Appendix e-1

Surgical procedures

All patients were bilaterally implanted in the subthalamic nucleus (STN) with macroelectrodes for deep brain stimulation (DBS, model 3389 Medtronic, Minneapolis, MN, USA). The STN was targeted by direct visualization through a CT-MRI fusion-based technique before surgery, as extensively reported elsewhere. The STN position was estimated by matching the CT-MRI fused images with a digitized stereotactic atlas. During surgery, the implant position was assessed by microrecordings from exploratory microelectrodes and by clinical assessment of the effects induced by stimulation through the same exploratory microelectrodes and also finally from the implanted macroelectrodes. The implanted 3389 Medtronic electrode has four cylindrical contacts (1.27 mm in diameter, 1.5 mm in length, placed 2 mm apart, center-to-center) denominated 0–1–2–3, beginning from the more caudal contact. According to neuroimaging and intraoperative clinical and neurophysiological tests, for all nuclei studied the position of contact 1 was consistent with placement within the STN area. After DBS surgery the electrode leads were externalized to enable electrode access in recording and stimulation modes, before the connection to the subcutaneous pulse generator, which was implanted in a second operative procedure few days later.

We included all patients who did not experience any surgical complications, and excluded patients who lacked a significant peak in beta LFP activity (11-35 Hz) during a screening recording session (see below). In this experiment, the beta peak was needed to adapt aDBS parameters and, as shown by the literature, not all patients display a clear LFP beta peak. All patients were studied unilaterally. The choice of the side is detailed in the next sections.

Experimental protocol

The experimental protocol included two sessions (one per day), that took place on day 5 (Day 1 of the experimental session) and day 6 (Day 2 of the experimental session) after the electrode implantation surgery.

On the first day (Day 1), before beginning the experimental session, a calibration session was performed in order to:

1. Verify the presence of a significant beta peak and of an adequate electrode impedance in at least one side.
(2) Establish the best side for recording LFPs and testing aDBS, according to neurophysiological recordings, clinical examination, and feedbacks from intra-surgery stimulation tests.

(3) Define the stimulation threshold (i.e., the stimulation voltage that would have been used to deliver cDBS) and the therapeutic window defined as the stimulation range comprised between the voltage eliciting the minimum detectable effect on cardinal symptoms and the voltage at which side effects occur.

All this information was needed in order to calibrate the aDBS external prototype used in the experimental sessions to record LFPs and deliver aDBS.

After that, the patient wore the aDBS external prototype in a pouch. On the Day 1, the external prototype was set only in “recording” mode (i.e., no stimulation delivered, LFP recordings on). On Day 2, the external prototype delivered beta power-driven unilateral aDBS on the selected side and, at the same time, recorded and stored LFPs. Both experimental sessions begun after 12 hours of medication withdrawal. Each experimental session lasted 7 to 8 hours, during which the patient underwent the following assessments performed by an experienced neurologist:

1- Baseline assessment: after 12 hours medication withdrawal, Unified Parkinson’s Disease Rating Scale, part III, motor part (UPDRS III), and Unified Dyskinesias Rating Scale (UDysRS), part 3 and 4;

2- Peak dose/Med ON 1: when the first administration of the patient’s usual morning medication was effective (about 45-60 minutes after medication intake depending on patient’s response), UPDRS III and UDysRS, part 3 and 4.

3- End Dose/Med OFF 1: at the end of the effect of the first administration of the patient’s usual morning medication, UPDRS III and UDysRS, part 3 and 4.

4- Peak dose/Med ON 2: when the usual second levodopa dose was effective (about 45-60 minutes after medication intake), UPDRS III and UDysRS, part 3 and 4.

5- End dose/Med OFF 2: at the end of the usual second levodopa dose, UPDRS III and UDysRS, part 3 and 4.

During the time between assessments, the patient was free to move and to carry out his/her normal activities (e.g., walking, eating, watching TV, sleeping, …), while the aDBS external prototype was comfortably placed in a pouch. Adverse events (AEs) were collected and
categorized as mild, moderate, or severe, and classified as causally related to the experimental procedure, the device, or the stimulation treatment ⁴.

**Calibration session**

On Day 1, after 12 h of withdrawal of antiparkinsonian medication, we recorded LFPs at rest (without stimulation and without medication; stim OFF/med OFF) from electrode contact pairs 0-2, 0-3, and 1-3. LFPs were first recorded from the side contralateral to that of disease onset and then recorded from the ipsilateral side. The signals were acquired using a standard amplifier (gain 80 db, passband 1-100 Hz, notch ON) (Model Grass ICP511, Astromed, USA) and then digitalized via a Micro1401-3 unit (Cambridge Electronic Design, UK). The signals were analysed using the Spike 2 software (Cambridge Electronic Design, UK) and the Matlab Software (version 7.10; The MathWorks, Natick, USA). Electrode impedances were acquired at 30Hz using an impedance meter (Model EZM 4; Grass, USA).

We qualified the oscillatory activity in the frequency domain by analysing the power spectral density (PSD). We calculated the intrinsic background noise (neural and specific device electronic noise) and used it as threshold for significant oscillatory activity. If the beta activity had a peak exceeding this threshold, we defined the maximum PSD value within the 11-35 Hz band as the patient-specific beta peak and the frequency band ranging ±2 Hz around the beta peak as patient-specific beta band. If we identified two significant beta peaks we chose the lowest one (i.e., low-beta). The patients that exhibited significant beta activity in at least one of the recorded signals were enrolled in the study. We selected the hemisphere and the electrode contact pair that exhibited the highest beta peak for recording and used the contact located between these contacts for stimulation.

After that, an experienced neurologist assessed the patient to define the therapeutic window ²: the threshold for the clinical effect (upper limb rigidity improvement on the side contralateral to stimulation by at least 60% without side effects), and the threshold for side effects. These two thresholds were used to personalize aDBS settings, so that the maximum stimulation voltage delivered remain within the therapeutic window, thus ensuring treatment safety.

**Beta power recording and aDBS delivery: the aDBS external prototype**

The aDBS prototype used in this study is fully described elsewhere ⁵, and was already used in a clinical study approved by the Italian Ministry of Health ⁶. In brief, the external prototype is a portable device (dimensions: 12 x 7 x 2.5 cm; weight: 150g) that can be placed in a pouch so that the patient was allowed to move freely. The device was developed according to the
requirements for signal amplification and stimulation artifact suppression previously defined. The recording chain allowing artifact-free recordings when DBS is turned ON, is composed of three stages, namely the pre-amplification, the analogue filtering, and the final amplification stage. The filtered analog signal is digitized and processed to extract the normalized power of the beta band (10-35 Hz), that is then used to adapt stimulation voltage, thus implementing aDBS. The normalized beta power was exponentially smoothed with a 50 s time constant before being used as control variable to calculate the new stimulation voltage value. The control law was linearly dependent on the beta power values with a saturation threshold. The device was connected to the electrode on the side selected for recording and stimulation. In Day 1, the device was switched on in a recording-only mode, and did not deliver any stimulation throughout the entire 8-hours session. The device recorded continuously beta power data, in the personalized band defined for each patient, and stored them for further download and analysis. In Day 2, the device was switch to the aDBS mode, and was programmed to deliver a 130 Hz stimulation with a 60 µs pulse and an amplitude that linearly changed, according to beta power recorded, between 0 V and the effective stimulation amplitude (threshold for clinical effects increased by 10-20%, according to the length of the therapeutic window) calculated for each patient. The device recorded the stimulation amplitude delivered throughout the whole session. In addition, the device recorded and stored continuous beta power data (with the same configuration as in Day 1) that were used for the analysis. aDBS was switched on after baseline assessment and turned off at the end of the experimental session.

Outcomes and Data analysis

We had two types of outcomes, one technical, aimed to get insights on the aDBS device functioning over 8 hours sessions, and the other one clinical, aimed to assess beta-band based aDBS feasibility.

Technical outcomes

We collected beta band power continuously throughout the 8-hours session, and aDBS amplitude throughout the 8-hours session in Day 2. As a further verification, we calculated the average beta power in each of the clinical conditions (baseline, Peak dose 1, End dose 1, Peak dose 2, End dose 2), considering the time interval of [-10;+10] minutes around the UPDRS III assessment. We also calculated the percentage changes from baseline as:
\[
\text{BetaPower}\% = \frac{(\text{BetaPower}_t - \text{BetaPower}_{\text{baseline}})}{\text{BetaPower}_{\text{baseline}}}
\]

where \( t \) is the time point during the experimental session (Peak dose 1, End dose 1, Peak dose 2, End dose 2) and baseline is the first morning assessment after 12 hours overnight medication washout.

From the aDBS device we could also collect the stimulation voltage delivered throughout the entire session on Day 2. To allow comparisons, we normalized the stimulation voltage as percentage of the maximum voltage delivered to the patient in the session:

\[
\text{StimVolt}\% = \left(\frac{\text{StimVolt}_{\text{max}} - \text{StimVolt}}{\text{StimVolt}_{\text{max}}}\right) \times 100
\]

We also collected the patient’s physical activity using a wearable commercial bracelet equipped with a three-axis accelerometer with a sampling rate of 50 Hz. The device used was a Pebble Time smart-watch. It includes a three-axis accelerometer and a Bluetooth connection with a mobile device (Android Phone). A dedicated app was developed to both acquire and store data, and to provide a clinical diary to be filled in by the patient every 30 minutes. The patient has a personal ID and password to access the app (enforced by an initial login screen). An alert set into the app warned the patient when it was time to fill the diary in. The diary consisted in the choice of the most representative activity of the last 30 minutes (sleep, rest, walk, talk, eat, other). Using the accelerometer data, we calculated an “activity index”, representing the amount of motor activity corresponding to each activity type. To calculate the activity index, we considered the accelerometer data in the band of 0.5 to 4 Hz. More specifically we determined the mean of each accelerometer axis and then we divided the data in 10 minutes bins. For each sample of the bin we incremented a counter by one unit if at least one of the three axis value was above its axis total mean. The “activity index” was the resulting end value of the counter of all bins. The mean threshold was used to rule out tremor or other background conditions; the “counting index” was implemented to include every possible activity with the same significance regardless of the instantaneous intensity.

We verified (1) distribution normality through the single-sample Shapiro-Wilk test (\( p > 0.05 \)), to allow the use of parametric statistics, and (2) the lack of carry over effects between the two days by comparing the baseline values (Med OFF, Stim OFF) of the two days through a paired t-test (\( p < 0.05 \)). Both tests confirmed the hypotheses.

We then applied Bonferroni corrected t-tests for multiple comparisons (\( p < 0.0125 \)) to assess the difference between beta power in all experimental conditions compared to baseline both in Day 1 and Day 2.

We first tested whether the beta power was modulated by levodopa using non-supraliminar levodopa doses. To do so, a paired t-test between beta power in the MedOFF and beta power
in the MedON condition was applied (p<0.05). Then, we correlated the beta power to (1) the patient’s clinical state as measured by the UPDRS III using the Pearson’s correlation coefficient (p<0.05); and to (2) the specific physical activities as measured by the wearable bracelet using the Pearson’s correlation coefficient (p<0.05).

Clinical outcomes
We collected the total UPDRS III and the UDysRS (part 3 and 4) five times during the whole experimental session: once at baseline, twice when the patient was in peak dose (Med ON, about 45-60 minutes after medication intake), and twice when the effect of medication ended (Med OFF, about 60-90 minutes after the peak dose). We used these data to compare the clinical outcomes in the two days, and establish whether aDBS was effective.

As well as for technical outcomes, we verified (1) distribution normality through the single-sample Shapiro-Wilk test (p>0.05), to allow the use of parametric statistics, and (2) the lack of carry over effects between the two days by comparing the baseline values (Med OFF, Stim OFF) of the two days through a paired t-test (p<0.05). Both tests confirmed the hypotheses.

Three main factors could influence the values of clinical data: the day of the experimental session (Day 1, no aDBS; Day 2, with aDBS); the medication condition (Peak dose, Med ON vs End dose, Med OFF); and the levodopa administration (administration 1, first morning dose; administration 2, second daily dose). For this reason, we run a three-way Analysis of Variance (ANOVA) with factors “day” (2 levels, Day 1 and Day 2), “condition” (2 levels, Med ON and Med OFF), and “administration” (2 levels, administration 1 and administration 2). When the three-way ANOVA showed a significant effect (p<0.05) for the factors “therapy” and “condition” and for their interaction, we proceeded applying post-hoc tests (Tukey’s HSD, p<0.05 and Bonferroni corrected t-tests for multiple comparisons, p<0.0125). Values in the text are given as mean±SE.

Finally, to test the ability of our beta-band LFP-based aDBS to follow the patient’s clinical state, we (1) calculated the correlation between the beta band power and the UPDRS III score (Spearman’s correlation coefficient, p<0.05), and (2) we compared the percentage stimulation amplitudes delivered in the Med ON and in the Med OFF conditions, using a paired t-test (p<0.05). Statistical analyses were performed using Statistica Software (version 5·5, StatSoft Inc.).

Study conduct
This was an open-label, exploratory, investigator-initiated study and was not registered on clinicaltrials.gov. The objective of the study was to verify the possibility to deliver aDBS in freely-moving patients and for longer periods of time using an external prototype, and did not have any regulatory purpose. The study was approved by the Institutional Review Board of the Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico of Milan.

Even though specific monitoring procedures were applied, case report forms (CRFs) were completed for all subjects who signed Informed Consent, even if the subject failed to complete the study. No section of the CRFs was left blank without an appropriate explanation by the investigators. Paper-based CRFs are kept at the Neurophysiology Unit of the Fondazione IRCCS Ca’ Granda Ospedale Maggiore Policlinico of Milan, where the experiments were conducted. Adverse events (AEs) were collected and classified according to whether they were causally related to the experimental procedure, the device, or to aDBS. Each AE was recorded as being severe, moderate, or mild⁴.

The investigators were responsible for severe AE management and, in the case of unexpected serious AE, would have reported it to the Institutional Review Board.
References


