Theta-phase closed-loop stimulation induces motor paradoxical responses in the rat model of Parkinson disease

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ABSTRACT

Background

High-frequency deep brain stimulation (DBS) has become a widespread therapy used in the treatment of Parkinson's Disease (PD) and other diseases. Although it has proved beneficial, much recent attention has been centered around the potential of new closed-loop DBS implementations.

Objective

Here we present a new closed-loop DBS scheme based on the phase of the theta activity recorded from the motor cortex. By testing the implementation on freely moving 6-OHDA lesioned and control rats, we assessed the behavioral and neurophysiologic effects of this implementation and compared it against the classical high-frequency DBS.

Results

Results show that both stimulation modalities produce significant and opposite changes on the movement and neurophysiological activity. Close-loop stimulation, far from improving the animals' behavior, exert contrary effects to those of high-frequency DBS which reverts the parkinsonian symptoms. Motor improvement during open-loop, high-frequency DBS was accompanied by a reduction in the amount of cortical beta oscillations while akinetic and disturbed behavior during close-loop stimulation coincided with an increase in the amplitude of beta activity.

Conclusion

Cortical-phase-dependent close-loop stimulation of the STN exerts significant behavioral and oscillatory changes in the rat model of PD. Open-loop and close-loop stimulation outcomes differed dramatically, thus suggesting that the scheme of stimulation determines the output of the modulation even if the target structure is maintained. The current framework could be extended in future studies to identify the correct parameters that would provide a suitable

control signal to the system. It may well be that with other stimulation parameters, this sort of DBS could be beneficial.

KEYWORDS

Deep brain stimulation; Close/open-loop scheme; Rat model of Parkinson's disease; Motor deficit; Neurophysiological correlate of behavior

INTRODUCTION

Since Benabid reported the first successful case of thalamic stimulation as a chronic treatment of Parkinson's disease (PD) tremor [1], deep brain stimulation (DBS) has become a standard clinical routine to alleviate the motor symptoms of PD [2,3] and dystonia [4,5]. During the last years the use of DBS has been extended to treat other psychiatric and neurologic disorders such as obsessive compulsive disorder [6,7], resistant depression [7,8] or Alzheimer's disease [9]. The DBS therapy consists in the implantation of multi-contact electrodes in subcortical regions that deliver electrical impulses at a constant rate [10,11]. For the treatment of PD the electrodes are typically placed in the subthalamic nucleus (STN) [3,12,13] or internal globus pallidus (GPi) [3,14,15] and the stimulation frequency is set around 130 Hz.

Despite of its efficacy, the high-frequency stimulation (HFS) presents numerous shortcomings. Disruptive effects can go beyond the treatment of the disease symptoms producing cognitive, postural and behavioral side-effects [16–19], effectiveness may be reduced by the evolution of the disease and the battery discharge compels the patient to visit the hospital for adjustment [20].

To cope with this issues, alternative DBS approaches such as the closed-loop DBS have emerged during the last years [21]. By using a feedback signal, closed-loop stimulation delivers electrical impulses in an adaptive manner considering at every moment the patient's state. Although there are different options for their implementation, in all cases a control signal is required to decide when the electrical impulses should be released. In this context, physiological signals represent a good candidate to serve as feedback signal to control the DBS [22,23] and first experimental works show promising results [24–27]. Here, we introduce and test a new closed-loop approximation. Previous studies carried out by our laboratory have emphasized the importance of the phase of low-frequency oscillations such

as delta and theta in the modulation of brain activity [28,29]. The phase/amplitude coupling in physiological [29] and pathological conditions [30] supports the idea of the low frequencies relevance as a coordinator of high frequency activity. In this way, the phase of theta activity has been demonstrated to mediate in cognitive processes and the communication between distant brain structures [31,32]. Following this, we designed an adaptive closed-loop system using a specific phase of the theta cortical activity to control the stimulation. We set out to test the effects of this closed-loop paradigm in a rat model of PD and demonstrate that theta phase-locked DBS has significant neurophysiological and behavioral effects.

MATERIALS AND METHODS

Animals

Two groups of adult male Wistar rats were used (250-300 gr), including 15 hemi-Parkisonian and 18 control rats. In addition group of four control rats (same strain, sex and weight) were used to assess the specificity of the changes induced by close-loop vs. open-loop theta stimulation schemes. Animal care and surgery procedures were approved by the animal ethics committee; Comité de Ética para la Experimentación Animal, Universidad de Navarra, approval CEEA132-12.

Hemi-Parkinsonian rat model

The hemi-Parkinsonian rat model was induced by unilateral injection of 6-hydroxydopamine (6-OHDA) into the left medial forebrain bundle (MFB). The stereotaxic coordinates were calculated using Paxinos atlas [33]: AP: -4.5mm, L: 1.2mm and V: -7.9mm from bregma. Before the surgery, rats were pretreated with pargyline (50mg/kg, Sigma-Aldrich) to inhibit monoamine oxidase and desipramine (25mg/kg Sigma-Aldrich) to protect noradrenergic neurons. Surgeries were carried out under inhalatory anaesthesia (oxigen flow 0.7 l/min, 2% isoflurane). Once the animals were anesthesized, an injection of 6-OHDA together with acid ascorbic was performed using a microliter syringe (Hamilton, Switzerland). A total of 6µl were injected at the speed of 1µl/min. After the surgery, animals were returned to the animal facilities. Control rats underwent the same surgery but instead of 6-OHDA, saline was injected.

Surgical electrode implantation

Before the electrode implantation surgery hemi-parkisonian rats were left eight weeks of lesion evolution. Oscillatory activity was recorded using a stainless-steel screw placed in the skull (1.6mm diameter, Plastics One, USA, ref 363). The screw was implanted in the left

primary motor cortex (CxM1). Reference and ground screws were placed over the cerebellum. Unilateral electrical stimulation was delivered through a bipolar electrode (SNE-x100 Kopf Instruments, California) implanted in the left STN [34,35]. All coordinates were selected according to previous studies [34,36].

Experiment protocol

The experiment started five days after the electrode implantation. First, the threshold for direct motor activation was calculated by following the procedure described in [34]. Specifically, biphasic square pulse trains (130Hz, 60μs-width) were delivered and amplitude of train stimuli successively increased from 20μA in 20μA steps until observing a motor response from the animals (characterized by a stereotypic rotational response). At this point, the amplitude value determines the motor threshold for DBS stimulation on each animal and defines the amplitude of the DBS stimulation for the forthcoming experiments, that is fixed at 80% of the threshold. Animals showing contralateral muscle contraction elicited by the electrical stimulation (4 control and 2 lesioned) were rejected to discard the possibility of having electrodes placed near or into the internal capsule.

The day after, animals were connected to the recording/stimulation cables and a grid test with no stimulation was carried out. Animals were then moved to a custom-made arena (60 cm x 60 cm) and let free to move for 20 minutes. After the habituation period, animals were recorded continuously during 15 minutes; 5 minutes of pre-stimulation, 5 minutes under electrical stimulation and 5 minutes after stimulation (post-stimulation). Animals undertook two different sessions that were delivered separately; one under a classic DBS scheme, and another following the proposed closed-loop implementation. After each session, a grid test under DBS stimulation was performed. Sessions were separated at least 6 hours to let animals to recover from the previous session. To avoid any time effects, the order of the sessions was randomized between animals.

Electrophysiological recordings

Brain signals were recorded using a multichannel cable (Ref:363/441/6W/Spring, Plastics One, USA) connecting a headstage (unity gain, Plexon Inc., USA) with a differential amplifier (PBX system, filters:0.3-8000Hz and gain1000, Plexon Inc., USA). Signals were digitalized at 25000 Hz and stored for offline analysis using a CEDpower1401 A/D together with Spike2 software (CED, UK).

Electrical Stimulation

We coined the term *classic* stimulation to the traditional way DBS is performed in PD patients. The stimulation consists on delivering biphasic pulses, 60µs-width, at 130Hz. These parameters have been widely used by previous studies [34,36,37] and are similar to the ones used for clinical routine [10,30]. In this study, electrical stimulation was delivered using a square pulse S88K stimulator together with two PSIU6 constant current units (GRASS, USA) to achieve the generation of biphasic pulses.

Closed-loop stimulation

Our closed-loop system was designed to stimulate the STN during the peaks of the theta oscillatory activity (4–8 Hz) recorded from the CxM1. To do that, the activity recorded from the CxM1was filtered *online* in the delta/theta band range and depending on the phase of the signal, an activation pulse was sent to the SK88 stimulator that released a short train of three pulses (130Hz, 60µs, biphasic). The activation pulse was only sent when two criteria were met: (1) the phase moment of the theta activity was the peak, and (2) the amplitude of the peak was above one standard deviation of the average theta activity. This allowed the system to stimulate only when genuine theta activity existed, avoiding spurious activations (Fig. 1). The frequency of the theta activity was determined from the spectrum of the CxM1 activity recorded during the pre-stimulation period. Complimentary, and in order to assess the specificity of the changes induced by the close-loop stimulation of the STN during the peaks

of the theta oscillatory activity from CxM1, we ran a control test where short trains of 3 pulses (130Hz, 60μs, biphasic) were delivered at a constant rate of 7Hz, with independence of the CxM1 activity. These experiments were carried out in a separate group of 4 control rats.

Behavioral assessment

Locomotor Activity

Animal's movement was tracked using a webcam placed on the top of the arena. Videos were analyzed automatically with custom-made tracking software running under Matlab (Mathworks, USA). The program allowed us to detect the center of the body coordinates of the animals in every single moment of the recording sessions. Apart from the total distance travelled, three additional indexes were calculated: time spent in mobility, time spent in fine movements and time spent in motionlessness [36]. Mobility was defined as any movement that produced ambulation, motionlessness was any period where the animals did not move and finally, any other movement such as head waiving was classified as fine movement.

Grid Test

We used the grid test [34,38] to measure the degree of catalepsy. In this test the forepaws of the animals are placed extended on a grid angled 45° from the horizontal ground and the time that the animals remain in that position is counted. If the animals did not move after 30 seconds, they are removed from the grid and the next trial starts. We performed five consecutive trials per animal and study condition.

Histological Verification

To proceed with the histological verification, animals were anaesthetized (ketamine, 75mg/kg

and xylacine, 11mg/kg) and intracardially perfused with a solution of paraformaldehyde (PAF, 4%), dissolved in phosphate buffer solution (PBS, 0.1M, pH7.4). After perfusion, the brain was taken out and post-fixed during 24h in PAF. Then it was passed to PBS-sacarose for at least 24h. Brains were cut in coronal axis using a cryotome. Slices of 40μm were obtained and processed to assess electrodes location and lack of damage due to electrical stimulation. The slides were stained with thionine and then observed in a microscope to determine the location of the stimulation electrodes.

To assess the dopaminergic denervation induced by 6-OHDA lesion, the sections containing the substantia nigra pars compacta (SNpc) were processed by tyrosine hydroxylase (TH) immunohistochemistry. In this case, free floating sections were thoroughly washed with PBS and left for incubation with a primary antibody for one night. The next day, the slices were cleaned with PBS for incubation with the second antibody for two hours and then stained with DAB peroxidase (Sigma). Finally, the total number of remaining dopaminergic neurons was quantified by stereology. Animals with a dopaminergic denervation < 90% (1 rat) were excluded from the study.

Spectral analysis

Power spectra estimation during basal (no DBS) condition was performed by means of the Welch Periodogram [39] (4s Hanning window, 0.25Hz/bin). This analysis served to detect/define the value of the theta peak that was then used in the close-loop setup.

Stimulation artifact during DBS was removed by applying a median filter implemented in Matlab. Using a median filter with a specific window length (in our case 4ms; for an example, see Fig. A.1) results in very convenient way of removing short duration spiky artefacts (as is the case in the DBS). Nonetheless, we restricted our spectral analyses to the [1 90] Hz frequency range; were no significant effects of the -already filtered- stimulation

artefacts would be expected.To account for the oscillatory changes exerted during DBS stimulation, we obtained a continuous estimation of the instantaneous amplitude of the recorded activity from 5 minutes before to 5 minutes after stimulation. To do that, we computed the analytic signal of the recorded activity through the estimation of the Hilbert transform and obtained the instantaneous amplitude and phase of the signal within each frequency range of interest [40]. Specifically, we quantified the effect of DBS in the delta (1 – 4 Hz), theta (4 – 10 Hz), beta (12 – 30), low-gamma (LG, 40 – 60 Hz) and high-gamma (HG, 70 -90 Hz) ranges.

Statistics

Prior to any statistical analysis, all the variables were normalized by using the transformation described in [41]. The differences on the pre-stimulation oscillatory activity, movement and scores of the grid test between animal groups were computed by means of two sample t-test. To compare the effects of the classic or closed-loop DBS one way repeated measures ANOVA test was used (time factor). In all cases, multiple comparisons Tukey post-hoc tests were applied. For all the analyses that compare energy levels between stimulation periods (pre, stimulation and post), the comparison was performed using the mean of the energy for each of the periods. The study of the correlation between the energy of the oscillatory activity and the movement was carried out using the Pearson correlation coefficient.

RESULTS

Two different groups of animals were studied. A dopaminergic lesioned and sham lesion group serving as control. We generated the conventional hemi-Parkinsonian model by unilateral injection of 6-OHDA into the MFB. Both, successful dopaminergic lesion (nigral dopaminergic loss: 91.5% ± 2.3%, mean ± SEM) and position of stimulation electrodes were confirmed histologically (Fig. A.2). At the end, 12 hemi-Parkinsonian and 14 sham rats took part of the final analyses. 4 additional control animals were used to carry out a control test to assess the importance of locking the stimuli to the peak of the theta activity respect to just delivering trains at the same frequency.

Hemi-parkinsonian rats present motor deficit, higher beta power and lower DBS threshold for motor response.

Prior to any further investigation into the DBS effect, we first assessed the existence of behavioural and oscillatory differences between the sham and 6-OHDA groups. Animals from the control group showed a significantly higher degree of movement in the open arena than lesioned animals (Fig. 2A, $t=2.21$, $p<0.05$). A more detailed evaluation of the locomotion patterns allowed us to detect that animals from the control group spent significantly more time moving than 6-OHDA (12.7% ± 3.2% vs. 4.6% ± 1.5% respectively, $p<0.05$). No differences were found for immobility (82.3% ± 3.9% and 91.8 ± 2.6%) nor for fine movements (4.8% ± 0.9% and 3.5 ± 1.2%). Significant differences were also found in the grid test (Fig. 2B): sham animals moved from the grid almost instantaneously (0.32s ± 0.03s) while 6-OHDA showed more akinesia (6.6s ± 0.14s, $t=8.84$, $p<0.001$).

Next, we investigated the differences in the oscillatory activity and detected a significant increase of the beta power in the 6-OHDA group (Fig. 2C, $t=10.83$, $p<0.001$). No significant differences were found for the other bands.

We also found differences in the threshold for the intensity of stimulation. Comparison of the intensities needed to elicit a motor response in the animals revealed that the control animals required a significantly higher level of intensity respect to the hemi-parkinsonian group (Fig. 2D, $t=2.19$, $p<0.05$).

Effects of classic DBS and closed-loop DBS on the locomotor activity

Next, we quantified the effects of DBS on motor performance. Classic DBS improved the mobility of the hemi-parkinsonian rats (Fig. 3A). As soon as the stimulation was delivered, the animals started to move and the beneficial effects disappeared right after the stimulator was turned off ($F_{2,12}=4.96$, $p<0.01$; post-hoc tests, $p_{stim/pre}<0.01$, $p_{stim/post}<0.01$). The detailed study of the movement patterns under DBS showed that the lesioned animals significantly augmented their time spent in mobility (25.3% ± 4.9%, $p<0.001$) and fine movements (8.5% ±1.2%, $p<0.05$) but reduced the periods spent with no-movements (66% ± 5.7%, $p<0.01$, Fig. 3B). Akinetic behavior in the grid test was fully reverted by the HFS were escape times dropped significantly (0.44s ± 0.33s, $p<0.001$, Fig. 3C).

Control animals also showed an increase in their degree of movement ($F_{2,14}=5.34$, $p<0.01$, Fig. 3D) with similar patterns on the time spent in mobile episodes (29.9% ± 5%, $p<0.01$), fine movements (9.2% ± 1.2%, $p<0.05$) and immobility (60.6% ± 5.6%, $p<0.01$, Fig. 3E). In the grid test, control animals maintained the low values of latency of escape (0.54s ± 0.1s) and no significant differences were found (Fig. 3F).

In a different session, we delivered the electrical stimulation following the closed-loop scheme based on the detection of the theta peak. For these session, the same periods were used: 5 mins pre-stimulation period, 5 mins closed-loop DBS and 5 mins after DBS. Contrary to the pattern observed during classical stimulation, under the effect of closed-loop stimulation hemi-parkinsonian animals did not show any differences in the degree of movement ($F_{2,12}$=0.1, p=0.9, Fig. 3A). On the contrary, when applied to the control group, the theta-peak-based closed-loop stimulation quantitatively reduced the degree of movement of the animals ($F_{2,14}$=6.23, p=0.012; post-hoc tests, $p_{stim/pre}$<0.01, $p_{stim/post}$<0.01, Fig. 3D). Control animals spent significantly less time in movement (4.7% ± 1.4%, p<0.05) and more time in immobility (91.5% ± 2.2%, p<0.05). No significant effects in fine movements were found (3.76% ± 0.8%, Fig. 3E). In the 6-OHDA group, mobility parameters remained at similar levels to those found in basal condition: time spent in mobility (5 % ± 2.2%), fine movements (3.89% ± 1.26%) and no-movement (91.02% ± 3.36%, Fig. 3B). In the grid test, closed-loop stimulation increased the degree of akinesia in both groups (sham: 1.43s ± 0.81s, 6-OHDA: 7.29s ± 2.53s), nevertheless, differences only reached statistical significance in the control group (p<0.05, Fig. 3F).

The control test designed to check whether stimulation with a train at the same frequency would have the same effects revealed that indeed, locking the pulses to the peak of the theta oscillation exerts a different effect than just delivering pulses according to an open-loop theta stimulation with the same parameters but in the absence of a feedback signal. Behavioural analysis showed that while theta-based closed-loop stimulation elicited a reduction in the amount of movement in the 4 test animals (-56.5% ± 20%, mean ± std; compared to no-stimulation condition), under open-loop stimulation at 7 Hz the effects were heterogeneous: three out of four animals showed different degrees of movement while the fourth suffered a significant reduction. As a result, the high inter-subject variability in the responses of the

animals (43.2% ± 45.3%, mean ± std) did not suggest a consistent behavioural effect as it does for the case of the closed-loop implementation.

Effects of classic DBS and closed-loop DBS on oscillatory activity

Next, we investigated the changes exerted by classic DBS and closed-loop DBS on the oscillatory activity recorded from CxM1. To do so, we computed the instantaneous energy for each of the oscillatory bands and followed the evolution of the energy bands through all the recording session.

In the delta and theta ranges, no significant effects were detected for the 6-OHDA group ($F_{2,12}$=2.32, p=0.116 and $F_{2,12}$=0.3851, p=0.684, respectively; Fig. 4A). Interestingly, classic stimulation did produce a significant decrease of the energy on the beta band during the stimulation period ($F_{2,12}$=6.09, p=0.006; post-hoc test p<0.01). Stimulation also had a significant effect increasing the energy of both gamma ranges (LG: $F_{2,12}$=4.54, p=0.02 and HG: $F_{2,12}$=9.26, p<0.001; post-hoc test p<0.01). The analysis of the activity on the control animals displayed very similar results (Fig. 4B) except for the delta band, were an energy rebound was observed when the HFS was turned off ($F_{2,14}$=5.71, p=0.007; post-hoc test p<0.01). The theta band did not show any significant effect ($F_{2,14}$=0.31, p=0.73), beta energy decreased significantly ($F_{2,14}$=10.61, p<0.001; post-hoc test, p<0.01) and both gamma ranges augmented their energies (LG: $F_{2,14}$=13.22, p<0.001 and HG: $F_{2,14}$=10.68, p<0.001; post-hoc test, p<0.01).

Using the same scheme as for the classic DBS modality, we analysed the effects of the close-loop implementation. The hemi-parkinsonian group had a remarkable increase in the energy of the beta ($F_{2,14}$=9.67, p<0.001), LG ($F_{2,14}$=19.63, p<0.001) and HG ($F_{2,14}$=35.7, p<0.001) bands together with a less pronounced change in the theta range ($F_{2,14}$=7.66, p=0.002). In all these cases, Tukey post-hoc analyses evinced a very significant increase of the energy

($p<0.01$) under the action of closed-loop DBS. Energy in the delta range was not altered ($F_{2,12}=2.46$, $p=0.103$).

Close-loop DBS had similar effects on the oscillatory activity of the control group. Stimulation elicited a significant increase on the energies of beta ($F_{2,14}=13.54$, $p<0.001$), LG ($F_{2,14}=13.38$, $p<0.001$) and HG ranges ($F_{2,14}=14.77$, $p<0.001$). Low-frequency bands (delta and theta) also showed significant effects ($F_{2,14}=6.32$, $p=0.008$; $F_{2,14}=4.1$, $p=0.025$). Delta energy increased after switching off the stimulation ($p<0.05$) while the theta energy increased only under the effect of the stimulation ($p<0.05$).

Finally, and to relate the aforementioned behavioral and oscillatory effects induced by DBS, we estimated the Pearson correlation coefficient to assess interactions between the different energy levels found during the three study conditions (non-stimulation, HFS and closed-loop DBS) and the degree of movement for each of the study groups. We found a significant negative association between the energy values of beta and the amount of movement. This was especially noticeable on the 6-OHDA group, where the correlation reached statistical significance (Table 1).

DISCUSSION

Here we introduce a closed-loop DBS paradigm that uses the activity recorded from the primary motor cortex as the parameter to control the stimulation. By targeting in real-time the phase of the theta activity we exert significant behavioral and oscillatory changes in the rat model of PD. When compared against HFS, the outcome of the closed-loop DBS differed dramatically, thus suggesting that the scheme of stimulation determines the output of this modulation even if the target structure is the same. Although there has been much interest in closed-loop stimulation from the theoretical point of view, this is one of the few studies that have successfully implemented on an animal model

We first assessed the existence of behavioral and oscillatory effects on the 6-OHDA model. The hemi-parkinsonian group showed increased levels of energy the beta band (12–30 Hz), a lower degree of movement in the open field and more akinesia in the grid test. These differences have already been reported in previous studies using the same model [42–47] and are in concordance with the findings in humans, where anomalous beta oscillations are recorded on the STN of PD patients and is attenuated when dopaminergic medication improves the clinical symptomatology [48–50].

Following previous studies -and in the same way that it is adjusted in human beings-, we estimated the motor threshold for DBS and detected that hemi-parkinsonian animals show a lower threshold when compared to control animals. This has already been reported on other experimental studies targeting the pedunculopontine nucleus [51] and aligns with studies of transcranial magnetic stimulation that have shown a decrease of the motor threshold in PD patients due to a state of hyperexcitability of the motor cortex [52,53].

DBS has proven to be an effective treatment for PD as it ameliorates one of their most characteristic symptoms: the alteration of the motor control [54]. In this work, and similarly to other experimental studies, animals from the 6-OHDA group showed a deficit on

locomotion that was restored to control levels under the action of HFS [36,55]. We also quantified the degree of akinesia by using the grid test. Results showed that lesioned animals spend significantly more time on the grid and that HFS completely reversed this deficit; thus confirming that the dopaminergic lesion produces akinesia and that HFS DBS can ameliorate this deficit [56,57].

We observed that HFS also increases the degree of movement shown by the control animals in basal condition. As in the lesioned group, DBS on the control animals following the classic scheme increased the time spent in mobility and fine movements. No significant changes were detected in the akinesia-related grid test, as control animals already showed an almost-zero latency of scape from the grid.

Interestingly, we observed that behavioral changes were consistently accompanied by a decrease of the energy in the beta range in both groups. All this agrees with previous studies showing a reduction of the power on the beta band during HFS [36,37,58–60]. Gamma power also increased under DBS and was accompanied by an increase on mobility. In this line, our group and others have already reported these gamma activity increase following motor improvement under the effect of dopaminergic agonist both, in experimental models and PD patients [30,61,46,62].

Despite of effectiveness, classic DBS provides with an invariant and constant train of electrical pulses that are delivered to the brain. This invariant approach does not account for the dynamic changes occurring in the brain and could therefore cause side effects or jeopardize its applicability [12,63]. In this way, new closed-loop DBS therapies represent the next frontier in the neurostimulation field. Close-loop based schemes are conceived to deliver stimulation in a selective way as they are programed to stimulate only under specific circumstances. When designing a closed-loop DBS approximation, the selection of the control signal becomes crucial. Signals such as local field potentials (LFP) can provide with

relevant information that would allow to perform an adaptive stimulation [23,64]. To date, only few studies have designed closed-loop approaches to treat PD [24,25,27] and essential tremor [26,65]. Rosin's study was one of the firsts testing the potential beneficial effects of closed-loop DBS in the MPTP-treated primate model and reported that closed-loop stimulation of the GPi based on the ongoing activity in M1 was more efficient in alleviating parkinsonian motor symptoms than the standard continuous HFS GPi DBS paradigm. In humans, Little et. al showed that an effective closed-loop DBS approximation can be obtained in PD patients by triggering the HFS stimulator by thresholding the online filtered amplitude of the abnormal beta activity recorded [24].

Although with a different goal, the idea of performing closed-loop DBS using the phase of theta oscillations has been previously tested on the hippocampus of freely moving mice [66].Here we present a close-loop approximation that considers the phase of the oscillatory activity recorded from the motor cortex. This choice is inspired by (i) previous studies from our laboratory showing that the phase of low-frequency oscillations determines the amplitude of faster activities and promotes/interferes with movement [28–30], and (ii) other investigations suggesting an important role of the cortex on the action of STN-DBS via antidromic activation [34,36]. This closed-loop scheme produced very different effects to the ones evinced by the classic DBS. Strikingly, control animals significantly reduced their amount of movement in the open field test and increased the latency of scape in the grid. Although worsened at some degree, movement differences on the hemi-parkinsonian group did not reach statistical significance, possibly due to the reduced level of mobility that they already show. In contrast with the HFS results, oscillatory changes induced by closed-loop DBS gave rise to a notable increment of the energy on the beta and gamma bands. This effect on the beta oscillations is totally opposite to that observed during HFS action and accompanies the reduction of the degree of movement and the difficulties to escape from the

grid. Indeed, we assessed the existence of a negative correlation between the amount of energy in this frequency range and the amount of movement. All these results enforce the hypothesis that beta activity can be causally related to the motor impairment of PD [67].

In summary, here we have introduced and tested a novel schema for closed-loop DBS based on the phase of the theta activity recorded from the cortex. Results suggest that this implementation of close-loop DBS has an opposite effect to that of the classic HFS DBS. Far from improving the motor symptoms in the hemi-parkinsonian animals, this modality worsened the motor abilities in the control group. Nevertheless, these results do not invalidate this approximation but rather demonstrates that it is possible to modulate some specific components of the oscillatory activity and that by doing so, it is possible to exert significant behavioral and electrophysiological changes. By acting over a specific frequency we have demonstrated that it is possible not only to alter the behavioral state of the animals, but also to modulate the activity in other frequency ranges, thus opening a new line of work focused into identifying the correct parameters to provide the right feedback to the closed-loop system. It may well be that using other phases of the theta activity, the phase of other activities or other stimulation patterns, this sort of DBS could be beneficial.

FIGURE LEGENDS

Fig. 1 - Theta-phase closed-loop deep brain stimulation scheme.

A – Scheme of the designed closed-loop stimulation system: The activity is recorded from the motor cortex (blue arrows) and processed in real time (red arrows) to decide whether DBS is delivered or not. To do that, the raw signal is filtered into the theta range and the peak of the phase detected; if conditions are met, the trigger releases the stimulation. B – Schematic comparison between classical and closed-loop DBS. During classical stimulation, electrical pulses are delivered constantly while the closed-loop DBS only stimulates at the peak of the theta phase.

Fig. 2 – Hemi-parkinsonian rats present motor deficit, higher beta power and lower DBS threshold for motor response.

A – Comparison of the total movement between sham (blue) and hemi-parkinsonian animals (red). The hemi-parkinsonian group shows a deficit in locomotion when compared with the intact group ($p<0.05$). B – Hemi-parkinsonian animals (red) spent score more time in the grid test than control animals ($p<0.001$). C – Hemi-parkinsonian animals (red) show a higher beta power when compared to control animals (blue) in basal conditions ($p<0.05$). D – The control group shows a significant higher motor threshold ($p<0.05$).

Fig. 3 – Comparison of open-loop vs. close-loop stimulation on motor performance

A, B, C – Bar plots of the movement and grid test scores for every stimulation under no stim (empty bar), classic (stripped bar) and closed-loop DBS (dotted bar) for the hemi-parkinsonian group. HFS restores the motor deficit and improves the grid test scores. D, E, F – Same as before for the control group. Closed-loop DBS significantly reduces the movement and worsens the grid test scores.

Fig. 4 – Comparison of open-loop vs. close-loop stimulation on oscillatory activity

Energy evolution of the frequency bands of study during the recording session: pre-stimulation (0-5 mins), stimulation (5 – 10 mins) and post-stimulation (10 – 15 mins) on the hemi-parkinsonian (A) and control group (B) for HFS (blue) and closed-loop stimulation (red). A sharp decrease on the beta energy is produced when the HFS is delivered together with a lower increase on gamma levels. These effects instantly disappear when the HFS stops. On the contrary, the closed-loop DBS produces an increase of the beta energy together with a raise on gamma levels.

TABLES

	6-OHDA	Control
Delta	r = -.0194	r = -.259
Theta	r = .03	r = .08
Beta	r = -.451 **	r = -.195
LG	r = .27	r = .08
HG	r = .316	r = .169

Table 1 – Linear correlations of energy band values and amount of movement for the 6-OHDA and control animals (p<0.01).**

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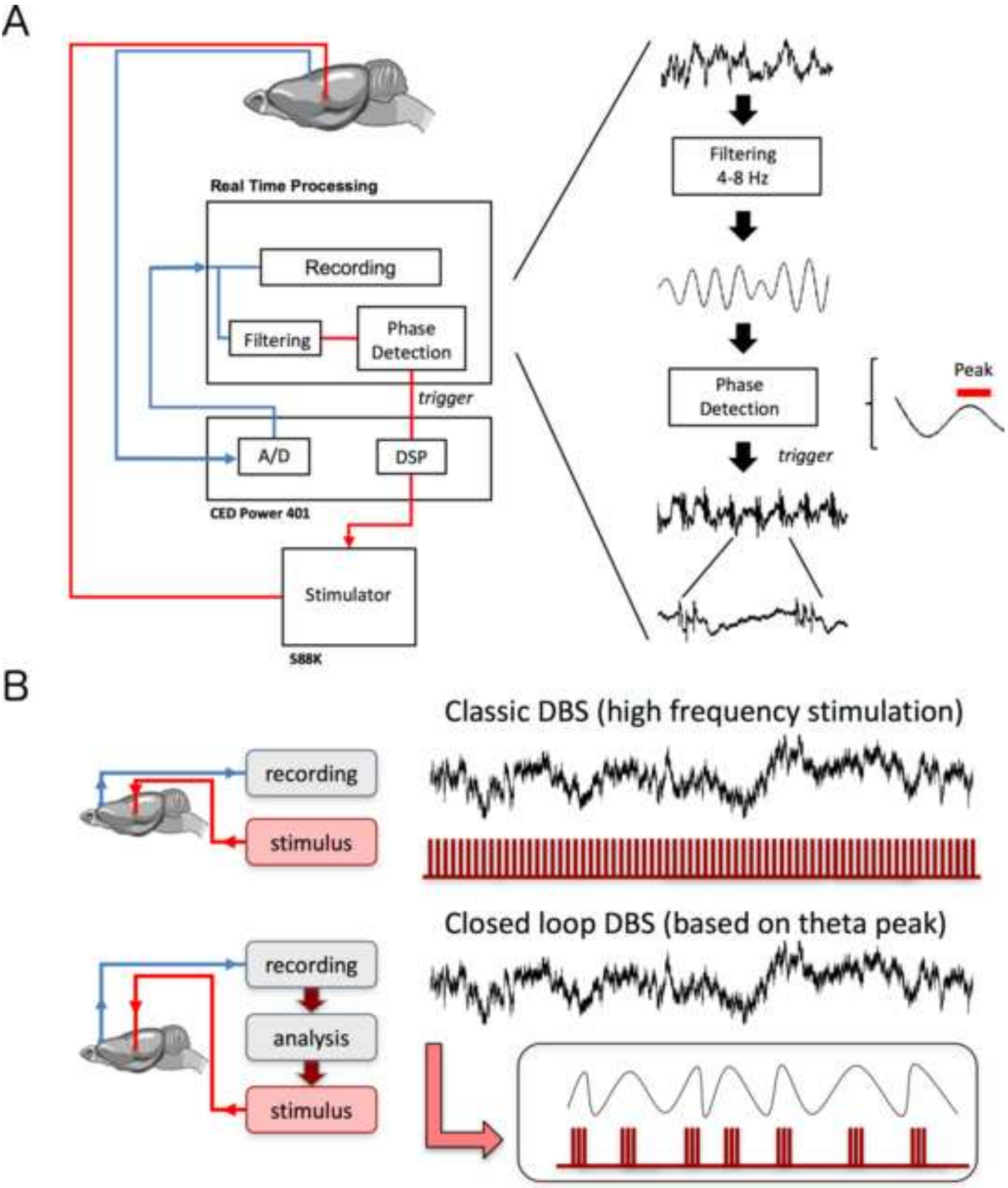

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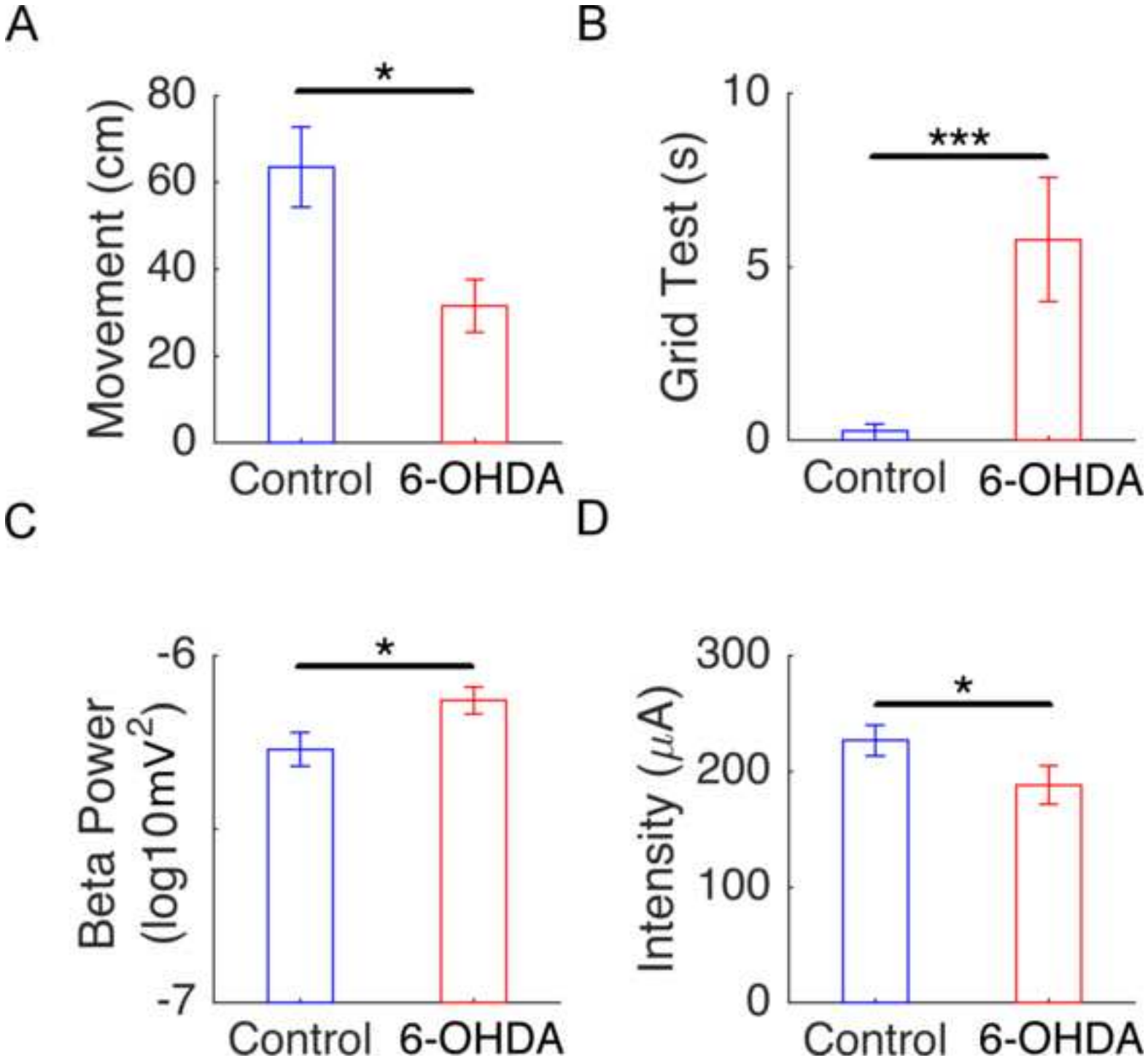

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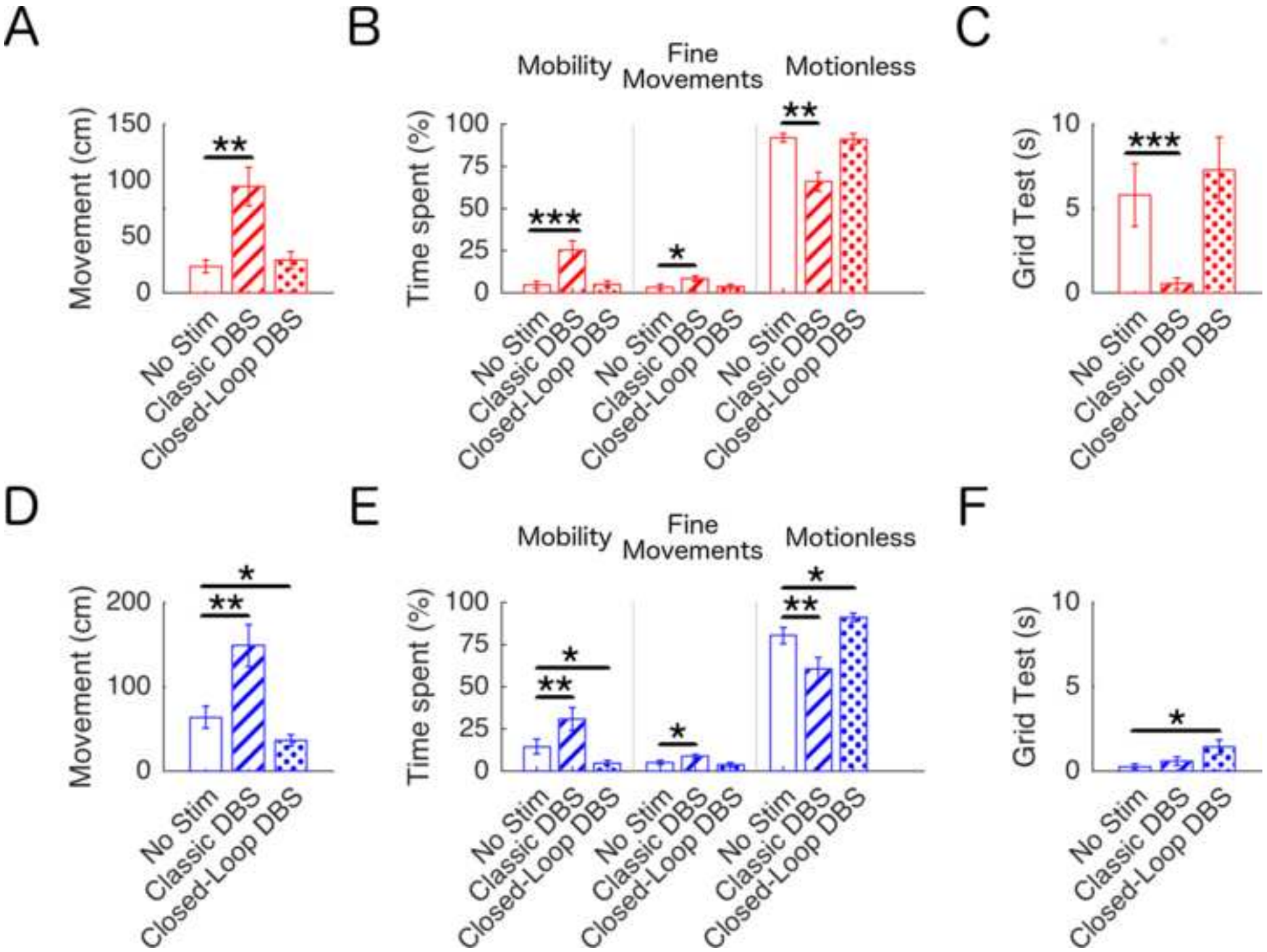

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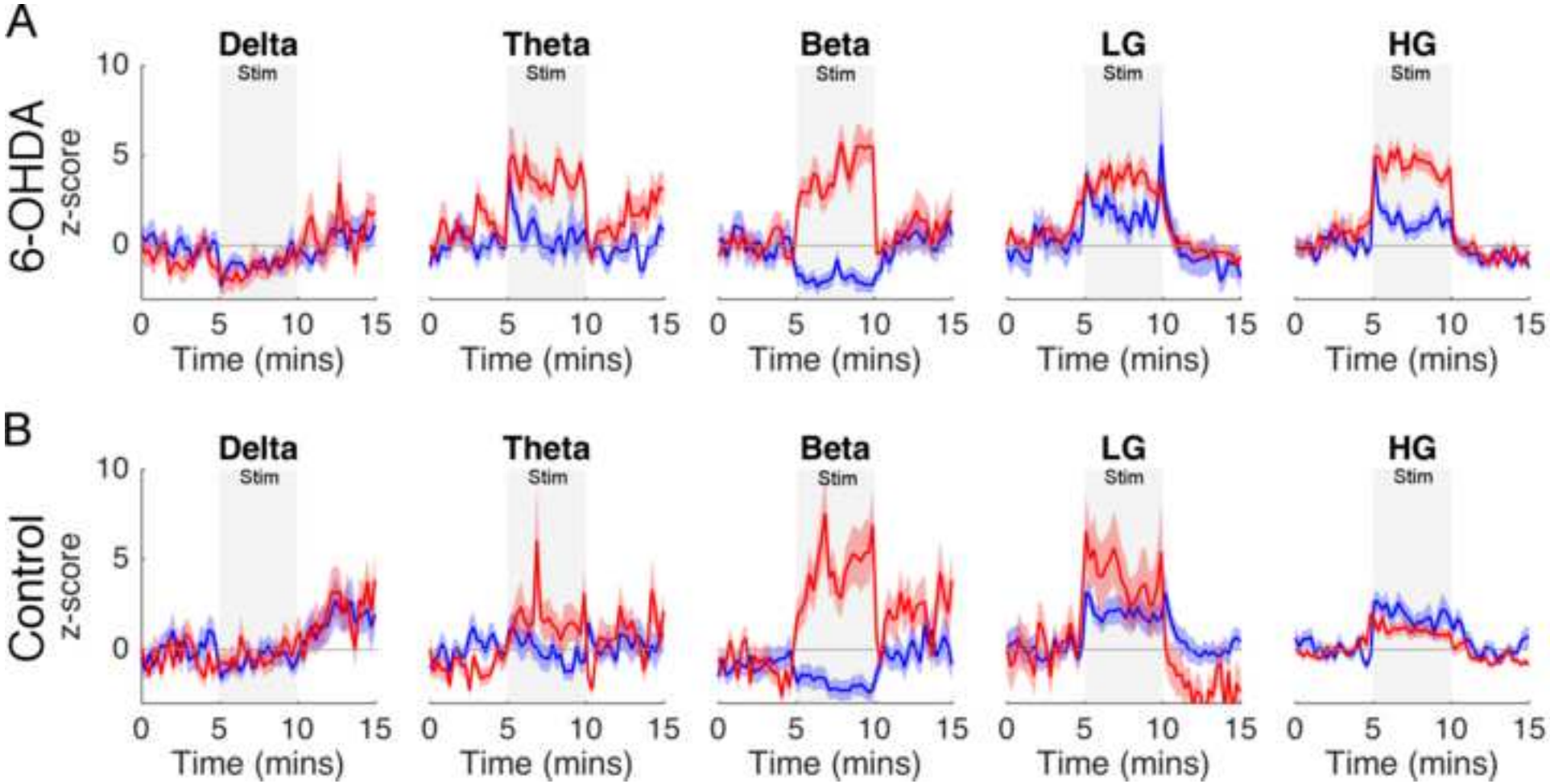

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