In vivo local transcranial static magnetic field stimulation alters motor behavior in normal rats

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Abstract

Transcranial static magnetic field stimulation (tSMS) has inhibitory neuromodulatory effects on the human brain. Most of the studies on static magnetic fields have been performed in vitro. To further understand the biological mechanisms of tSMS, we investigated the effects of in vivo tSMS on motor behavior in normal awake rats. The skull of a male Wistar rat was exposed and a polyethylene tube was attached to the skull using dental cement at the center of the motor cortex (n = 7) or the other cortex (n = 6). By attaching a cylindrical NdFeB neodymium magnet into the tube, in vivo tSMS (REAL) was performed. For SHAM, we applied a similar size non-magnetic stainless-steel cylinder. All rats received twice each SHAM and REAL stimulation every two days using a crossover design, and motor function was measured during the stimulation. Activity level and asymmetry of forelimb use were not affected, but less accurate movements in the horizontal ladder test were found in REAL stimulation of the motor cortex. This study shows that in vivo tSMS has inhibitory neuromodulatory effects on motor behavior depending on the stimulated region on the rat cortex.

Keyword:

Static magnetic fields, Neuromodulation, Cortex, Behavior, Inhibition

1. Introduction

Noninvasive brain stimulation (NIBS), representatively transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS), has been focused on as a potent neuromodulation modality [1]. Low-frequency repetitive TMS (rTMS) induces the suppression effect on neuronal activity and has been applied to the intact hemisphere of a stroke patient to suppress interhemispheric inhibition (IHI) from the intact hemisphere to the damaged hemisphere, and to facilitate motor functional recovery [2–4]. In the last decade, it has been reported that transcranial static magnetic field stimulation (tSMS) as a new type of NIBS also induced a suppression effect on neuronal activity in humans [5–9]. Thus, tSMS, which has a suppression effect, can be expected as a new NIBS for stroke rehabilitation such as low-frequency rTMS.

Static magnetic fields (SMFs) have physiological modifications following eddy current by displacement, Lorentz's force, magnetic force, magnetic torque, and effects on radicals, that can affect the central and peripheral nerves [10,11]. As a biological mechanism of the suppression effect of SMFs, it has been hypothesized that magnetic torque induces the molecular reorientation of the cell membrane and ion channels, affecting the transmembrane ion flux [12]. However, the mechanism is not clear and discussions are ongoing.

tSMS also showed neuromodulatory effects on motor function in humans [13,14]. Most of the studies targeting the biological effects of SMFs have been performed in vitro. In vivo studies have also been reported, but most experiments were performed under the irradiation of SMFs in a special cage, under restraint, under anesthesia, or using a chronically fixed magnet on the skull [15–20]. These experiments naturally had a gap from tSMS research in humans. To clarify this gap and further understand the effect of

tSMS on motor function, it is necessary to establish a novel method that can accommodate SMFs in conditions where the magnet is detachable and experimental animals are awake and move freely.

Therefore, we tried to establish an in vivo and detachable tSMS for normal rats, and investigated the effect of its application on motor behavior in awake and free moving conditions and whether the effect depended on the region of stimulation.

2. Materials and methods

2.1 Animals

Thirteen 7-week-old male Wistar rats were housed in a single cage in a temperature- and humidity-controlled room on a 12-h light/dark cycle. Food and water were available ad libitum. Animals were divided into two groups as follows: motor region stimulation (Motor, n = 7) and non-motor region stimulation (Non-Motor, n = 6). All study procedures were approved by the ethics committee for animal research of Hokkaido University in Japan and conducted according to the guidelines of the committee.

2.2 In vivo local transcranial static magnetic field stimulation

Animals were under deep anesthesia with three types of mixed anesthetic agents (0.15 mg/kg of medetomidine, 2.0 mg/kg of midazolam, and 2.5 mg/kg of butorphanol) intraperitoneal injection (i.p.) and placed in a stereotaxic frame. The skull was exposed and a polyethylene tube (diameter 6 mm, height 3 mm) was attached to the skull with dental cement at the center of the right (n = 4) and left (n =3) primary motor cortex (M1) (1.5 mm anterior to the bregma, 2.5 mm laterally to the midline: Motor group) according to the brain atlas [21,22]. By attaching a cylindrical NdFeB neodymium magnet (Model

N52, diameter 5 mm, height 5 mm, surface magnetic flux density 528 mT; NeoMag, Chiba, Japan) into the tube, in vivo tSMS (REAL stimulation) was performed. North polarity of the magnet was set on the skull side. For SHAM stimulation, we used a similar size non-magnetic stainless-steel cylinder (Fig.1-A). To investigate the region effects of tSMS, an additional Non-motor group was set. There was no difference in the behavior described later between stimulating either the left or right motor cortex by preliminary analysis in the Motor group (data not shown), so the polyethylene tube for all animals in the Non-Motor group was attached to the center of the right non-motor area, which was separated from the M1 and included partly from the sensory to visual cortex (4.0 mm posterior to the bregma, 4.0 mm laterally to the midline) according to the brain atlas [21,22]. On the day following the final behavior assessments, rats were euthanized by an overdose of 4% chloral hydrate (25 mL/kg, i.p.). During the experiment, the polyethylene tube did not fall off from the skull in any of the rats.

2.2 Behavioral assessments

All rats were habituated to the assessment device before surgery. Three days postoperatively, we assessed the motor behavior of all rats for the control condition (CON), which did not apply either a magnet or stainless steel, that is, no REAL or SMAM stimulation. Then, the rats received twice each SHAM and REAL stimulation every two days using a crossover design, and motor behavior was measured during the stimulation (Fig.1-B). In the SHAM and REAL conditions, the rats were allowed 10 min free moving in the open arena after attaching a magnet or stainless-steel. Subsequently, the cylinder test and the horizontal ladder test were performed with each stimulation (Fig.1-C). For the REAL and SHAM conditions, the average value of two trials (A and B) was used as

the representative value in each behavioral assessment.

The symmetry of forelimb use was evaluated using a cylinder test [23]. We videotaped the free movement of the rats in a Plexiglas cylinder (20 cm in diameter and 40 cm in height) and evaluated the function of the forelimb which was contralateral to the stimulated area of the brain, that is, the stimulated forelimb. We counted the number of times a rat touched the wall and whether the rat independently used either forelimb or simultaneously used both forelimbs. The percentage of stimulated forelimb use was calculated using the following formula: [(the stimulated forelimb contacts + 1/2 bilateral contacts)/total contacts] × 100.

Forelimb motor function during walking was evaluated using the horizontal ladder test [24]. We videotaped the rats walking across a ladder at irregular intervals from 1 to 3 cm. We counted the number of total steps, correct placement steps, and error steps at each trial. The test was performed three times with varying intervals in each trial. We calculated the percentage of correct placement steps (correct ratio) and error steps (error ratio) at each trial, and the average value was used as the representative value.

During the behavioral testing, the magnet or stainless steel did not come off from the tube in any of the rats.

2.3 Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM). Two-way repeated-measures analysis of variance (ANOVA) was used with Condition (CON, SHAM, REAL) as the within-subject factor and Region (Motor, Non-Motor) as the between-subjects factor. In case of significant interaction effects, post hoc analyses were performed by Student's paired-samples t-test and Student's t-test with a Bonferroni

correction for multiple comparisons. Statistical analyses were performed using SPSS Statistics software (ver. 26.0, IBM, USA), and the criterion for significance was set at $p < 0.05$.

3. Results

Figure 2 shows the results of the cylinder test. For the number of wall touches (Fig.2-A), two-way repeated-measures ANOVA showed no significant effect of Condition ($F_{2,22} = 0.818$, $p = 0.454$, $\eta p^2 = 0.07$), Region ($F_{1,11} = 0.313$, $p = 0.587$, $\eta p^2 = 0.03$), and Condition (CON, SHAM, REAL) × Region (Motor, Non-Motor) interactions were not detected in the number of wall touches ($F_{2,22} = 0.706$, $p = 0.505$, $\eta p^2 = 0.06$). For the ratio of stimulated forelimb use (Fig.2-B), two-way repeated-measures ANOVA showed no significant effect of Condition ($F_{2,22} = 0.572$, $p = 0.573$, $\eta p^2 = 0.05$), Region ($F_{1,11} = 2.568$, $p = 0.137$, $\eta p^2 = 0.29$), and Condition × Region interactions ($F_{2,22} = 1.921$, $p = 0.170$, $\eta p^2 = 0.15$). These results indicated that tSMS had no effect on the activity level or symmetry of forelimb use.

Figure 3 shows the results of the horizontal ladder test. For the error ratio of the stimulated forelimb (Fig.3-A), two-way repeated-measures ANOVA showed no significant effect of Condition ($F_{2,22} = 1.804$, $p = 0.188$, $\eta p^2 = 0.14$), Region ($F_{1,11} = 0.460$, $p = 0.512$, $\eta p^2 = 0.04$), and Condition × Region interactions ($F_{2,22} = 2.186$, $p = 0.136$, $\eta p^2 = 0.17$). For the error ratio of the unstimulated forelimb (Fig.3-B), two-way repeated-measures ANOVA showed no significant effect of Condition ($F_{2,22} = 1.728$, $p = 0.201$, $\eta p^2 = 0.14$), Region ($F_{1,11} = 0.126$, $p = 0.729$, $\eta p^2 = 0.01$), and Condition × Region interactions ($F_{2,22} = 0.208$, $p = 0.814$, $\eta p^2 = 0.02$). For the correct ratio of the stimulated forelimb (Fig.3-C), two-way repeated-measures ANOVA showed a significant effect of

Condition ($F_{2,22}$ = 11.313, $p < 0.001$, $\eta p^2 = 0.51$), but no significant effect of Region ($F_{1,11}$ = 1.378, $p = 0.265$, $\eta p^2 = 0.11$). Significant Condition × Region interactions were observed ($F_{2,22}$ = 15.645, $p < 0.001$, $\eta p^2 = 0.59$). In the Motor group, the post hoc test showed that the correct ratio of stimulated forelimb in the REAL condition was significantly lower than those in the CON ($p < 0.001$) and SHAM ($p < 0.001$) conditions. In the Non-Motor group, there were no significant differences between any conditions. Furthermore, in the REAL condition, the post hoc test showed that the correct ratio of stimulated forelimb in the Motor group was lower than that in the Non-Motor group ($p = 0.001$). There were no significant differences between the Motor and Non-Motor groups in CON ($p = 0.394$) and SHAM ($p = 0.308$) conditions. For the correct ratio of the unstimulated forelimb (Fig.3-D), two-way repeated-measures ANOVA showed no significant effect of Condition ($F_{2,22}$ = 0.272, $p = 0.764$, $\eta p^2 = 0.02$), Region ($F_{1,11}$ = 0.502, $p = 0.493$, $\eta p^2 = 0.04$), and Condition× Region interactions ($F_{2,2}2$ = 0.298, $p = 0.745$, $\eta p^2 = 0.03$). These results indicated that tSMS induced a decrease in dexterity function in the stimulated forelimb, specifically during M1 stimulation.

4. Discussion

This is the first study as we know to show that tSMS specifically over M1 has an inhibitory neuromodulatory effect on motor behavior, especially in the stimulated forelimb dexterity function, in normal rats by using a new stimulation method.

A human brain model study showed that the spatial gradient of the tSMS B-field was peak directly below a magnet in the cortex, suggesting the possibility that tSMS could stimulate specific localized regions of brain to some extent [25]. Indeed, tSMS over M1 affected motor learning in humans [13,14]. In addition, an animal study showed that

SMFs over the visual cortex altered the visuospatial function [20]. The stimulation site used in this study was determined by referring to a previous study using the same type of rat [22]. According to the study, the brain region where the magnet was set predominantly consisted of the motor area. The present study showed that tSMS over M1 reduced the dexterity of forelimb motor function, but not when the non-motor area was stimulated. Therefore, this tSMS system could selectively and locally stimulate at least two target brain regions (motor and non-motor). However, this study lacked data on how the magnetic fields were distributed upon stimulation, and it was a limitation of this study. Moreover, the magnet used in this study was large relative to the rat head, considering the ratio of magnet size to human head size as used in human studies, although notably the surface magnetic flux densities are comparable. Therefore, brain regions adjacent to M1 might have been affected, including the sensory cortex and subcortical areas such as the basal ganglia. Future studies simulating the strength or extent of magnetic fields and investigating whether tSMS is localized to M1 are warranted.

In human tSMS studies, counterbalancing the weight of the magnet is sometimes required to prevent head deviation [26]. Head deviation was not observed in any of the conditions in this study. Verification under conditions with stainless steel (SHAM), which had a weight similar to that of the magnet, as well as without the magnet and stainless steel (CON), was performed. The SHAM and CON conditions did not affect motor function, suggesting that the weight of the magnet or stainless steel did not influence head deviation or behavior in this study.

The number of wall touches in the cylinder test was not changed by tSMS, indicating that tSMS did not affect voluntary movement in rats. The cylinder test is used for assessment of forelimb function in animals with unilateral brain injury [23]. We

hypothesized that the symmetry of forelimb use would change due to inhibition of unilateral neural activity by SMS, similar to the unilateral neurological dysfunction induced by brain injury; however, this was not the case, unlike the horizontal ladder test. The inhibitory effect of SMS on the brain might not have been strong enough to induce neurological dysfunction such as brain injury. From the view of the sensitivity of the assessments, the horizontal ladder test might be more suitable for detecting slight behavioral changes than the cylinder test in stroke model rats [27,28]. Therefore, it is suggested that motor behavioral changes were detected specifically in the horizontal ladder test. However, there was a significant effect on the correct ratio of stimulated forelimbs, but not on the error ratio. Correct steps were defined as those in which the rats placed the center of the forelimb on the ladder [24]. If they gripped the ladder with their fingers or placed their wrist on the ladder, the steps were defined as partial steps. Therefore, correct steps might have detected forelimb dexterity more rigorously and sensitively than error steps in normal rats. Since the error steps were manifested in central nervous system disease model animals, the inhibitory effects of SMS on the brain might not have been strong enough to induce neurological dysfunction. Here, we should consider the time-dependent effects of tSMS. Previous studies have shown that not only the higher intensity [5,29] but also the longer [5,20] stimulation showed higher suppression effects. In our study, we subsequently analyzed two behavioral tests, and the order was not changed during the experiment. Rats were exposed to the SMFs for 10 min at the start of the cylinder test, and for 20 min at the start of the horizontal ladder test. Thus, it was reasonable to expect that the rats could be exposed to SMFs for tSMS in the horizontal ladder test longer than in the cylinder test. However, the limitation of this study was that we have no information of the actual and simulation data on the surface magnetic

flux density and how it spreads into the cortex. Therefore, we cannot discuss the influence of the intensity of tSMS in this study. Furthermore, it is unclear whether the sensitivity of the rat brain to SMFs is equal to that of the human brain. Therefore, further studies, including verification of the magnet size and stimulation time, are necessary.

We investigated the online effect of tSMS on motor behavior under stimulation by using a crossover design in the same animal, and the suppression effect was shown only in Real stimulation, but not in SHAM stimulation. Thus, the biological mechanisms of the suppression effect by tSMS found in this study were unlikely to be the structural changes of brain tissue or protein expression in the brain. Previous studies have shown that molecular reorientation of the cell membrane and ion channels (including sodium, calcium, and potassium channels) can be induced by magnetic torque, and that the transmembrane ion flux was affected in in vitro studies [12,29–31]. Furthermore, one study points out the changes in the GABAergic system in the human brain [6]. Altogether, dynamic changes in neuronal activity and related factors might have occurred during the SMS in this study. Further investigations are needed for the elucidation of the mechanisms of the suppression effect by tSMS.

Recently, tSMS over the unilateral M1 in human showed remote neuromodulatory effects on the contralateral M1 such as decreasing of intracortical facilitation [32] or the facilitation of the cortical excitability [33]. Moreover, tSMS over the M1 reduced IHI from the stimulated M1 to the unstimulated one [33]. If the cortical excitability of contralateral M1was facilitated with suppression of IHI by tSMS, the motor function corresponding to the unstimulated M1 may also be affected. However, the motor function of unstimulated forelimb was not affected in this study. It might be because a remote neuromodulatory effect on contralateral M1 was not enough to induce changes in

motor function in the normal rats. A low-frequency rTMS over the unaffected hemisphere induced motor functional recovery of a paralyzed hand in stroke patients [3]. Therefore, changes in motor function of unstimulated forelimb caused by a facilitative effect on neuronal activity in the contralateral M1 by tSMS might be shown in stroke model animals in which the cortical activity between hemispheres becomes imbalanced [34,35].

Finally, we did not select the method of attaching a magnet or stainless-steel to the skull directly, as in a previous report [19], and fitted them into the tube attached to the skull for detachable tSMS in this study. Thus, this method made the performance of time-course experiments possible using the same animal under various conditions, such as with or without stimulation, and SHAM or REAL stimulation. It might be used in chronic experiments in which the stimulation is repeated only for a certain period of time in a day.

5. Conclusions

For a preliminary trail to understand the biological mechanisms of tSMS, we investigated the feasibility of in vivo tSMS under awake and free moving conditions and its effect on motor behavior in normal rats. We found it feasible and showed that in vivo tSMS has inhibitory neuromodulatory effects on motor behavior depending on the stimulation region in normal rats.

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Declaration of Competing Interest

The authors report no declarations of interest.

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Figure legends

Fig. 1. (A) The image of in vivo and detachable static magnetic stimulation. (B) Experimental timeline by the cross over design. (C) Behavioral tests and the timeline.

Fig. 2. The figures show the number of total wall touches (A) and the ratio of stimulated forelimb use (B) assessed by the cylinder test. Data are shown as the mean ± SEM and each value.

Fig. 3. The figures show the error ratio of stimulated forelimb (A), the error ratio of unstimulated forelimb (B), the correct ratio of stimulated forelimb (C) and the correct ratio of unstimulated forelimb (D) assessed by horizontal ladder test. Data are shown as the mean ± SEM and each value. **** indicates $p < 0.001$ for comparison of Condition (CON, SHAM, REAL) as within-subject factor. ### indicates $p < 0.005$ for comparison of Region (Motor, Non-Motor) as between-subjects factor.

Fig.1

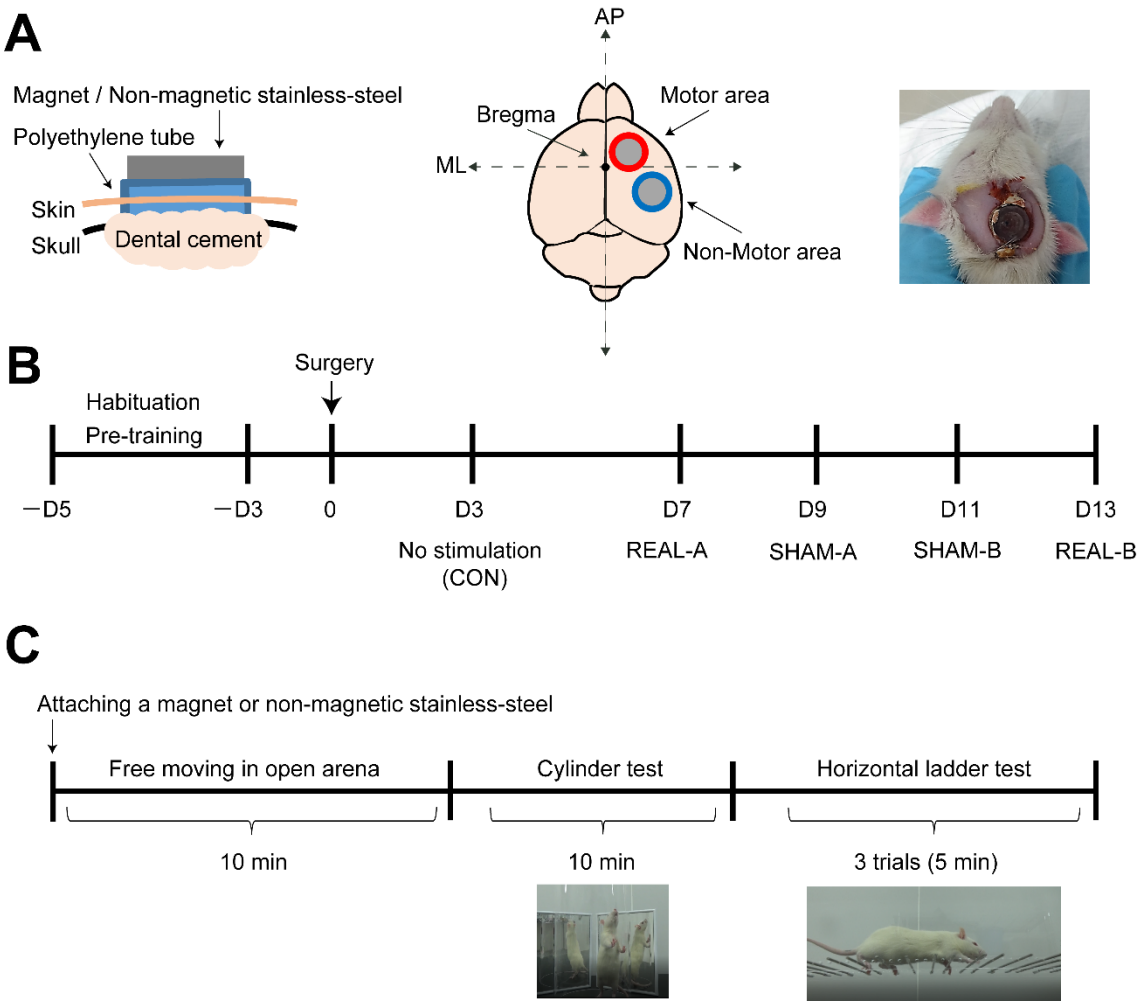

A
Magnet / Non-magnetic stainless-steel
Polyethylene tube
Skin
Skull
Dental cement
AP
Bregma
Motor area
ML
Non-Motor area
B
Surgery
Habituation
Pre-training
−D5
−D3
0
D3
No stimulation
(CON)
D7
REAL-A
D9
SHAM-A
D11
SHAM-B
D13
REAL-B
C
Attaching a magnet or non-magnetic stainless-steel
Free moving in open arena
10 min
Cylinder test
10 min
Horizontal ladder test
3 trials (5 min)

Fig.2

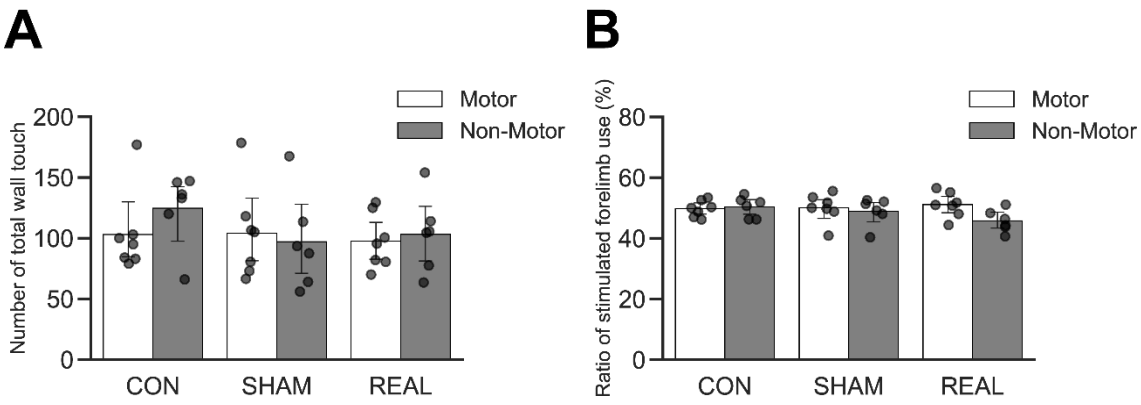
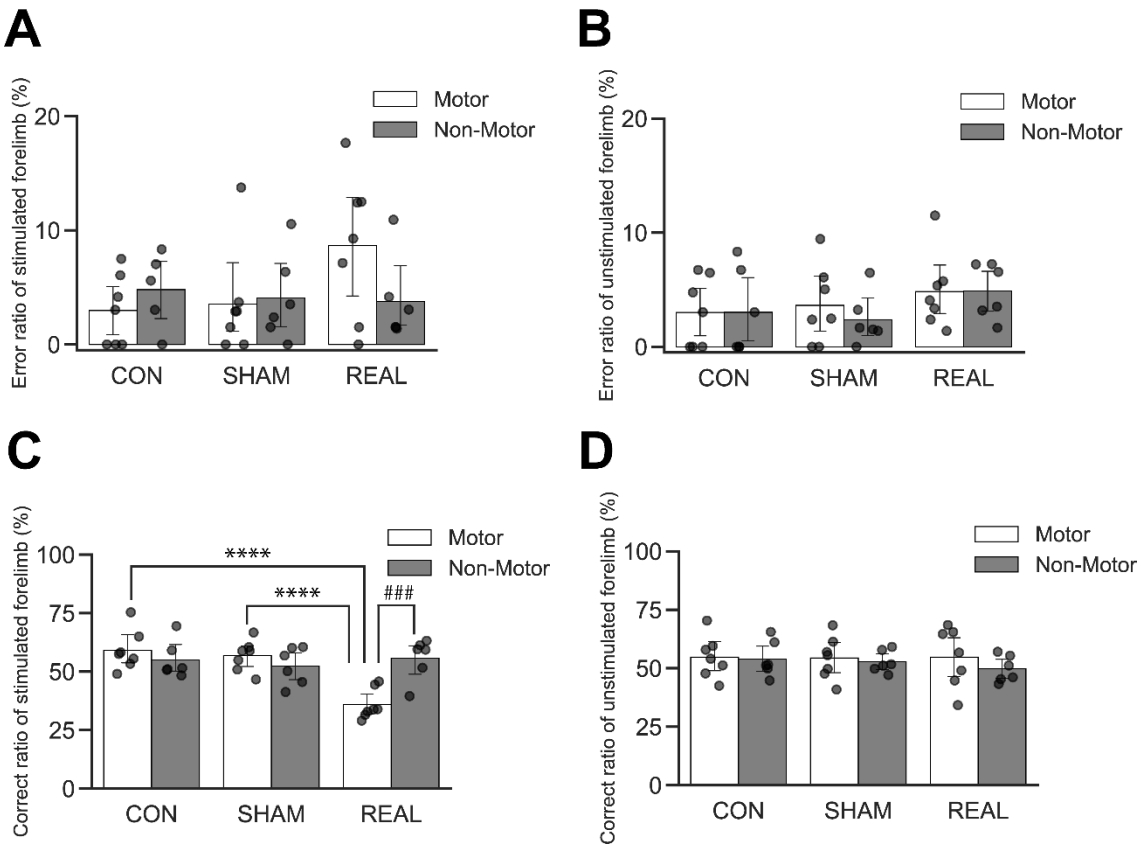

A
Motor
Non-Motor
Number of total wall touch
200
150
100
50
0
CON
SHAM
REAL
B
Motor
Non-Motor
Ratio of stimulated forelimb use (%)
80
60
40
20
0
CON
SHAM
REAL

Fig.3


A
Error ratio of stimulated forelimb (%)
20
10
0
Motor
Non-Motor
CON
SHAM
REAL
B
Error ratio of unstimulated forelimb (%)
20
10
0
Motor
Non-Motor
CON
SHAM
REAL
C
Correct ratio of stimulated forelimb (%)
100
75
50
25
0
****
****
###
Motor
Non-Motor
CON
SHAM
REAL
D
Correct ratio of unstimulated forelimb (%)
100
75
50
25
0
Motor
Non-Motor
CON
SHAM
REAL