of SD is different of the one that it could be use in animals. Without taking in account

drug-induced insomnia, there are a huge of activities that can be use to keep awake people. SD in animals is adulterated by a degree of stress. At the same time, we would need to look for new protocols in order to prevent stress or adverse conditions (Colavito et al., 2013). These obviously self-evident points are applicable if we compare experimental SD in humans and laboratory animals.

A positive effect of exposure to TMS on spatial learning and memory was observed on all the cognitive procedures explored (RAM, BM and NOR). We showed that sleep-deprived animals had better cognitive performance as assessed by RAM, when groups were treated with one and seven days of TMS treatment. As well, we showed that normal sleep animals with 1 and 7 TMS had better cognitive performance than untreated normal animals (Fig. 2 B). These findings are consistent with previous studies suggesting an improvement in social recognition memory after 1 mT extremely low frequency magnetic fields (ELF MF) exposure for 2 hours daily during 9 days (Pascual-Leone, 2002; Vazquez-Garcia et al., 2004). Therefore, behavioral and neurophysiological alterations have been described after exposure to ELF-MF in both animals and humans (Capone et al., 2009). The physiological support of these facts is still inadequately comprehended. After a chronic ELF-MF treatment, it has been described an accurate impact in social recognition memory and spatial learning, indicating an important role of the duration of the exposure (He et al., 2011). In particular, it is not known specially which stimulation parameters (frequency, intensity, duration, and number of pulses) are needed for an optimal answer. For instance, TMS has shown to evoke a diversity of different responses depending on the stimulated site or intensity (Rizzo et al., 2004) (Yang et al., 2012) (Houdayer et al., 2008). We might not rule out that probably appreciable modifications can take place in nervous system during the exposure time, but nevertheless ultimately leading to a more efficient transmission of neural signals, tolerating greater capacity to moderate the cognitive dysfunction induced by SD, as shown by the BM and RAM approaches (Figs. 2 and 3).

It is worth suggesting that it has been corroborated that applying a magnetic pulse over a cortical zone of the brain has no consequence at all on the normal

answer. The magnetic stimulation could meddle with the task performance even though when the treatment is triggered during cognitive recruitment (Cowey, 2005) (Silvanto and Pascual-Leone, 2008). Furthermore, it has been showed that a single TMS pulse can produce significant differences in cortical response depending on how activated the cortex is at the moment the pulse is applied (Hanakawa et al., 2009). This should be taken in account when talking about inhibitory or excitatory effects. Hippocampus is an important area in the formation of recognition memory in both human and animals (Norman and Eacott, 2004) and perirhinal cortex has also been suggested that could play an important function in object recognition (Nardone et al., 2012). In addition, the favorable effects of no invasive stimulation may be enhanced if we stimulate a cortical area which is implicated in the execution of a training task. A TMS treatment induces a transient electromagnetic field over the scalp. This treatment is going to change magnetic fields which cause trans-synaptic depolarizations of groups of neurons that are placed in the external cortical stratum. The potential ability of TMS to neutralize a brain function with a poor condition, is a presumable application in cognitive rehabilitation (Nardone et al., 2012).

We showed that sleep-deprived animals had worse cognitive performance as assessed by RAM, BM and NOR. However, when groups were treated with only one session of TMS, it also showed a great improvement in some specific variables of paradigms used to examine spatial learning and memory (Fig. 2 and 3). TMS mechanisms have been elucidated by animal studies (Wang et al., 1996) and demonstrated its security (Russell et al., 1994) (Post et al., 1999). When *O. degus* were exposed to seven days of TMS, they showed significant decreases in the escape latency, in the total time and in both working and reference memory errors at the test day. The total time they spent in perform the task was significantly lower in animals that were treated with only one session of TMS. This point that the dose we used, are physiologically relevant in a therapeutic range with no toxicity. Moreover, in novel object recognition test, the more TMS sessions were administrated, the higher recognition index was obtained. Our data showed that in 4 TMS animal dose had a greater recognition index than 1 TMS animals (Fig. 4). Our data suggest that in relation

with working memory, only one session of TMS is not enough and it is necessary a chronic treatment with TMS in order to counteract the transient cognitive impairment. TMS is able to modulate the cortical excitability (Pascual-Leone et al., 1998). As well, it can produce lasting after effects on cortical function and it may be due to the first period of synaptic potentiation / depression (Rothwell, 2012). Despite its proven significance, the mechanism through which TMS exerts its beneficial action on neuronal function, as we said above, are badly understood (Tasset et al., 2013). TMS represents a technique that induces electrical currents within the cerebrum and this function can be applied in order to caused a briefly disrupt of a brain area, map cortical area or assess cortical excitability, as well it may change cortical activity. However, there is also some difference of opinion regarding whether TMS can cause activation or inhibition of cortical excitability. Furthermore, the practicability and reproducibility of TMS in the rat have been valued (Luft et al., 2001). The lateralized TMS advancing to asymmetric brachioradialis activation is achievable according to a study with traditional TMS equipment in rats (Rotenberg et al., 2010). In addition, it has been demonstrated the use of TMS as an electroconvulsive therapy in rats with anti- depressive activity and without side effects (Zyss et al., 2000). Different studies found that TMS improves affective and motor symptoms in patients with depression and Parkinson's disease (Anderkova and Rektorova, 2014) (Lefaucheur et al., 2014) (Kamble et al., 2014). The data suggest that these benefits effects may be in relationship with changes in dopamine levels. Dopamine is a catecholamine implicated in the maintenance of sleep and wake. In addition, data obtained in works of experimental models similar to depression, Parkinson's or Huntington's disease shown that TMS provokes enhancement of dopamine levels in nervous tissue (Tasset et al., 2012) (Arias-Carrion et al., 2006) (Heumann et al., 2014). A recent study provides indications on effects of TMS on sleep and vigilance in humans. These effects depend on the protocols, design and models we used (Mensen et al., 2014). These studies highlight the therapeutic potential of the induction of TMS in *O. degus.* Taking into consideration that this rodent constitutes a "spontaneous" model of neurological disorders such as Alzheimer´s disease (Tarragon et al., 2013), it holds a significant promise to progress in the comprehension of brain mechanisms and to originate novel

rehabilitation approaches improving the prognosis of people with neurodegenerative disorders. Acute treatment of TMS is positive for improving cognitive performance after sleep deprivation, mainly spatial memory, but best recovering is obtained in spatial learning and in both spatial and working memory after a chronic TMS treatment. This treatment has a therapeutic potential significantly improving cognitive performance in *O. degus* after sleep deprivation.

Acknowledgements

This work was supported by grants from the Spanish Ministry of Science and Innovation (FIS PI13 01293), *Fundación Séneca* (FS/14902/PI/10), University Jaume I (13I004.01/1), "*Prediction of cognitive properties of new drug candidates for neurodegenerative diseases in early clinical development*" (European Community's Seventh Framework Programme (FP7/2007-2013) for the Innovative Medicine Initiative under Grant Agreement No 115009) and CIBERNED (*Centro de Investigación Biomédica en Red sobre Enfermedades Neurodegenerativas*).

Legends

Figure 1. Design of experimental tests. The first test after training was performed under normal sleep condition (No SD), whereas sleep deprivation (SD) was conducted before the second test. A) Design of RAM and B) BM. For both paradigms, RAM and BM, animals were trained for seven days. In 1 TMS groups, animals were treated for two hours before they performed the test. In chronic TMS groups, animals were treated for a period of seven days during the training period. C) NOR test. In 1 TMS groups, animals were treated for two hours before they performed the test. Previous four days before performing the NOR test, animals were treated with TMS as a chronic treatment, being applied for 2 hours in the morning and 2 hours in the afternoon. D) Representative picture of technique for noninvasive magnetic stimulation.

Figure 2. Effect of 1 session of TMS and 7 days of TMS in latency, total time, working memory errors and reference memory errors in RAM (n=30). A) Latency time in first arm (seconds), B) Total time of entrance into all arms (seconds) in animals, ## $p \leq 0.01$ versus normal and SD 1 TMS and 7 TMS groups. C) Working memory errors, ** $p \leq 0.01$ versus normal control group, ## $p \leq 0.01$ versus SD 1 TMS and 7 TMS groups. D) Reference memory errors, ** $p \leq 0.01$ versus normal control group, ## $p \leq 0.01$ versus SD 1 TMS and 7 TMS groups.

Figure 3. Effect of 1 session of TMS and 7 days of TMS in latency, total time, working memory errors and reference memory errors in BM (n=30). A) Latency time in first arm (seconds), ** $p \leq 0.01$ with respect to normal control group, ## $p \leq 0.01$ with respect to SD 1 TMS and 7 TMS groups, B) Total time of entrance into all arms (seconds), ** $p \leq 0.01$ with respect to normal control group, ## $p \leq 0.01$ with respect to SD control group, # $p \leq 0.05$ with respect to SD 1 TMS group, C) Working memory errors, ** $p \leq 0.01$ with respect to normal control group; ## $p \leq 0.01$ with respect to normal SD control group, # $p \leq 0.05$ with respect to SD 1 TMS group, D) Reference memory errors, ** $p \leq 0.01$ with respect to normal control group, ## $p \leq 0.01$ with respect to SD 1 TMS and 7 TMS groups.

Figure 4. Effect of 1 session of TMS and 4 days of TMS. A) Familiarization, B) NLR IR% of untreated, 1 session TMS and 4 days of TMS of normal and SD

groups during the novel local recognition (n=30) ** p≤0.01 versus normal group, ## p≤0.01 versus SD control group, # p≤0.05 versus SD 1 TMS group, C) NOR IR% of untreated, 1 session TMS and 4 days of TMS of normal and SD groups during the novel object recognition (n=30) ** p≤0.01 versus normal group, ## p≤0.01 versus SD control and 1 TMS group, # p≤0.05 versus normal 1 TMS group.

References

Adam, K., Oswald, I., 1977. Sleep is for tissue restoration. J R Coll Physicians Lond. 11**,** 376-88.

Aleisa, A. M., et al., 2011. Acute nicotine treatment prevents REM sleep deprivation-induced learning and memory impairment in rat. Hippocampus. 21**,** 899-909.

Alhaider, I. A., et al., 2011. Sleep deprivation prevents stimulation-induced increases of levels of P-CREB and BDNF: protection by caffeine. Mol Cell Neurosci. 46**,** 742-51.

Alzoubi, K. H., et al., 2012. The neuroprotective effect of vitamin E on chronic sleep deprivation-induced memory impairment: the role of oxidative stress. Behav Brain Res. 226**,** 205-10.

Anderkova, L., Rektorova, I., 2014. Cognitive effects of repetitive transcranial magnetic stimulation in patients with neurodegenerative diseases - clinician's perspective. J Neurol Sci. 339**,** 15-25.

Arias-Carrion, O., et al., 2006. Neuronal precursors within the adult rat subventricular zone differentiate into dopaminergic neurons after substantia nigra lesion and chromaffin cell transplant. J Neurosci Res. 84**,** 1425-37.

Benington, J. H., Frank, M. G., 2003. Cellular and molecular connections between sleep and synaptic plasticity. Prog Neurobiol. 69**,** 71-101.

Berger, R. J., Phillips, N. H., 1995. Energy conservation and sleep. Behav Brain Res. 69**,** 65-73.

Birks, J., 2006. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev. CD005593.

Blissitt, P. A., 2001. Sleep, memory, and learning. J Neurosci Nurs. 33**,** 208-15.

Bryan, K. J., et al., 2009. Transgenic Mouse Models of Alzheimer's Disease: Behavioral Testing and Considerations.

Capone, F., et al., 2009. Does exposure to extremely low frequency magnetic fields produce functional changes in human brain? J Neural Transm. 116**,** 257-65.

Colavito, V., et al., 2013. Experimental sleep deprivation as a tool to test memory deficits in rodents. Front Syst Neurosci. 7**,** 106.

Cowey, A., 2005. The Ferrier Lecture 2004 what can transcranial magnetic stimulation tell us about how the brain works? Philos Trans R Soc Lond B Biol Sci. 360**,** 1185-205.

Dang-Vu, T. T., et al., 2006. A role for sleep in brain plasticity. Pediatr Rehabil. 9**,** 98-118.

De Koninck, J., et al., 1990. Language learning efficiency, dreams and REM sleep. Psychiatr J Univ Ott. 15**,** 91-2.

Dudchenko, P. A., 2004. An overview of the tasks used to test working memory in rodents. Neurosci Biobehav Rev. 28**,** 699-709.

Everson, C. A., 1993. Sustained sleep deprivation impairs host defense. Am J Physiol. 265**,** R1148-54.

Forest, G., Godbout, R., 2000. Effects of sleep deprivation on performance and EEG spectral analysis in young adults. Brain Cogn. 43**,** 195-200.

Fregni, F., et al., 2006. Predictors of antidepressant response in clinical trials of transcranial magnetic stimulation. Int J Neuropsychopharmacol. 9**,** 641-54.

Gais, S., et al., 2007. Sleep transforms the cerebral trace of declarative memories. Proc Natl Acad Sci U S A. 104**,** 18778-83.
Graves, L., et al., 2001. Sleep and memory: a molecular perspective. Trends Neurosci. 24**,** 237-43.
Graves, L. A., et al., 2003. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem. 10**,** 168-76.
Guan, Z., et al., 2004. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res. 1018**,** 38-47.
Guzman-Marin, R., et al., 2008. Rapid eye movement sleep deprivation contributes to reduction of neurogenesis in the hippocampal dentate gyrus of the adult rat. Sleep. 31**,** 167-75.
Guzman-Marin, R., et al., 2003. Sleep deprivation reduces proliferation of cells in the dentate gyrus of the hippocampus in rats. J Physiol. 549**,** 563-71.
Hairston, I. S., et al., 2005. Sleep restriction suppresses neurogenesis induced by hippocampus-dependent learning. J Neurophysiol. 94**,** 4224-33.
Hanakawa, T., et al., 2009. Stimulus-response profile during single-pulse transcranial magnetic stimulation to the primary motor cortex. Cereb Cortex. 19**,** 2605-15.
Harrison, Y., Horne, J. A., 2000. Sleep loss and temporal memory. Q J Exp Psychol A. 53**,** 271-9.
He, L. H., et al., 2011. Effects of extremely low frequency magnetic field on anxiety level and spatial memory of adult rats. Chin Med J (Engl). 124**,** 3362-6.
Heumann, R., et al., 2014. Dyskinesia in Parkinson's disease: mechanisms and current non-pharmacological interventions. J Neurochem. 130**,** 472-89.
Houdayer, E., et al., 2008. The effects of low- and high-frequency repetitive TMS on the input/output properties of the human corticospinal pathway. Exp Brain Res. 187**,** 207-17.
Huber, R., et al., 2004. Local sleep and learning. Nature. 430**,** 78-81.
Joo, E. Y., et al., 2012. Adverse effects of 24 hours of sleep deprivation on cognition and stress hormones. J Clin Neurol. 8**,** 146-50.
Jugovac, D., Cavallero, C., 2012. Twenty-four hours of total sleep deprivation selectively impairs attentional networks. Exp Psychol. 59**,** 115-23.
Kamble, N., et al., 2014. Therapeutic applications of repetitive transcranial magnetic stimulation (rTMS) in movement disorders: a review. Parkinsonism Relat Disord. 20**,** 695-707.
Karkada, G., et al., 2012. Nardostachys jatamansi extract prevents chronic restraint stress-induced learning and memory deficits in a radial arm maze task. J Nat Sci Biol Med. 3**,** 125-32.
Kas, M. J., Edgar, D. M., 1999. Circadian timed wakefulness at dawn opposes compensatory sleep responses after sleep deprivation in Octodon degus. Sleep. 22**,** 1045-53.
Kim, E. Y., et al., 2005. REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. Neurosci Lett. 388**,** 163-7.
Lefaucheur, J. P., et al., 2014. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS). Clin Neurophysiol.

Luber, B., et al., 2013. Applications of transcranial magnetic stimulation and magnetic seizure therapy in the study and treatment of disorders related to cerebral aging. Dialogues Clin Neurosci. 15**,** 87-98.
Luft, A. R., et al., 2001. Transcranial magnetic stimulation in the rat. Exp Brain Res. 140**,** 112-21.
Maquet, P., 2001. The role of sleep in learning and memory. Science. 294**,** 1048-52.
McCoy, J. G., Strecker, R. E., 2011. The cognitive cost of sleep lost. Neurobiol Learn Mem. 96**,** 564-82.
McEwen, B. S., 2006. Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. Metabolism. 55**,** S20-3.
Meerlo, P., et al., 2009. New neurons in the adult brain: the role of sleep and consequences of sleep loss. Sleep Med Rev. 13**,** 187-94.
Mensen, A., et al., 2014. The effects of theta-burst stimulation on sleep and vigilance in humans. Front Hum Neurosci. 8**,** 420.
Mirescu, C., et al., 2006. Sleep deprivation inhibits adult neurogenesis in the hippocampus by elevating glucocorticoids. Proc Natl Acad Sci U S A. 103**,** 19170-5.
Mueller, A. D., et al., 2008. Sleep deprivation can inhibit adult hippocampal neurogenesis independent of adrenal stress hormones. Am J Physiol Regul Integr Comp Physiol. 294**,** R1693-703.
Nahas, Z., et al., 2004. Safety and benefits of distance-adjusted prefrontal transcranial magnetic stimulation in depressed patients 55-75 years of age: a pilot study. Depress Anxiety. 19**,** 249-56.
Nardone, R., et al., 2012. Effect of transcranial brain stimulation for the treatment of Alzheimer disease: a review. Int J Alzheimers Dis. 2012**,** 687909.
Norman, G., Eacott, M. J., 2004. Impaired object recognition with increasing levels of feature ambiguity in rats with perirhinal cortex lesions. Behav Brain Res. 148**,** 79-91.
Palchykova, S., et al., 2006. Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. Physiol Behav. 87**,** 144-53.
Pascual-Leone, A., 2002. Handbook of transcranial magnetic stimulation. Arnold ;
Oxford University Press distributor, London
New York, NY.
Pascual-Leone, A., et al., 1998. Study and modulation of human cortical excitability with transcranial magnetic stimulation. J Clin Neurophysiol. 15**,** 333-43.
Poirrier, J. E., et al., 2008. Proteomic changes in rat hippocampus and adrenals following short-term sleep deprivation. Proteome Sci. 6**,** 14.
Post, A., et al., 1999. Repetitive transcranial magnetic stimulation in rats: evidence for a neuroprotective effect in vitro and in vivo. Eur J Neurosci. 11**,** 3247-54.
Rajapakse, T., Kirton, A., 2013. Non-Invasive Brain Stimulation in Children: Applications and Future Directions. Transl Neurosci. 4.
Rizzo, V., et al., 2004. Shaping the excitability of human motor cortex with premotor rTMS. J Physiol. 554**,** 483-95.
Rotenberg, A., et al., 2010. Lateralization of forelimb motor evoked potentials by transcranial magnetic stimulation in rats. Clin Neurophysiol. 121**,** 104-8.

Rothwell, J. C., 2012. Clinical applications of noninvasive electrical stimulation: problems and potential. Clin EEG Neurosci. 43**,** 209-14.
Russell, G. B., et al., 1994. Preservation of neurogenic motor-evoked potentials during isoflurane electroencephalographic burst suppression in rats. Spine (Phila Pa 1976). 19**,** 2632-6.
Silvanto, J., Pascual-Leone, A., 2008. State-dependency of transcranial magnetic stimulation. Brain Topogr. 21**,** 1-10.
Srikumar, B. N., et al., 2006. The involvement of cholinergic and noradrenergic systems in behavioral recovery following oxotremorine treatment to chronically stressed rats. Neuroscience. 143**,** 679-88.
Sterpenich, V., et al., 2007. Sleep-related hippocampo-cortical interplay during emotional memory recollection. PLoS Biol. 5**,** e282.
Tarragon, E., et al., 2013. Octodon degus: a model for the cognitive impairment associated with Alzheimer's disease. CNS Neurosci Ther. 19**,** 643-8.
Tarragon, E., et al., 2014. Memantine prevents reference and working memory impairment caused by sleep deprivation in both young and aged Octodon degus. Neuropharmacology. 85**,** 206-14.
Tasset, I., et al., 2012. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density. Neuroscience. 209**,** 54-63.
Tasset, I., et al., 2013. Extremely low-frequency electromagnetic fields activate the antioxidant pathway Nrf2 in a Huntington's disease-like rat model. Brain Stimul. 6**,** 84-6.
Tononi, G., Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med Rev. 10**,** 49-62.
Vazquez-Garcia, M., et al., 2004. Exposure to extremely low-frequency electromagnetic fields improves social recognition in male rats. Physiol Behav. 82**,** 685-90.
Walker, M. P., Stickgold, R., 2004. Sleep-dependent learning and memory consolidation. Neuron. 44**,** 121-33.
Wang, H., et al., 1996. LTD and LTP induced by transcranial magnetic stimulation in auditory cortex. Neuroreport. 7**,** 521-5.
Webster, S. J., et al., 2013. Comprehensive behavioral characterization of an APP/PS-1 double knock-in mouse model of Alzheimer's disease. Alzheimers Res Ther. 5**,** 28.
Wu, C. S., et al., 2009. The association between dementia and long-term use of benzodiazepine in the elderly: nested case-control study using claims data. Am J Geriatr Psychiatry. 17**,** 614-20.
Yang, Y., et al., 2012. Acute neuroprotective effects of extremely low-frequency electromagnetic fields after traumatic brain injury in rats. Neurosci Lett. 516**,** 15-20.
Yoo, S. S., et al., 2007. A deficit in the ability to form new human memories without sleep. Nat Neurosci. 10**,** 385-92.
Youngblood, B. D., et al., 1999. The effects of paradoxical sleep deprivation and valine on spatial learning and brain 5-HT metabolism. Physiol Behav. 67**,** 643-9.
Zagaar, M., et al., 2012. The beneficial effects of regular exercise on cognition in REM sleep deprivation: behavioral, electrophysiological and molecular evidence. Neurobiol Dis. 45**,** 1153-62.

Zyss, T., et al., 2000. [The behavioral effects of the transcranial magnetic brain stimulation in rat: the comparison with electroshock]. Psychiatr Pol. 34**,** 111-28.

Figure 1

Click here to download high resolution image

Figure 1

A. RAM: learning & spatial memory

Days	0	1 - 7	8	9
Control (n=10)	Habituation	RAM Cognitive training	RAM test	RAM test
1TMS (n=10)	Habituation	RAM Cognitive training	TMS session RAM test	TMS session RAM test
7TMS (n=10)	Habituation	RAM Cognitive training + daily TMS	TMS session RAM test	TMS session RAM test

B. BM: learning & spatial memory

Days	0	1 - 7	8	9
Control (n=10)	Habituation	BM Cognitive training	BM test	BM test
1TMS (n=10)	Habituation	BM Cognitive training	TMS session BM test	TMS session BM test
7TMS (n=10)	Habituation	BM Cognitive training + daily TMS	TMS session BM test	TMS session BM test

C. NOR: working memory

Days	1 – 4	5	6
Control (n=10)	————	NOR test	NOR test
1TMS (n=10)	————	TMS session NOR test	TMS session NOR test
4TMS (n=10)	———— + daily TMS	TMS session NOR test	TMS session NOR test

Figure 2

Click here to download high resolution image

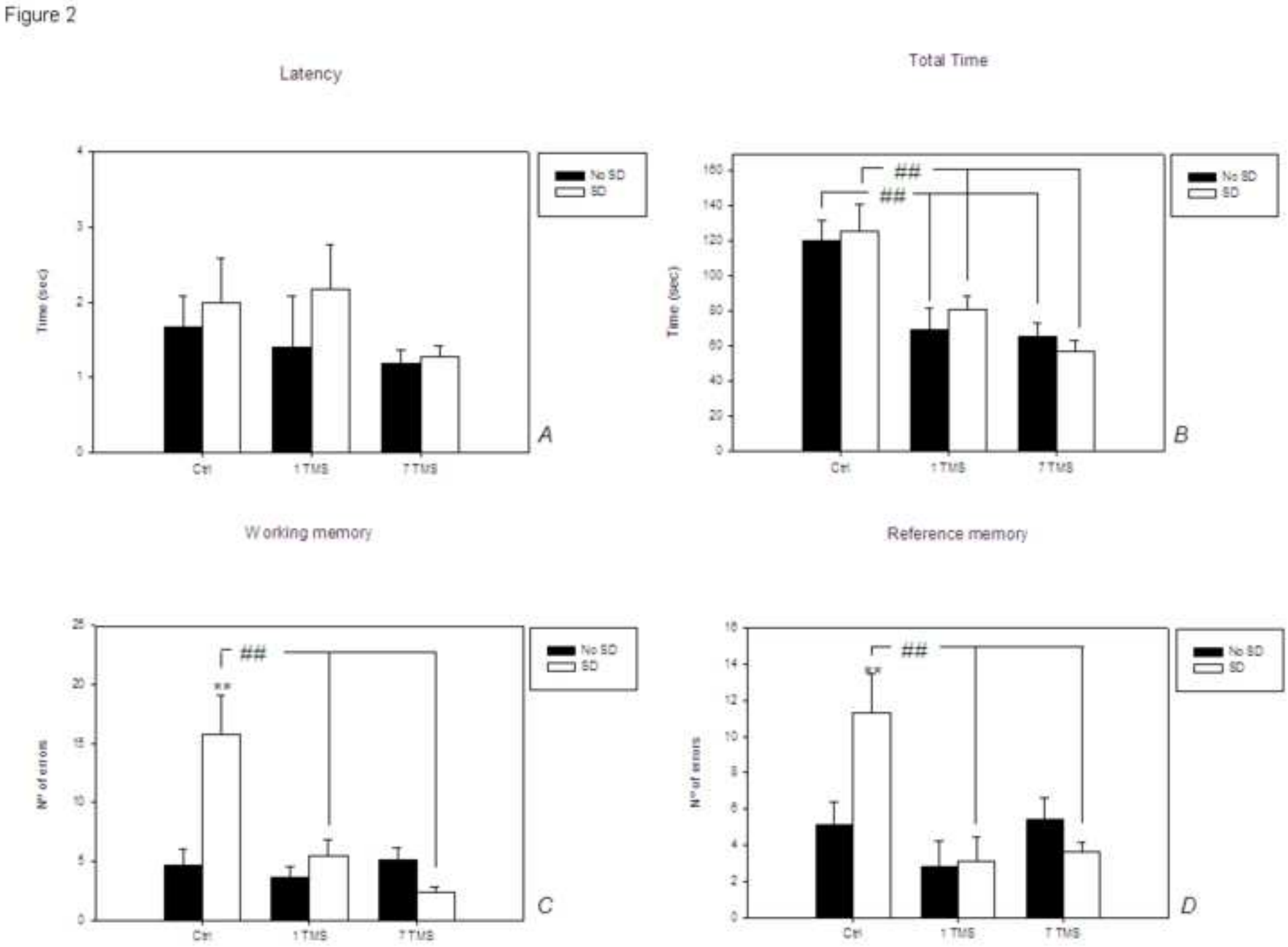

Figure 3

Click here to download high resolution image

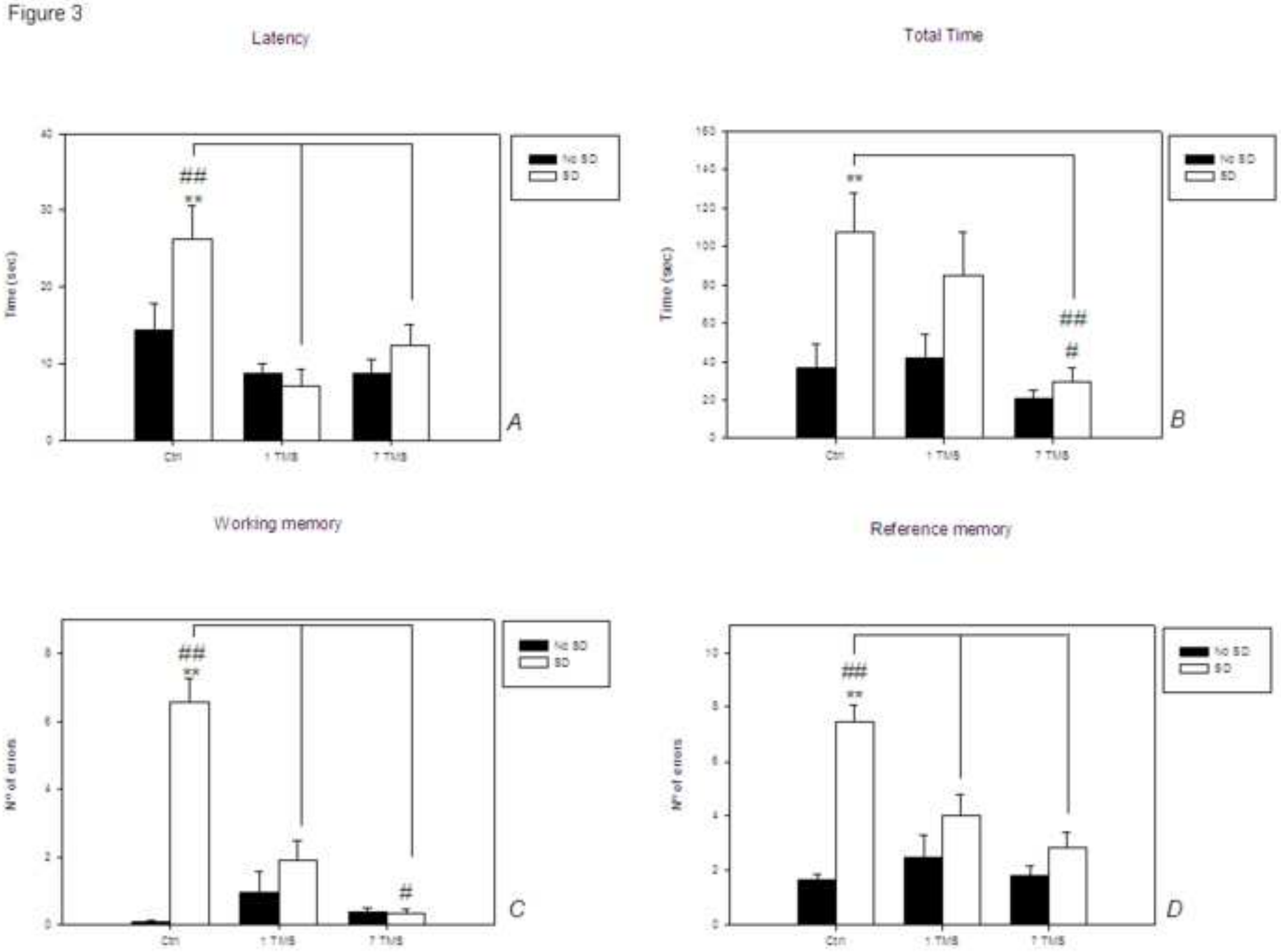

Figure 4

Click here to download high resolution image