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Repetitive Transcranial Magnetic Stimulation leads to higher metabolic efficiency in spatial memory

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Abstract:	Repetitive transcranial magnetic stimulation is a non-invasive neuromodulation technique that allows generating causal-based interferences between brain networks and cognitive or behavioral responses. It has been used to improve cognition in several disease models. However, although its exploration in healthy animals remains essential to attribute its pure effect in learning and memory processes, studies in this regard are scarce. We aimed to evaluate whether repetitive transcranial magnetic stimulation leads to memory facilitation in healthy rats, and explore the brain-related oxidative metabolism. We stimulated Wistar healthy rats during three consecutive days with high-frequency (100 Hz) and low-intensity (0.33 T), and evaluated its effect on the performance of an allocentric spatial reference learning and memory task. The stimulation was performed after training in order to address active brain networks. Following the last day of learning, we assessed oxidative brain metabolism through quantitative cytochrome c oxidase histochemistry. Results showed that transcranial magnetic stimulation leads to a slight behavioral improvement reflected by higher target quadrant entries, and more marked reduced latencies across training in comparison to non-stimulated rats. Moreover, the behavioral outcome was accompanied by a cytochrome c oxidase reduction in the prefrontal, retrosplenial, parietal, and rhinal cortices, as well as in the striatum, amygdala, septum, mammillary bodies, and the hippocampus brain metabolism. In conclusion, the magnetic stimulation leads to a facilitation of spatial learning processes, with a highly efficient use of the brain metabolism of task-related areas.

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Abstract

Repetitive transcranial magnetic stimulation is a non-invasive neuromodulation technique that allows generating causal-based interferences between brain networks and cognitive or behavioral responses. It has been used to improve cognition in several disease models. However, although its exploration in healthy animals remains essential to attribute its pure effect in learning and memory processes, studies in this regard are scarce. We aimed to evaluate whether repetitive transcranial magnetic stimulation leads to memory facilitation in healthy rats, and explore the brain-related oxidative metabolism. We stimulated Wistar healthy rats during three consecutive days with high-frequency (100 Hz) and low-intensity (0.33 T), and evaluated its effect on the performance of an allocentric spatial reference learning and memory task. The stimulation was performed after training in order to address active brain networks. Following the last day of learning, we assessed oxidative brain metabolism through quantitative cytochrome c oxidase histochemistry. Results showed that transcranial magnetic stimulation leads to a slight behavioral improvement reflected by higher target quadrant entries, and more marked reduced latencies across training in comparison to non-stimulated rats. Moreover, the behavioral outcome was accompanied by a cytochrome c oxidase reduction in the prefrontal, retrosplenial, parietal, and rhinal cortices, as well as in the striatum, amygdala, septum, mammillary bodies, and the hippocampus brain metabolism. In conclusion, the magnetic stimulation leads to a facilitation of spatial learning processes, with a highly efficient use of the brain metabolism of task-related areas.

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1. Introduction

Transcranial magnetic stimulation (TMS) is a non-invasive method that delivers electromagnetic energy through the scalp with the use of an induction coil (Barker et al., 1985; Burke et al., 2019). The brain activity changes induced by TMS can last beyond the stimulation period and can therefore translate into therapeutical application (Lefaucheur et al., 2020; Leon-Sarmiento et al., 2015; Nardone et al., 2020; Zorzo et al., 2019a), in addition to be used as a neurophysiological tool (Borghetti et al., 2008; Meng et al., 2020). In fact, repetitive TMS (rTMS) –a stimulation application approach usually employed for clinical purposes– has reached a definite level of efficacy in depression, neuropathic pain, and the post-acute stage of stroke (Lefaucheur et al., 2020).

The use of rTMS to treat several neurological, psychiatric, and psychological conditions is growing (for further details see (Lefaucheur et al., 2020)), as well as its perceived value to enhance memory functions (Kim et al., 2019). Nevertheless, when rTMS is used to improve cognition, it is usually to address a deficit that occurred as part of a certain disease or a reflection of brain injury (Begemann et al., 2020; Cantone et al., 2014; Doeltgen et al., 2015; Kim et al., 2019; Yin et al., 2020). It is important to emphasize that changes in the brain structure and function usually occur under these conditions (Harro et al., 2014). Therefore, in order to attribute rTMS effects to learning and memory processes, research with healthy subjects remains essential.

Memory function, and particularly, the spatial cognition component, is commonly explored in rodents using the Morris Water Maze (MWM) task (Morris, 1984). This task allows the assessment of the allocentric component of spatial navigation, *i.e.*, the use of visual distal cues, to establish a cognitive mapping that enables orientation in the surrounding environment (Epstein et al., 2017). Although the rTMS effect on spatial memory has been examined in various disease models (Chen et al., 2019; Hong et al., 2020; Yang et al., 2019), among others, there are only two studies that aim to decipher the electromagnetic induction impact on spatial memory function in normal rats (Li et al., 2007; Shang et al., 2016).

Our objective was to determine whether rTMS can generate an improvement on spatial cognition in healthy rats and explore the underlying brain oxidative metabolic activity.

To do this, we stimulated Wistar rats during three consecutive days with a high-frequency (100 Hz) and low-intensity (0.33 T) rTMS protocol. It was concomitant with the first three days of the MWM spatial training, which lasted five days and relied on the allocentric strategy to solve the task. Afterwards, brain-related function was assessed through a quantitative cytochrome c oxidase (CCO) histochemistry. CCO is a mitochondrial enzyme that catalyzes oxygen consumption during cellular respiration, and is actively involved in ATP production (Gonzalez-Lima and Cada, 1994; Wong-Riley, 1989). Thus, CCO quantification reveals changes in the brain metabolic capacity of healthy rats which are related to spatial memory processes (Méndez-López et al., 2013; Zorzo et al., 2020) and stimulation therapies (Arias et al., 2016; Zorzo et al., 2019b).

2. Results

2.1. Spatial learning and memory task

2.1.1. Time spent: quadrants, focal zone, and periphery

The control rats showed differences in time spent between quadrants from day two (D1: $H_{(3)}$= 1.151, *P*= .679; D2: $F_{(3, 36)}$= 7.364, *P*< .001; D3: $H_{(3)}$= 22.200, *P*< .001; D4: $H_{(3)}$= 22.728, P< .001; D5: $H_{(3)}$= 19.987, *P*< .001), revealing a higher time spent in D in comparison with the remaining A, B, and C quadrants (D2, D3, D4, D5: *P*< .05) (Figure 1A). The stimulated rats also showed differences between quadrants from day one of the task (D1: $F_{(3, 36)}$= 2.919, *P*> .050; D2: $H_{(3)}$= 14.399, *P*= .002; D3: $F_{(3,36)}$= 53.357, *P*< .001; D4: $H_{(3)}$= 25.229, *P*< .001; D5: $H_{(3)}$= 22.655, *P*< .001). Post-hoc analysis exhibited a higher time spent in quadrant D when compared to A and B on day two (*P* < .05), but not when compared to C (*P* > .05), and an increased D permanence in comparison with A, B, and C quadrants across the remaining days of the test (D3, D4, D5: *P*< .05) (Figure 1B).

Regarding focal zone permanence, there were no differences between CO and rTMS groups across days (D1: *U*= 32.000, n_1 = 10, n_2 = 10, *P* = .571; D2: *U*= 47.000, n_1 = 10, n_2 = 10, *P* = .850; D3: *U*= 32.000, n_1 = 10, n_2 = 10, *P* = .186; D4: t_{18} = .162, *P* = .873; D5: *U*= 48.000, n_1 = 10, n_2 = 10, *P* = .910) (Figure 2A). Similar results were obtained for the periphery, with no differences between groups (D1: t_{18} = -.848, *P* = .408; D2: t_{18} = .364, *P* = .720; D3: t_{18} = 1.305, *P* = .208; D4: *U*= 36.000, n_1 = 10, n_2 = 10, *P* = .304; D5; *U*= 46.000, n_1 = 10, n_2 = 10, *P* = .791) (Figure 2B).

2.1.2. Frequency: quadrants and focal zone

The CO group displayed differences in the number of total entries on day four (D4: $H_{(3)}$= 22.763, *P*< .001) but not on the other days of the task (D1: $F_{(3, 36)}$= .682, *P*= .569; D2: $F_{(3,36)}$= 1.585, *P*= .210; D3: $H_{(3)}$= 6.523, *P*= .089; D5: $H_{(3)}$= 9.224, *P*= .050). Dunn's method revealed differences between quadrant C in comparison with quadrant B and D (*P*< .05) (Figure 3A). Regarding the rTMS group, differences were found from day two (D1: $H_{(3)}$= .788, *P*= .852; D2: $H_{(3)}$= 15.502, *P*= .001; D3: $F_{(3, 36)}$= 5.324, *P*= .004; D4: $H_{(3)}$= 13.461, *P*= .004; D5: $H_{(3)}$= 9.848, *P*= .02), being the mentioned differences between D and C (*P*< .05) on day two, four and five, and quadrant D in comparison to A and C (*P*< .05) on day three (Figure 3B).

2.1.3. Latencies

The CO latencies to reach the platform showed an escape latency reduction across learning days ($F_{(4,36)}$= 3.293, *P*= .021), particularly, between day one in comparison with four and five (*P*< .05) (Figure 4A). Similar results were found in the group which received stimulation ($F_{(4,36)}$= 8.268, *P*< .001), with significant differences between day one in comparison with four and five (*P*< .05), but also between day two when compared to four and five (*P*< .05) and between day three and five (*P*< .05) (Figure 4B).

2.2. Cytochrome c oxidase activity

The analysis of CCO activity revealed a decrease of metabolic activity in the rTMS group in regard to controls. This reduction was found in the prefrontal cortex (CG: $t_{(16)}$= 2.488, *P*= .0243; PL: $t_{(17)}$= 2.888, *P*= .0102; IL: $t_{(17)}$= 2.789, *P*= .0126) (Figure 5A), retrosplenial cortex (RSG: $t_{(17)}$= 5.048, *P*< .001; RSA: $t_{(17)}$= 4.756, *P*< .001) (Figure 5B), parietal cortex (PAR: $t_{(17)}$= 3.665, *P*< .001) (Figure 5C), rhinal cortex (PRH: $t_{(15)}$= 4.763, *P*< .001; ENT: $t_{(15)}$= 5.726, *P*< .001) (Figure 5D), striatum (STR: $t_{(18)}$= 3.543, *P*= .0023; AcC: $t_{(18)}$= 3.747, *P*= .0015; AcSh: $t_{(18)}$= 4.041, *P*< .001) (Figure 6A), septum (MS: $t_{(17)}$= 2.918, *P*< .001; LS: $t_{(17)}$= 3.534, *P*= .0026) (Figure 6B), thalamus (ADT: $t_{(15)}$= 3.673, *P*= .0023; AVT: $t_{(15)}$= 4.611, *P*< .001; MDT: $t_{(15)}$= 5.040, *P*< .001) (Figure 6C), amygdala (CeA: *U*= 18.000, n_1 = 9, n_2 = 10, *P*= .030; LaA: *U*= 23.000, n_1 = 10, n_2 = 10, *P*= .045; BLA: $t_{(18)}$= 3.423, *P*= .0030) (Figure 6D), mammillary bodies (SuM: $t_{(14)}$= 2.578, *P*= .0219; MMM: $t_{(14)}$= 2.594, *P*= .0212; MML: $t_{(18)}$= 4.041, *P*< .001; ML:

$t_{(14)}$= 2.355, *P*= .0336) (Figure 6E) and hippocampus (CA1-D: $t_{(18)}$= 5.087, *P*< .001; CA3-D: $t_{(18)}$= 4.991, *P*< .001; DG-D: $t_{(18)}$= 6.176, *P*< .001; CA1-I: *U*= 10.000, n_1 = 9, n_2 = 10, *P*= .005; CA3-I: $t_{(17)}$= 3.621, *P*= .0021; DG-I: *U*= 12.000, n_1 = 9, n_2 = 2, *P*= .013; CA1-V: $t_{(17)}$= 4.728, *P*< .001; CA3-V: *U*= 18.000, n_1 = 9, n_2 = 10, *P*= .030; DG-V: *U*= 12.000, n_1 = 9, n_2 = 10, *P*= .008) (Figure 7). However, there were no differences between groups in DLG ($t_{(17)}$= -.488, *P*= .632) and Au1 ($t_{(17)}$= 1.435, *P*= .170).

3. Discussion

The purpose of this study was to explore the impact of rTMS on the performance of an allocentric spatial reference learning and memory task in healthy rats, and assess the brain-related function through oxidative metabolism analysis across many brain limbic structures traditionally linked to spatial learning. Three days of high-frequency and low intensity rTMS application led to a greater persistence to enter the target quadrant, in addition to a pronounced reduction of latencies. No differences were found in terms of accuracy when searching for the platform. Finally, a reduced CCO activity in the rTMS group was found across the prefrontal, retrosplenial, parietal, and rhinal cortices, as well as the striatum, amygdala, septum, mammillary bodies, and hippocampus. Nevertheless, there are no differences in auditory cortex and geniculate nucleus.

The rTMS application could be considered a promising treatment to improve cognitive abilities when they are compromised by a certain illness, although its administration is also recently gaining attention in regard to enhancing cognition in individuals not affected by a disease (Kim et al., 2019). Cognitive facilitation on healthy subjects has been reported on both working (Bagherzadeh et al., 2016; Beynel et al., 2019) and episodic memory (Gagnon et al., 2011; Yeh and Rose, 2019) and evaluating the effects of rTMS on healthy animals' learning and memory undoubtedly provides a valuable tool to understand behavioral and brain functions, which can be useful for further clinical analysis.

A valuable way to explore the spatial memory function in rodents is through the MWM task (Vorhees and Williams, 2014), a task which also allows comparative studies with humans due to the development of the virtual MWM version (Schoenfeld et al., 2017). Regarding permanencies in the reinforced zone, the results show that both groups managed to reach the learning criteria [defined as higher time spent in the target

quadrant in comparison with others (Gutiérrez-Menéndez et al., 2019; Haidar et al., 2019)] with slight differences, given that control rats learned the task from day two whereas the stimulated group from day three. However, both groups revealed the same rate of learning after the rTMS application had ended, that is, from day four. The number of entries in each MWM quadrant provides further information about spatial learning (Ma et al., 2017). It is interesting to note that the study of frequencies in each quadrant revealed that the rTMS group displayed a higher platform search persistence. Therefore, these results suggest that applying rTMS after spatial training during three consecutive days leads to a slight facilitation in terms of persistence when trying to enter the quadrant in which the platform is located, but does not trigger differences regarding total time spent searching for the platform in the right quadrant. Literature results are diverse: higher permanencies in the target quadrant at the end of the task on the stimulated group (Shang et al., 2016), or a memory decline (Li et al., 2007), suggesting that both the stimulation parameters and behavioral training are important. Our results differ from (Li et al., 2007) in terms of the stimulation protocol (low frequency) and slight training differences. Our training protocol was similar to (Shang et al., 2016), and although we used different rTMS frequencies, both are considered higher. Nevertheless, the moment of stimulation differs in our study given that we administered rTMS for three days following the behavioral task, whereas (Shang et al., 2016) delivered it 10 days prior to MWM testing.

We explored the time spent in the focal zone, defined as a circle around the platform double in diameter, to assess the rat's accuracy in searching for the platform. There were no differences between groups across the five days of the task, suggesting that the rTMS does not cause a differential accuracy rate, contrary to other authors who observed more platform crossings at the end of the last acquisition day (Shang et al., 2016). In regard to latencies to reach the platform, a modest improvement due to magnetic induction was observed. The time it took control animals to reach the platform from day one to the last days of the task decreased, while the rTMS group showed a reduced latency form the first three days of the task in comparison with days four and five. These results suggest that the quickness to locate the platform occurs when the rTMS application has finished, similar to (Shang et al., 2016), suggesting that the potential beneficial effect of rTMS needs a time-interval to become significant at the behavioral level.

Brain functioning was assessed by a CCO histochemistry, which reflects changes in tissue metabolic capacity that are induced by sustained energy requirements, such as those derived from learning (Méndez-López et al., 2013). We have previously shown that rTMS administrations trigger higher CCO activity under basal conditions (Pernia et al., 2020; Zorzo et al., 2019b). However, here we have observed a continuous decrease of CCO activity in response to the spatial task along many brain areas, including the prefrontal, retrosplenial, parietal, and rhinal cortices, as well as the striatum, amygdala, septum, mammillary bodies, and hippocampus. These differences were no found in areas that are not directly related to the task, such as auditory cortex or geniculate nucleus. The effects generated by the rTMS are influenced not only by the selection of stimulation parameters, *e.g.*, frequency, pattern of stimulation, intensity, or coil shape (Klomjai et al., 2015), but are also dependent on biological states such as individual levels of excitability prior to the rTMS application (Tuñez Fiñana and Pascual-Leone, 2014). In this study, rTMS addresses active brain networks at a cellular level, as its application followed the training, which is when synaptic consolidation occurs, a process that appears shortly after memory encoding and relies on changes in synaptic and cellular nodes that allow the transformation of information into its long-term form (Dudai et al., 2015). Hence, rTMS has been shown to modulate neuronal excitability from outside the skull, and high frequencies have been linked to inducing long-term potentiation-like plasticity (Klomjai et al., 2015; Tang et al., 2015).

The brain metabolic results derived from this study are related to the last days of training, when there is a well consolidated memory, and consequently, the energy cost to solve the task may be lower in comparison with the initial training (Méndez-López et al., 2013). The CCO reduced activity in the rTMS group indicates that stimulated rats employ lower energy consumption in order to successfully solve the allocentric spatial memory task, with slight improvements in relation to controls, suggesting a higher brain metabolic efficiency. Accordingly, it has been shown that reduced neuronal resources are achieved with the use of cognitive enhancers (Volkow et al., 2008), and a CCO activity decrease has been linked to a faster acquisition of a spatial reference memory task (Banqueri et al., 2017). Cognitive reduced demands lead to brain network reorganization, with higher modularity and a smaller long-distance interaction, which can trigger the minimization of the metabolic cost (Bullmore and Sporns, 2012). The metabolic efficiency observed as a response of rTMS administration occurs across all

areas that actively participate in spatial memory, such as the hippocampus and neocortex as well as subcortical structures traditionally linked to spatial processing (Aggleton, 2012; Hunsaker and Kesner, 2018; Rolls and Wirth, 2018).

4. Conclusions

Three days of high frequency and low intensity rTMS delivered concomitant with spatial learning within its initial phase leads to a modest behavioral improvement in healthy male rats, reflected by higher target quadrant entries and more marked reduced latencies across training in comparison to non-stimulated rats. The behavioral outcome was accompanied by a highly efficient use of the prefrontal, retrosplenial, parietal, and rhinal cortices, as well as the striatum, amygdala, septum, mammillary bodies, and hippocampus, suggesting a brain network reorganization of task-related areas.

5. Material and methods

5.1. Animals

A total of 20 male Wistar rats were used (220-300 grams, 12 weeks old at the start of the experiment). They had ad libitum access to food and tap water and were maintained at constant room temperature (20-22 ºC), with a relative humidity of 65-70% and an artificial light-dark cycle of 12 h (08:00-20:00h on/20:00-08:00h off). Rats were housed in groups of four per cage until the end of the experiment. Behavioral and stimulation procedures were performed between 8:00 and 14:00 h.

This study was approved by the local committee for animal studies (Agriculture Council of the Principality of Asturias) and all the experimental procedures were carried out according to the European Communities Council Directive (2010/63/UE) and the Spanish legislation related to the protection of animals used for experimentation and other scientific purposes (Royal Decree 53/2013).

5.2. Experimental design

Rats were randomly assigned to two groups: rTMS (repetitive transcranial magnetic stimulated group), which received active stimulation and CO (control group), which was submitted to sham stimulation. Prior to the behavioral testing and stimulation application, all rats were handled on a daily basis for seven days to get them used to the immobilization required by rTMS. The groups were then submitted to the MWM spatial

task and rTMS (sham or active) was applied during days one to three of training after the behavioral task (Figure 8).

5.3. Behavioral procedure

5.3.1. Apparatus

Rats were trained in the MWM (Morris, 1984), a circular swimming pool which is 150 cm in diameter and 40 cm high, supported on a 35 cm high platform. The MWM was filled with 21 ± 1 °C tap water until a level of 30 cm. It was located in a 16 m^2 room with dimmed lights and there were black panels with five allocentric visual cues with different volumes and color patters surrounding the pool, 30 cm away from it. The pool was divided into four imaginary quadrants (A, B, C, and D), three non-reinforced (A, B and C) and one of them (D), reinforced with a hidden platform that allowed rats to escape from the water during learning. The platform was located 2 cm below the water surface and was 10 cm in diameter and 28 cm high. To record behavior, we employed a computerized video-tracking system (Ethovision XT 14.0, Noldus Information Technologies, Wageningen, The Netherlands).

5.3.2. Spatial learning and memory

The spatial learning and memory task began with one habituation day, in which rats were subjected to 4 trials with a visible platform that protruded 2 cm above water located in the center of the pool. During the learning phase, which lasted 5 consecutive days, rats performed 2 training trials in which the platform was hidden and placed in the center of quadrant D. They then received one probe trial in which the escape platform was removed, and the rat was introduced from the opposite quadrant to where the platform had been located in previous trials. Finally, rats were submitted to an additional trial to avoid a possible extinction of learning as a result of the probe trial, which included the hidden platform. Across trials, rats were released from each quadrant facing the pool wall in a pseudo-randomized sequence. The trial-interval was 30 s (rats were place on a black bucket) and each trial had a maximum duration of 60 s. When rats found the platform, they remained there for 15 s. If rats failed to reach it during the training sessions and the additional trial to avoid extinction, they were gently guided toward the platform. We registered time in each quadrant, in the focal zone (20 cm circle around the platform) and in the periphery (%) during the probe trial,

frequency (number of entries) in each quadrant during the probe trial and latencies to reach the platform (s) during the training trials.

5.4. Repetitive transcranial magnetic stimulation

5.4.1. Apparatus

The functioning of the rTMS apparatus is explained in detail in Pernia et al. (2020). It consists of a half-bridge converter that generates a train of pulses with a frequency of 100 Hz, input capacitors (C_f=1500μF) to transfer the pulse into the half-bridge converter which is controlled by a microcontroller, and a stimulation coil. The coil consists of a small magnetic head made of nanocrystalline material (Vitroperm 500F) to adjust to the small size of the rat's head so a magnetic transducer was needed to focus the magnetic field. The magnetic field reaches a field amplitude of 330 mT.

5.4.2. Repetitive transcranial magnetic stimulation application

The rTMS application was the same as in our previous studies (Zorzo et al., 2019b). The delivery was located on the upper part of the skull, near Bregma -3.96 mm (Paxinos and Watson, 2005), and the rTMS group received 10 min of 100 Hz trains lasting a total of 3000 pulses each min, with a 30 s interval, during three consecutive days. The CO group received sham stimulation. The rTMS application was well tolerated by all animals, with no sign of abnormal behavior or discomfort.

5.5. Cytochrome c oxidase histochemistry and quantification

Rats were decapitated 90 min after the end of the last spatial learning and memory session, the encephalon was removed, frozen in N-methyl butane (*Sigma-Aldrich, Germany*), and stored at -40 °C to make coronal sections of 30 μm thick in a cryostat at -20 °C (*Leica CM1900, Germany*) for the CCO histochemistry. Section slides were processed with quantitative CCO histochemistry, and quantified by optical densitometry as previously described (Higarza et al., 2019) The regions of interest and their distances in mm counted from the bregma were: +3.24 mm for the prefrontal cortex (cingulate (CG), infralimbic (IL), and prelimbic cortex (PL)); +1.92 mm for the striatum (STR), accumbens core (AcC) and accumbens shell (AcSh), +0.72 mm for the septum (medial septum (MS) and lateral septum (LS)); -1.44 mm for the thalamus (anterodorsal (ADT), anteroventral (AVT), and mediodorsal (MDT)); -2.28 mm for the amygdala (central

(CeA), basolateral (BLA), and lateral (LaA)); -3.48 mm for the dorsal hippocampus (CA1-D and CA3-D subfields and dentate gyrus (DG-D)), retrosplenial cortex (granular retrosplenial (RSG), agranular retrosplenial (RSA)), and parietal cortex (PAR); −4.56 mm for the mammillary bodies (supramammillary (SuM), Medial medial mammillary (MMM), Medial lateral mammillary (MML) and mammillary lateral (ML)), -4.80 mm for rhinal cortex (entorhinal (ENT) and perirhinal (PHR)), primary auditory cortex (Au1), dorso-lateral geniculate nucleus (DLG) and the intermediate hippocampus (CA1-I and CA3-I subfields and dentate gyrus (DG-I)); and -5.16 mm for the ventral hippocampus (CA1-V and CA3-V subfields and dentate gyrus (DG-V)).

5.6. Statistical analysis

Behavioral and CCO activity data were analyzed with the SigmaStat 12.5 program (*Systat, Richmond, USA*). Normality (Shapiro-Will test) and homoscedasticity (Levene test) assumptions were evaluated to select between parametric or non-parametric tests. The data's normal distribution and variances were equally distributed when $P > .05$. Time and frequency in each quadrant were compared by One Way ANOVA or Kruskal-Wallis One Way Analysis of Variance on Ranks. Latencies were evaluated thorough One-Way Repeated Measures ANOVA (Factor A: Day; Factor of Repetition: Subject). Latencies for the two trials per day were averaged. The Holm-Sidak post-hoc method was applied with parametric tests while Dunn's method was applied with non-parametric procedures. Differences between groups in time spent in the focal zone and in the periphery of the pool, frequency in the focal zone, and CCO measurements were explored with a t-test for independent samples or the Mann-Whitney U test. Statistical differences were considered significant at the .05 level. Finally, graphic representation of the results were performed with the SigmaPlot 12.5 software program (*Systat, Richmond, USA*). Data were expressed as mean ± standard error of mean (SEM).

Declarations of interest: none

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References

Aggleton, J.P., 2012. Multiple anatomical systems embedded within the primate medial temporal lobe: Implications for hippocampal function. Neurosci. Biobehav. Rev. 36, 1579–1596. https://doi.org/10.1016/j.neubiorev.2011.09.005

Arias, N., Méndez, M., Arias, J.L., 2016. Low-light-level therapy as a treatment for minimal hepatic encephalopathy: behavioural and brain assessment. Lasers Med. Sci. 31, 1717–1726. https://doi.org/10.1007/s10103-016-2042-4

Bagherzadeh, Y., Khorrami, A., Zarrindast, M.R., Shariat, S.V., Pantazis, D., 2016. Repetitive transcranial magnetic stimulation of the dorsolateral prefrontal cortex enhances working memory. Exp. Brain Res. 234, 1807–1818. https://doi.org/10.1007/s00221-016-4580-1

Banqueri, M., Méndez, M., Arias, J.L., 2017. Spatial memory-related brain activity in normally reared and different maternal separation models in rats. Physiol. Behav. 181, 80–85. https://doi.org/10.1016/j.physbeh.2017.09.007

Barker, A.T., Jalinous, R., Freeston, I.L., 1985. Non-invasive magnetic stimulation of human motor cortex. Lancet 1, 1106–07. https://doi.org/10.1016/S0140-6736(85)92413-4

Begemann, M.J., Brand, B.A., Curčić-Blake, B., Aleman, A., Sommer, I.E., 2020. Efficacy of non-invasive brain stimulation on cognitive functioning in brain disorders: A meta-analysis. Psychol. Med. https://doi.org/10.1017/S0033291720003670

Beynel, L., Davis, S.W., Crowell, C.A., Hilbig, S.A., Lim, W., Nguyen, D., Palmer, H., Brito, A., Peterchev, A. V., Luber, B., Lisanby, S.H., Cabeza, R., Appelbaum, L.G., 2019. Online repetitive transcranial magnetic stimulation during working memory in younger and older adults: A randomized within-subject comparison.

PLoS One 14, 1–19. https://doi.org/10.1371/journal.pone.0213707

Borghetti, D., Sartucci, F., Petacchi, E., Guzzetta, A., Piras, M.F., Murri, L., Cioni, G., 2008. Transcranial magnetic stimulation mapping: A model based on spline interpolation. Brain Res. Bull. 77, 143–148. https://doi.org/10.1016/j.brainresbull.2008.06.001

Bullmore, E., Sporns, O., 2012. The economy of brain network organization. Nat. Rev. Neurosci. 13, 336–349. https://doi.org/10.1038/nrn3214

Burke, M.J., Fried, P.J., Pascual-Leone, A., 2019. Transcranial magnetic stimulation: Neurophysiological and clinical applications, 1st ed, Handbook of Clinical Neurology. Elsevier B.V. https://doi.org/10.1016/B978-0-12-804281-6.00005-7

Cantone, M., Di Pino, G., Capone, F., Piombo, M., Chiarello, D., Cheeran, B., Pennisi, G., Di Lazzaro, V., 2014. The contribution of transcranial magnetic stimulation in the diagnosis and in the management of dementia. Clin. Neurophysiol. 125, 1509–1532. https://doi.org/10.1016/j.clinph.2014.04.010

Chen, X., Chen, S., Liang, W., Ba, F., 2019. Administration of repetitive transcranial magnetic stimulation attenuates A β 1-42 -induced Alzheimer's disease in mice by activating β-catenin signaling. Biomed Res. Int. 2019. https://doi.org/10.1155/2019/1431760

Doeltgen, S.H., Bradnam, L. V., Young, J.A., Fong, E., 2015. Transcranial non-invasive brain stimulation in swallowing rehabilitation following stroke - A review of the literature. Physiol. Behav. 143, 1–9. https://doi.org/10.1016/j.physbeh.2015.02.025

Dudai, Y., Karni, A., Born, J., 2015. The Consolidation and Transformation of Memory. Neuron 88, 20–32. https://doi.org/10.1016/j.neuron.2015.09.004

Epstein, R.A., Patai, E.Z., Julian, J.B., Spiers, H.J., 2017. The cognitive map in humans: Spatial navigation and beyond. Nat. Neurosci. 20, 1504–1513. https://doi.org/10.1038/nn.4656

Gagnon, G., Schneider, C., Grondin, S., Blanchet, S., 2011. Enhancement of episodic memory in young and healthy adults: A paired-pulse TMS study on encoding and

retrieval performance. Neurosci. Lett. 488, 138–142. https://doi.org/10.1016/j.neulet.2010.11.016

Gonzalez-Lima, F., Cada, A., 1994. Cytochrome oxidase activity in the auditory system of the mouse: A qualitative and quantitative histochemical study. Neuroscience 63, 559–578. https://doi.org/10.1016/0306-4522(94)90550-9

Gutiérrez-Menéndez, A., Banqueri, M., Méndez, M., Arias, J.L., 2019. How Does Maternal Separation Affect the Cerebellum? Assessment of the Oxidative Metabolic Activity and Expression of the c-Fos Protein in Male and Female Rats. Cerebellum 19, 68–77. https://doi.org/10.1007/s12311-019-01087-5

Haidar, M., Tin, K., Zhang, C., Nategh, M., Covita, J., Wykes, A.D., Rogers, J., Gundlach, A.L., 2019. Septal GABA and glutamate neurons express RXFP3 mRNA and depletion of septal RXFP3 impaired spatial search strategy and long-term reference memory in adult mice. Front. Neuroanat. 13. https://doi.org/10.3389/fnana.2019.00030

Harro, J., Kanarik, M., Kaart, T., Matrov, D., Kõiv, K., Mällo, T., Del Río, J., Tordera, R.M., Ramirez, M.J., 2014. Revealing the cerebral regions and networks mediating vulnerability to depression: Oxidative metabolism mapping of rat brain. Behav. Brain Res. 267, 83–94. https://doi.org/10.1016/j.bbr.2014.03.019

Higarza, S.G., Arboleya, S., Gueimonde, M., Gómez-Lázaro, E., Arias, J.L., Arias, N., 2019. Neurobehavioral dysfunction in nonalcoholic steatohepatitis is associated with hyperammonemia, gut dysbiosis, and metabolic and functional brain regional deficits, PLoS ONE. https://doi.org/10.1371/journal.pone.0223019

Hong, Y., Liu, Q., Peng, M., Bai, M., Li, J., Sun, R., Guo, H., Xu, P., Xie, Y., Li, Y., Liu, L., Du, J., Liu, X., Yang, B., Xu, G., 2020. High-frequency repetitive transcranial magnetic stimulation improves functional recovery by inhibiting neurotoxic polarization of astrocytes in ischemic rats. J. Neuroinflammation 17, 1–17. https://doi.org/10.1186/s12974-020-01747-y

Hunsaker, M.R., Kesner, R.P., 2018. Unfolding the cognitive map: The role of hippocampal and extra-hippocampal substrates based on a systems analysis of spatial processing. Neurobiol. Learn. Mem. 147, 90–119.

https://doi.org/10.1016/j.nlm.2017.11.012

Kim, T.D., Hong, G., Kim, J., Yoon, S., 2019. Cognitive enhancement in neurological and psychiatric disorders using transcranial magnetic stimulation (TMS): A review of modalities, potential mechanisms and future implications. Exp. Neurobiol. 28, 1–16. https://doi.org/10.5607/en.2019.28.1.1

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

Lefaucheur, J.P., Aleman, A., Baeken, C., Benninger, D.H., Brunelin, J., Di Lazzaro, V., Filipović, S.R., Grefkes, C., Hasan, A., Hummel, F.C., Jääskeläinen, S.K., Langguth, B., Leocani, L., Londero, A., Nardone, R., Nguyen, J.P., Nyffeler, T., Oliveira-Maia, A.J., Oliviero, A., Padberg, F., Palm, U., Paulus, W., Poulet, E., Quartarone, A., Rachid, F., Rektorová, I., Rossi, S., Sahlsten, H., Schecklmann, M., Szekely, D., Ziemann, U., 2020. Evidence-based guidelines on the therapeutic use of repetitive transcranial magnetic stimulation (rTMS): An update (2014–2018). Clin. Neurophysiol. 131, 474–528. https://doi.org/10.1016/j.clinph.2019.11.002

Leon-Sarmiento, F.E., Rizzo-Sierra, C. V., Leon-Ariza, J.S., Leon-Ariza, D.S., Sobota, R., Prada, D.G., 2015. A new neurometric dissection of the area-under-curve-associated jiggle of the motor evoked potential induced by transcranial magnetic stimulation. Physiol. Behav. 141, 111–119. https://doi.org/10.1016/j.physbeh.2015.01.014

Li, W., Yang, Y., Ye, Q., Yang, B., Wang, Z., 2007. Effect of chronic and acute low-frequency repetitive transcranial magnetic stimulation on spatial memory in rats. Brain Res. Bull. 71, 493–500. https://doi.org/10.1016/j.brainresbull.2006.11.002

Ma, J., Wang, J., Lv, C., Pang, J., Han, B., Gen, Y., Wang, M., 2017. The role of hippocampal structural synaptic plasticity in repetitive transcranial magnetic stimulation to improve cognitive function in male SAMP8 mice. Cell. Physiol. Biochem. 41, 137–144. https://doi.org/10.1159/000455982

Méndez-López, M., Méndez, M., Sampedro-Piquero, P., Arias, J.L., 2013. Spatial

learning-related changes in metabolic activity of limbic structures at different posttask delays. J. Neurosci. Res. 91, 151–159. https://doi.org/10.1002/jnr.23134

Meng, H.J., Zhang, L.L., Luo, S.S., Cao, N., Zhang, J., Pi, Y.L., 2020. Modulation of hand motor skill performance induced by motor practice combined with matched or mismatched hand posture motor imagery. Physiol. Behav. 225, 113084. https://doi.org/10.1016/j.physbeh.2020.113084

Morris, R.G., 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11, 47–60. https://doi.org/10.1016/0165-0270(84)90007-4

Nardone, R., Sebastianelli, L., Versace, V., Brigo, F., Golaszewski, S., Manganotti, P., Saltuari, L., Trinka, E., 2020. Repetitive transcranial magnetic stimulation in traumatic brain injury: Evidence from animal and human studies. Brain Res. Bull. 159, 44–52. https://doi.org/10.1016/j.brainresbull.2020.03.016

Paxinos, G., Watson, C., 2005. The rat brain in sterotaxic coordinates 367. https://doi.org/10.1017/CBO9781107415324.004

Pernia, A.M., Zorzo, C., Prieto, M.J., Martinez, J., Higarza, S., Mendez, M., Arias, J., 2020. Equipment for repetitive transcranial magnetic stimulation. IEEE Trans. Biomed. Circuits Syst. 14, 525–534. https://doi.org/10.1109/TBCAS.2020.2981012.

Rolls, E.T., Wirth, S., 2018. Spatial representations in the primate hippocampus, and their functions in memory and navigation. Prog. Neurobiol. 171, 90–113. https://doi.org/10.1016/j.pneurobio.2018.09.004

Schoenfeld, R., Schiffelholz, T., Beyer, C., Leplow, B., Foreman, N., 2017. Variants of the Morris water maze task to comparatively assess human and rodent place navigation. Neurobiol. Learn. Mem. 139, 117–127. https://doi.org/10.1016/j.nlm.2016.12.022

Shang, Y., Wang, X., Shang, X., Zhang, H., Liu, Z., Yin, T., Zhang, T., 2016. Repetitive transcranial magnetic stimulation effectively facilitates spatial cognition and synaptic plasticity associated with increasing the levels of BDNF and synaptic

proteins in Wistar rats. Neurobiol. Learn. Mem. 134, 369–378. https://doi.org/10.1016/j.nlm.2016.08.016

Tang, A., Thickbroom, G., Rodger, J., 2015. Repetitive Transcranial Magnetic Stimulation of the Brain: Mechanisms from Animal and Experimental Models. Neuroscientist 23, 82–94. https://doi.org/10.1177/1073858415618897

Tuñez Fiñana, I., Pascual-Leone, A., 2014. Estimulacón magnética transcraneal y neuromodulación: Presente y futuro en neurociencias.

Volkow, N.D., Fowler, J.S., Wang, G.J., Telang, F., Logan, J., Wong, C., Ma, J., Pradhan, K., Benveniste, H., Swanson, J.M., 2008. Methylphenidate decreased the amount of glucose needed by the brain to perform a cognitive task. PLoS One 3, 1–7. https://doi.org/10.1371/journal.pone.0002017

Vorhees, C. V., Williams, M.T., 2014. Assessing spatial learning and memory in rodents. ILAR J. 55, 310–332. https://doi.org/10.1093/ilar/ilu013

Wong-Riley, M.T.T., 1989. Cytochrome oxidase: an endogenous metabolic marker for neuronal activity. Trends Neurosci. 12, 94–101. https://doi.org/10.1016/0166-2236(89)90165-3

Yang, L.L., Zhao, D., Kong, L.L., Sun, Y.Q., Wang, Z.Y., Gao, Y.Y., Li, N., Lu, L., Shi, L., Wang, X.Y., Wang, Y.M., 2019. High-frequency repetitive transcranial magnetic stimulation (rTMS) improves neurocognitive function in bipolar disorder. J. Affect. Disord. 1, 851–856. https://doi.org/10.1016/j.jad.2018.12.102

Yeh, N., Rose, N.S., 2019. How can transcranial magnetic stimulation be used to modulate episodic memory?: A systematic review and meta-analysis. Front. Psychol. 10. https://doi.org/10.3389/fpsyg.2019.00993

Yin, M., Liu, Y., Zhang, L., Zheng, H., Peng, L., Ai, Y., Luo, J., Hu, X., 2020. Effects of rTMS Treatment on Cognitive Impairment and Resting-State Brain Activity in Stroke Patients: A Randomized Clinical Trial. Front. Neural Circuits 14, 1–12. https://doi.org/10.3389/fncir.2020.563777

Zorzo, C., Arias, J.L., Méndez, M., 2020. Retrieval of allocentric spatial memories is

preserved up to thirty days and does not require higher brain metabolic demands. Neurobiol. Learn. Mem. 175, 1–12. https://doi.org/10.1016/j.nlm.2020.107312

Zorzo, C., Banqueri, M., Higarza, S.G., Pernía, A.M., Arias, J.L., 2019a. Current state of transcranial magnetic stimulation and its use in psychiatry. Actas Esp. Psiquiatr. 47, 110–120.

Zorzo, C., Higarza, S.G., Méndez, M., Martínez, J.A., Pernía, A.M., Arias, J.L., 2019b. High frequency repetitive transcranial magnetic stimulation improves neuronal activity without affecting astrocytes and microglia density. Brain Res. Bull. 150, 13–20. https://doi.org/10.1016/j.brainresbull.2019.05.004

Figure legends

Figure 1. Time spent in reinforced and non-reinforced quadrants during the learning probe tests. (A) The CO group showed differences between the target quadrant and the rest of them from day two (*P< .05). (B) The rTMS group showed differences between the target quadrant and quadrants A and B on day two, and with all the non-reinforced quadrants from day three (*P< .05).

Figure 2. (A) Time spent in the focal zone during the learning probe tests. (B) Time spent in the periphery during the learning probe tests. There were no differences between groups (P > .05).

Figure 3. Total number of entries in reinforced and non-reinforced quadrants during the learning probe tests. (A) The CO group showed differences between quadrant C and quadrants B and D on day four (*P< .05). (B) The rTMS group showed differences between the target quadrant and C on day two, four, and five (*P< .05), and between the target quadrant and A and C on day three (*P< .05).

Figure 4. Mean latencies to reach the platform during the spatial learning and memory task. (A) The CO group showed differences between day one and days four and five (*P < .05). (B) The rTMS group exhibited differences between day one, two, and three compared to days four and five (*P < .05).

Figure 5. CCO activity in the CO and rTMS groups across cortical areas. Stimulated rats showed a reduced brain metabolism on (A) Prefrontal cortex, (B) Retrosplenial cortex, (C) Parietal cortex, (D) Rhinal cortex (*P< .05).

Figure 6. CCO activity in the CO and rTMS groups across subcortical areas. Stimulated rats showed a reduced brain metabolism on (A) Striatum, (B) Septum, (C) Thalamus, (D) Amygdala. (E) Mammillary bodies (*P< .05).

Figure 7. CCO activity in the CO and rTMS groups across dorso-ventral axis of hippocampus (*P< .05).

Figure 8. Experimental design. Rats were habituated to rTMS protocol for seven days. Afterwards, they were submitted the MWM procedure and received sham or active induction following training. Hab= Habituation.

Figure1

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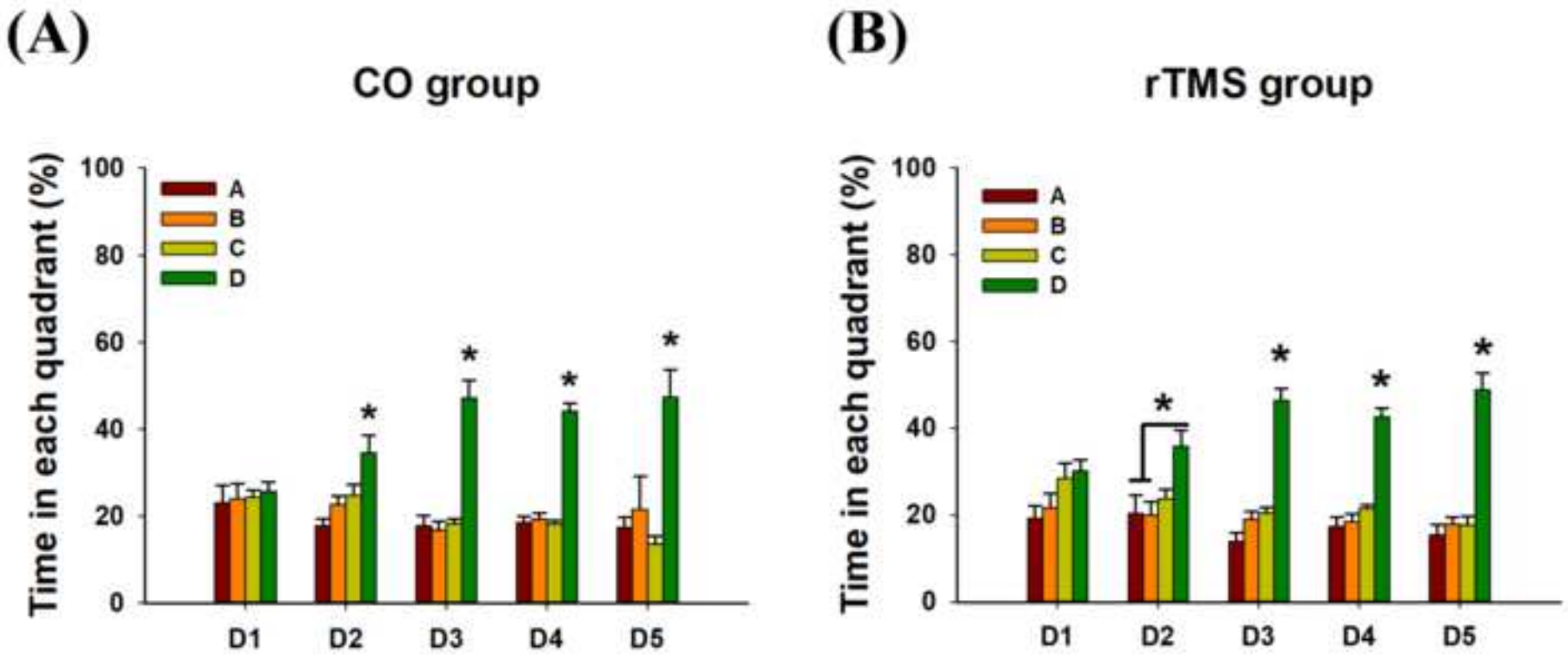

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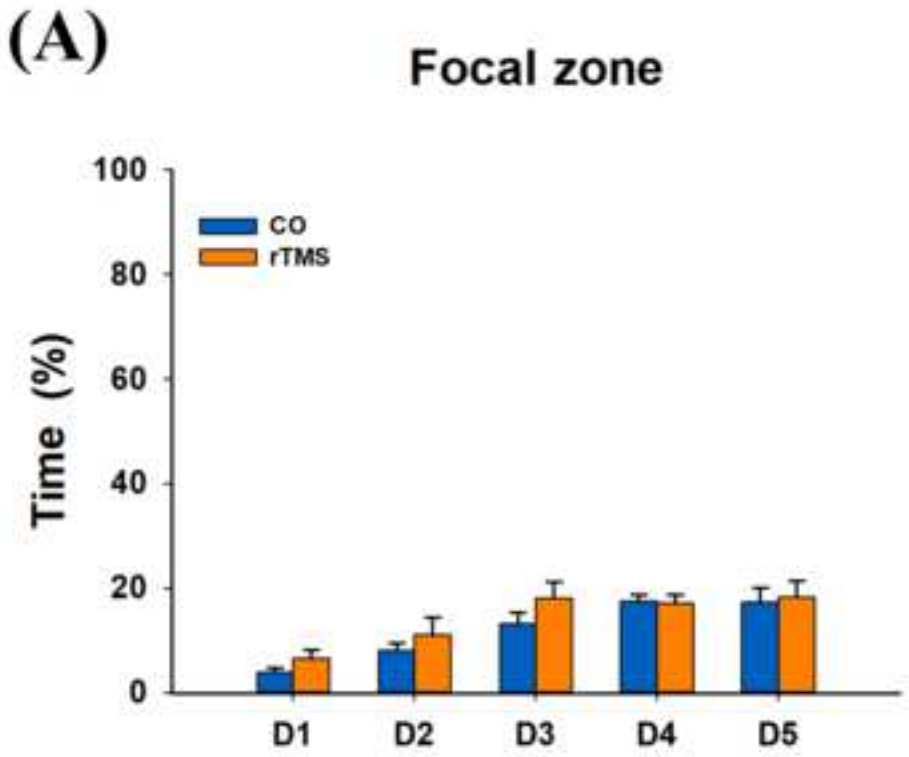

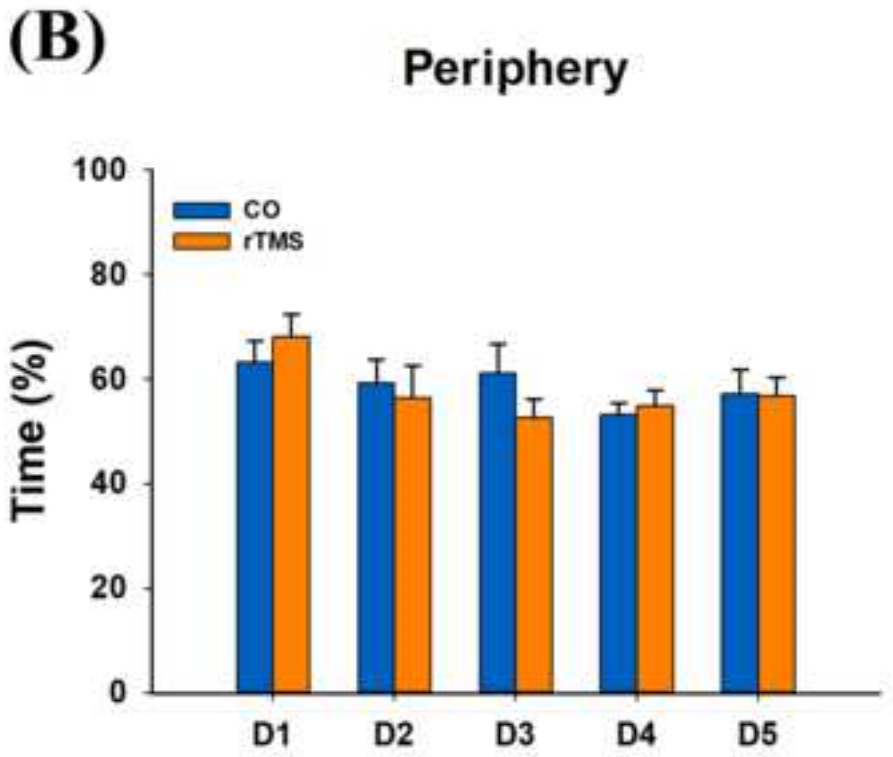

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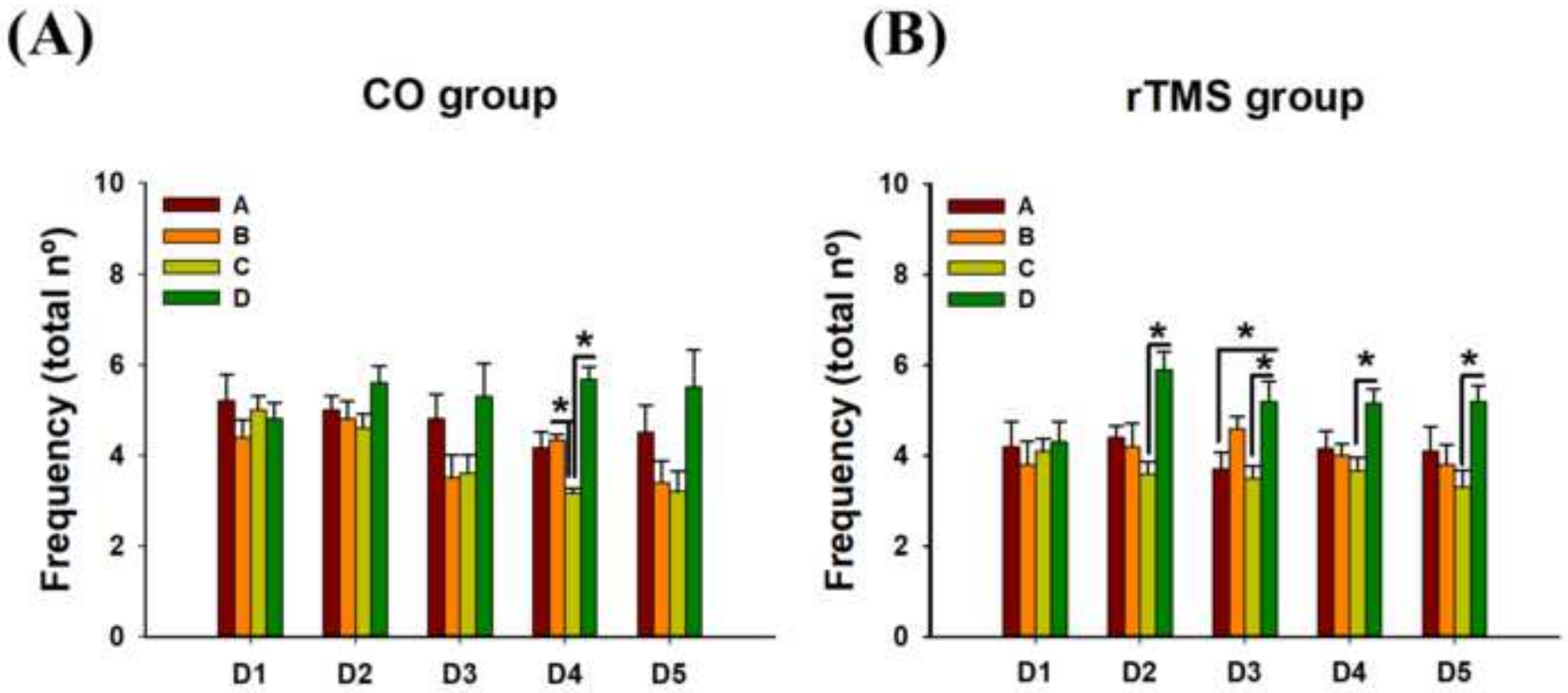

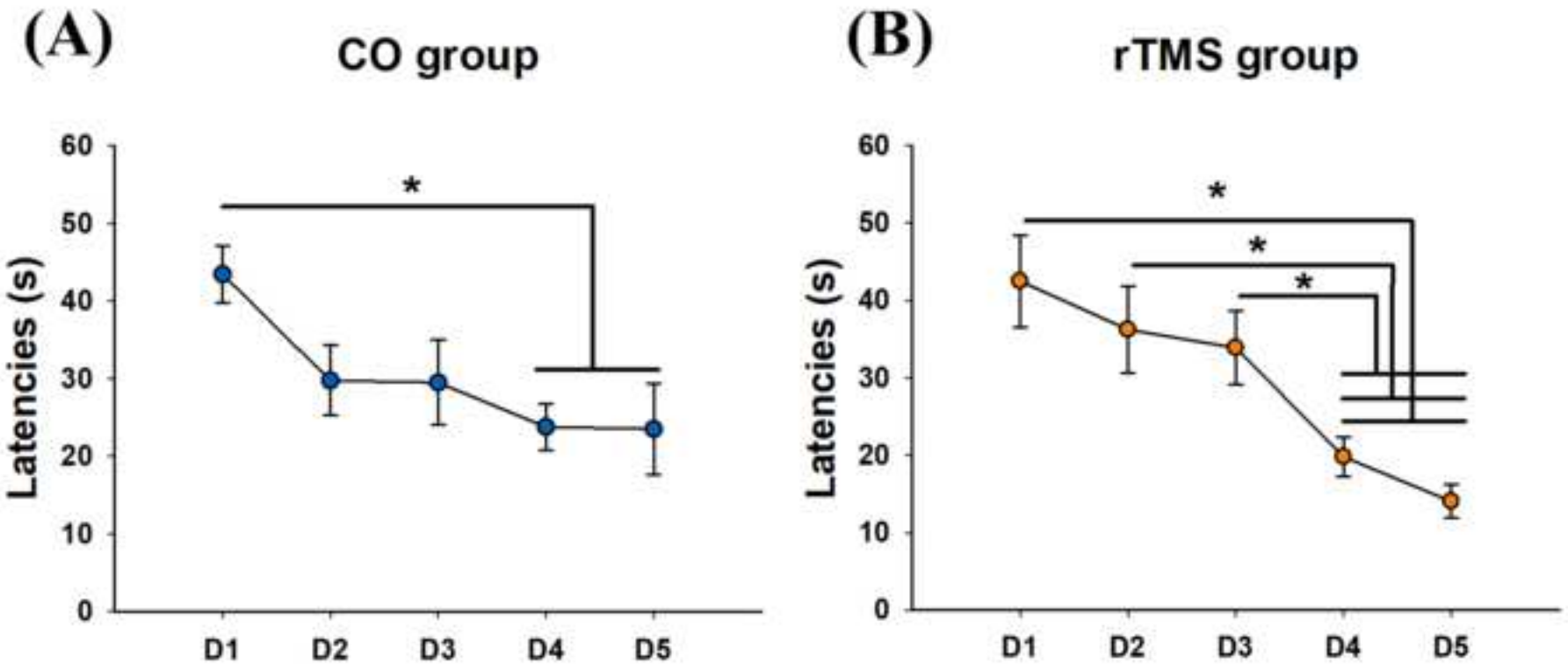

Figure5

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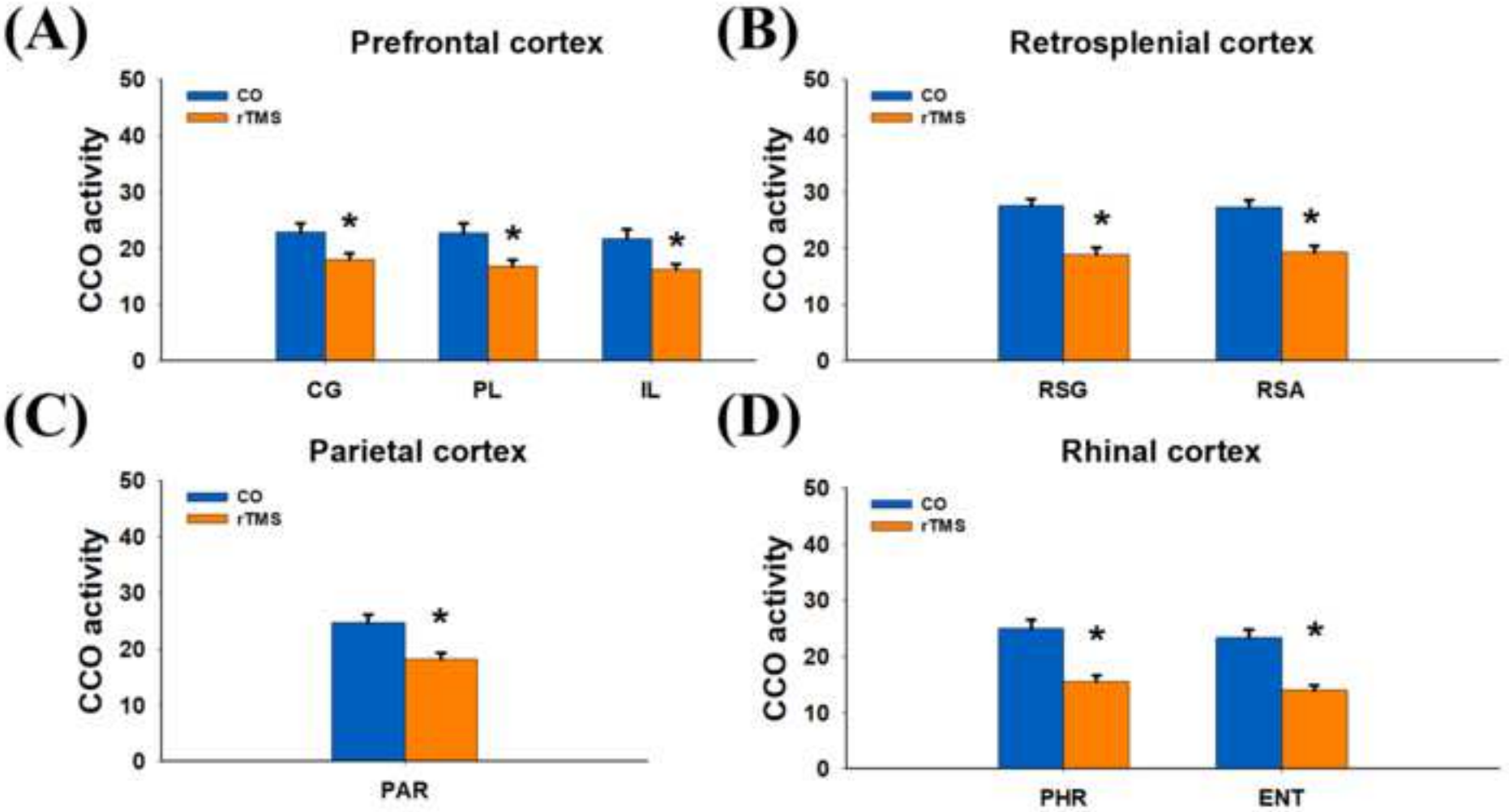

Figure6

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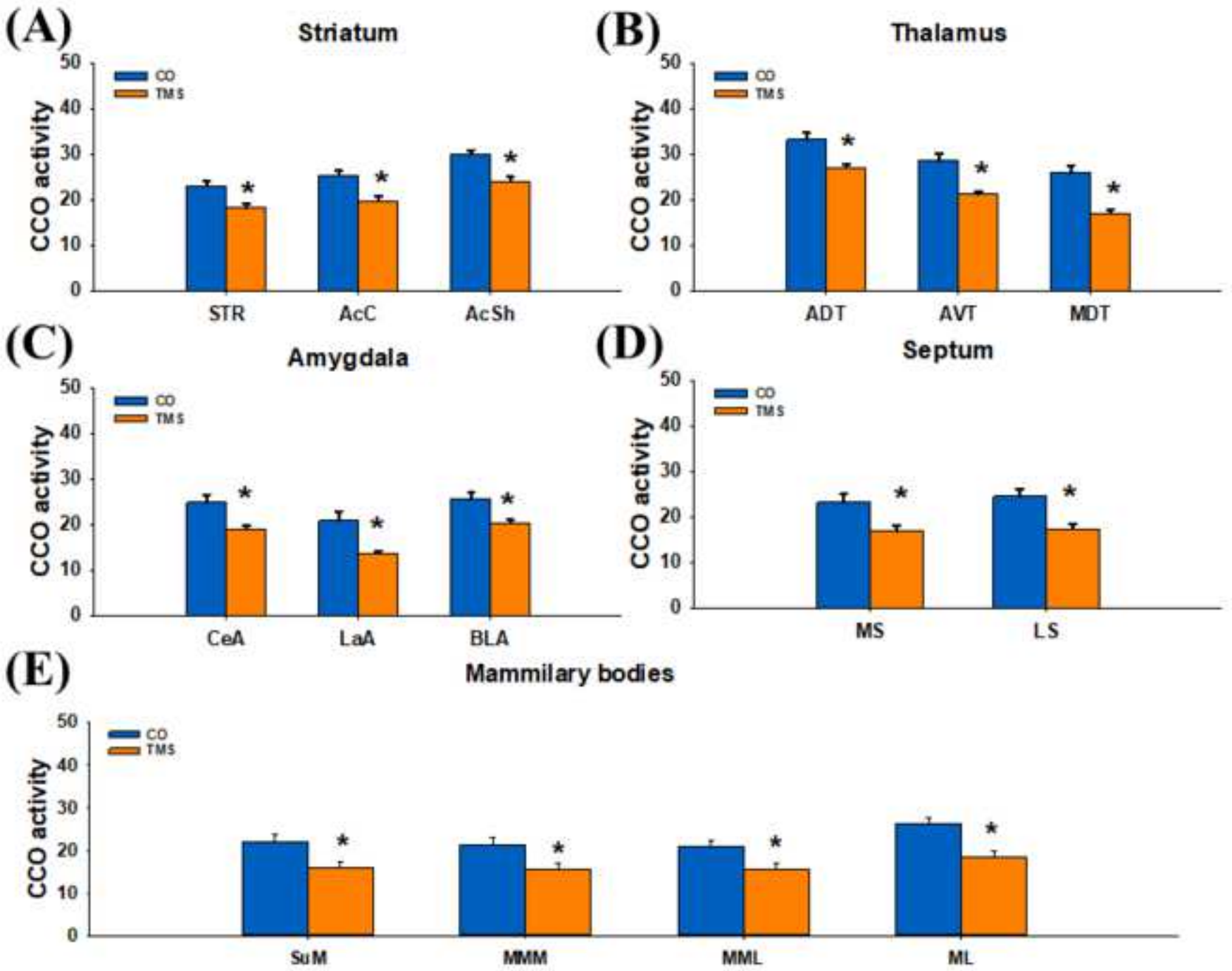

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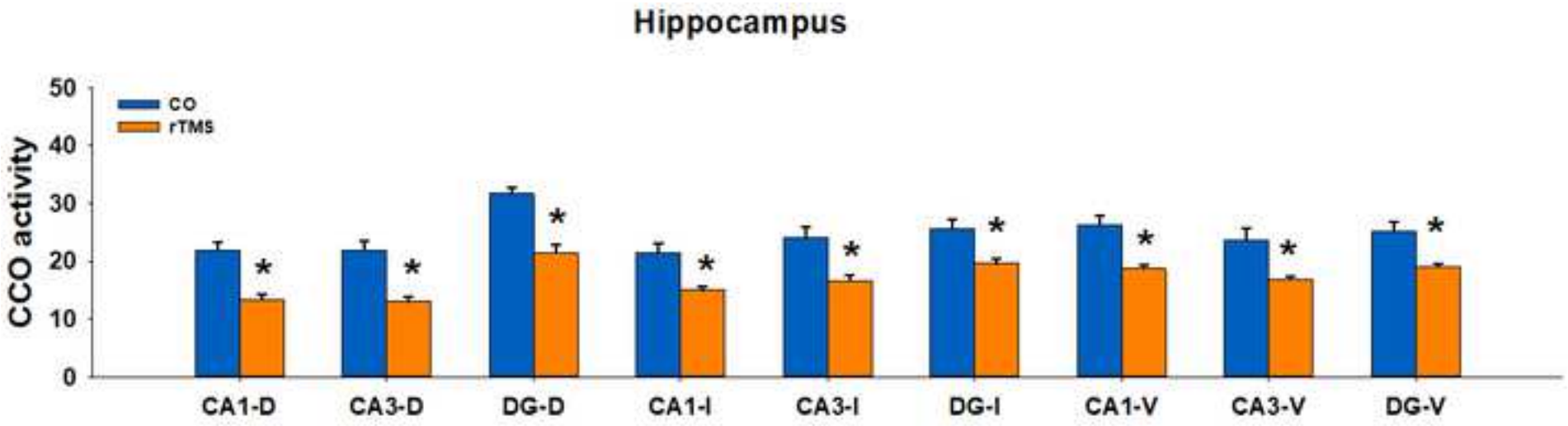
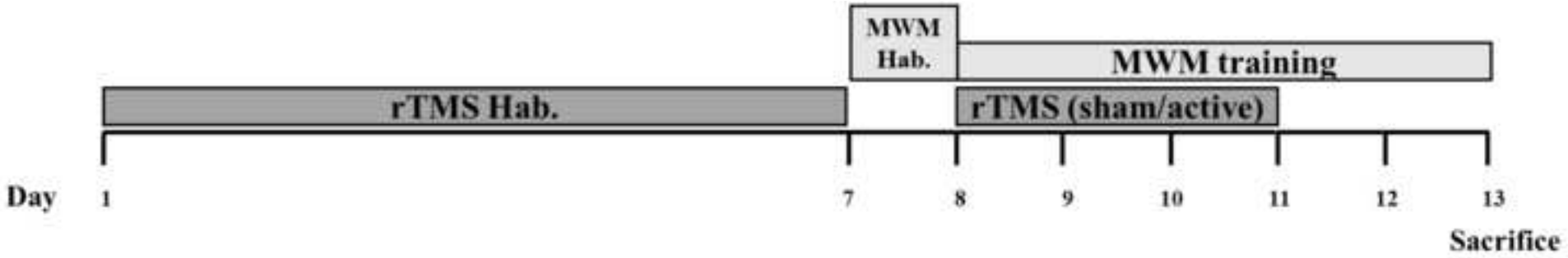


Figure8



Graphical Abstract