SUPPLEMENTAL MATERIALS AND METHODS

Experimental design

The aim of this study was to develop a model of STN DBS in hemiparkinsonian mice, and to evaluate the parameter space for effective STN DBS in this model. Our hypothesis, based on pilot studies, was that the parameter space would mirror the space described for human PD patients and depend largely upon the frequency of stimulation rather than pulse width or current amplitude. Experiments were designed based on power analyses that indicated a minimum of 10 mice would be necessary to detect behavioral differences between effective and ineffective stimulation parameters. Stimulation parameter order was randomized across all mice and predefined quantitative measures of motor performance were used to assess changes in parkinsonian symptoms. Though we tested 11 parkinsonian mice in this study, postmortem tissue for anatomical confirmation of the stimulation site could not be recovered from two mice, and thus their behavioral data was excluded from the study. To verify that 1- and 10-minute stimulation epochs evoked similar increases in movement, an additional cohort of 7 mice were evaluated. A third cohort of 5 mice was used in Figure 3B-D to determine how velocity scales with pulse width or current while all other parameters are held constant.

Surgical procedures

All surgical procedures were performed stereotactically on 3-6 month old C57BL/6 mice (Jackson Laboratory). Anesthesia was induced with intraperitoneal (IP) injection of ketamine/xylazine (0.1mL, 1mg/mL) and maintained with 0.5-1% inhaled isofluorane. The neurotoxin 6-hydroxydopamine (6-OHDA, 1μL, 5mg/mL in normal saline) was injected unilaterally in the medial forebrain bundle (MFB, -1.0 AP, -1.0 ML, 4.9 DV). Desipramine (0.2mL, 2.5mg/mL) was injected intraperitoneally (IP) just prior to surgery to reduce uptake by serotonergic and noradrenergic neurons in the MFB. This procedure resulted in near-complete loss of ipsilateral dopaminergic innervation of the striatum and severe hemiparkinsonism. After surgery, animals received analgesic agents and IP saline. During the first week of recovery, cages were placed on a heating pad and animals received supplementary IP saline, gel nutritional supplements, and softened food. A minimum of two weeks following dopamine depletion, mice were implanted with a 6-lead bipolar stimulating electrode array in the ipsilesional STN (-1.4 AP, -1.65 ML, 4.5 DV). Mice recovered for at least 1 additional week before stimulation testing began.

Electrode fabrication

Six-lead bipolar stimulating electrodes were constructed by twisting together and heating 6 stainless steel 76.2μm coated wires (A-M Systems). The six untwisted ends were then stripped using a razor blade and pressure fit into female Millmax connectors. Each electrode was tested for short circuits prior to implantation.

Electrical stimulation

An isolated constant current bipolar stimulator (WPI) was used to deliver electrical stimuli. The stimulator had a maximum voltage supply of >100 volts, a rise time of 6μs, and a built-in alarm indicating when the voltage limit was exceeded. We calculated that even at the highest settings used here we would not reach more than 80% of the total voltage available (ensuring accurate current delivery), a conclusion supported by the fact that the built-in voltage limit alarm did not sound during any of the experiments. The timing of stimuli was controlled by TTL input from an Arduino. Maximum current amplitude was set at 400μA, based on pilot experiments in which seizures occurred in some mice at or above this level.

Optimal stimulation electrode determination

Optimal stimulation electrode pairs were determined following STN DBS implantation based on the behavioral response of each pair in the open field setting. Each of the 3 electrode pairs were stimulated at standard settings (200μA, 60μs pulse-width, 120Hz) for 1 minute, flanked by 1 minute stim-off rest periods. The electrode pair eliciting the largest increase in velocity during stimulation epochs was used for all subsequent studies (Supplemental Figure 1D). In one mouse, a short developed between its optimal electrode pair in the interval between early and late stimulation phases, so the electrode pair was switched during the late phase to another pair that had shown similar efficacy to the optimal pair during initial characterization.

Open field behavior

Following optimal electrode pair selection, efficacy of STN DBS in parkinsonian mice was assessed in the open field (a transparent acrylic cylinder with a diameter of 25cm) during 11-minute trials in which 1-minute epochs of stimulation were alternated with 1-minute epochs of no stimulation (rest). Every trial began and ended with a rest epoch, resulting in a total of 5 stimulation epochs and 6 rest epochs per trial. The parameters of stimulation were

consistent within each trial and varied randomly between trials. Possible parameter combinations were chosen from a list of 81 parameters, half of which were pseudorandomly generated (current drawn from 0 to 300μA, frequency drawn from 0 to 200Hz, pulse width drawn from 0 to 120μs, all in integer increments) while the other half were deliberately selected. All mice received at least 1 trial with each parameter. A composite metric, p_i^{combo}, was calculated for each parameter combination using the formula p_i^{combo}= [current^2 * frequency * pulse width] (1).

Dyskinesia during stimulation and rest epochs was quantified using a modified version of the abnormal involuntary movements (AIM) scoring method (2) . Dyskinesia was monitored online by one unblinded rater, and a subset of videos (222 minutes) were re-scored offline by one blinded rater to ensure low inter-rater variability (average difference between raters' individual AIM scores = 0.05 +/- 0.009). Dyskinesia was quantified in one-minute increments during each trial, with axial, limb, and orofacial body segments rated on a scale of 0-3 each. A score of 0 indicates no abnormal movement, while a score of 3 indicates abnormal movements during the entire minute-long epoch. The scores are then summed, for a maximum score of 9 per epoch.

Following each trial, video-tracking software (Noldus Ethovision) was used to calculate the velocity, percent time moving, and rotational bias of each mouse (calculated as rotational bias = ipsilateral rotations / (ipsilateral rotations + contralateral rotations), and all metrics were compared between stimulation and rest epochs. As the dyskinesias observed in our study largely did not provoke changes in the center of mass of the mouse they did not contaminate the calculation of velocity and thus no additional processing was required to separate the two.

The onset of stimulation-induced changes in velocity was defined as the first time point during a stimulation bout in which the velocity was three standard deviations above the mean of the pre-stim period. The offset of stimulation-induced changes in velocity was defined as the first time point during each post-stimulation period in which velocity returned to the mean of the respective pre-stim period.

All mice initially experienced 31 of the 81 parameters within a 1 to 3-week period, approximately 1 month following implantation (early DBS). They then experienced the remaining 50 parameters during a 3-week period, approximately 3 months following implantation (late DBS), in order to assess changes in DBS efficacy.

For 10-minute stimulation epochs performed in a separate cohort of 7 mice (Supplemental Figure 1E), trials lasted for 30 minutes and consisted of a 10-minute pre-stim period, followed by a 10-minute stim period, and concluded with a 10-minute post-stim period. To account for habituation-related declines in spontaneous

movement for these longer sessions, velocity during 120Hz stimulation was normalized to the average velocity during a 30-minute no-stimulation trial. Standard error for this normalized velocity was calculated through propagation of error.

Locomotion in healthy mice

Open field locomotor data for healthy, nonparkinsonian controls was obtained from a separate cohort of mice with intracranial implants and headstage cables, so as to closely replicate the potential effects of tethering in STN DBS mice. Tracking data (Noldus) was analyzed in the same fashion as in parkinsonian mice: nonparkinsonian controls were only analyzed for movement metrics within the first 10 minutes of recording.

Human data

All human data were estimated from bar graphs provided by Moro, et al (3). In order to analyze human data in as similar a fashion as possible to the mouse data collected here, only parameter combinations in which all 12 patients had participated were used. Additionally, only parameters that fell within a range equivalent to those tested in mice were used (frequency between 0 and 200Hz and pulse width between 0 and 120μs). For determining voltage range (since our mice were tested using constant current), we used a range from 0V to twice as high as the patient average for current clinical settings (since our maximum tested current, 400μA, was twice our estimate for standard human parameters, 200μA, and since current and voltage scale linearly). Thus, voltages between 0 and 6.2V were considered. To adjust p_i^{combo} for constant voltage rather than constant current, we used the formula p_v^{combo}= [voltage^2 * frequency * pulse width].

DBS model

The models for DBS efficacy were developed using the regressions generated in Figure 3E (for Supplemental Figure 3A-D) and Supplemental Figure 2C (for Supplemental Figure 3E-H) to calculate predicted velocity for parameters within the space shown (current from 0-400μA in 20μA increments, frequency from 0-200Hz in 10Hz increments, and pulse width from 0-200μs in 10μs increments).

Statistics

All behavioral data recorded with video tracking (Noldus Ethovision) was exported to Matlab (Mathworks) for offline analysis. Statistical differences between stimulation parameters were assessed using one-way repeated-measures ANOVA, followed by post hoc comparisons using Tukey's Honest Significant Difference procedure. $P_{adjusted}$ reported for ANOVAs is a p-value with the most conservative lower bound adjustment, as calculated by Matlab. Linear correlations and adjusted R-squared values were calculated in Matlab by fitting data to a linear model. The generalized linear model was created in Matlab using a normal distribution.

Histology

Accurate targeting of STN DBS electrodes and successful depletion of dopaminergic projections to the striatum were confirmed histologically following perfusion. Two mice, out of the original cohort of 11, were excluded from analysis as postmortem tissue (confirming electrode localization) could not be recovered. Mice were deeply anesthetized with IP ketamine/xylazine (1 ml, 1mg/mL). In order to aid in locating STN DBS electrode tips in postmortem tissue, we electrolytically lesioned the two leads used for behavioral experiments with 150μA of monopolar direct current for 5 seconds, just prior to transcardial perfusion with paraformaldehyde (PFA). The brain was dissected from the skull, post-fixed overnight in 4% PFA, then stored in 30% sucrose at 4°C. Brains were then sliced in 30 μm sections on a freezing microtome (Leica) and dopamine depletion was verified via tyrosine hydroxylase (TH) immunohistochemistry. Briefly, sections were washed in PBS (5x10 minutes) and blocked in normal donkey serum (NDS)/0.1% Triton-X (1hr at room temperature, RT), followed by incubation in primary antibody (Pel-Freez rabbit anti-TH, 1:1000 at 4°C overnight) and secondary antibody (donkey anti-rabbit 647 nm, Jackson Immunoresearch, 1:500 in NDS at RT for 2hrs). Following mounting on glass slides (Vectashield Mounting Medium), sections were imaged in the Cy5 (excitation 650nm, emission 684nm) channel and stitched fluorescence images were taken on a Nikon 6D conventional wide-field microscope at 4-10X, using custom software (UCSF Nikon Imaging Center). Coordinates of mouse DBS electrodes were determined from histological images using a standard mouse brain atlas (Paxinos and Franklin). Center coordinates for the dorsal border pyramidal tract were determined in a similar way, and distance between this point and the coordinates of the electrode for each mouse was calculated using Euclidean distance.

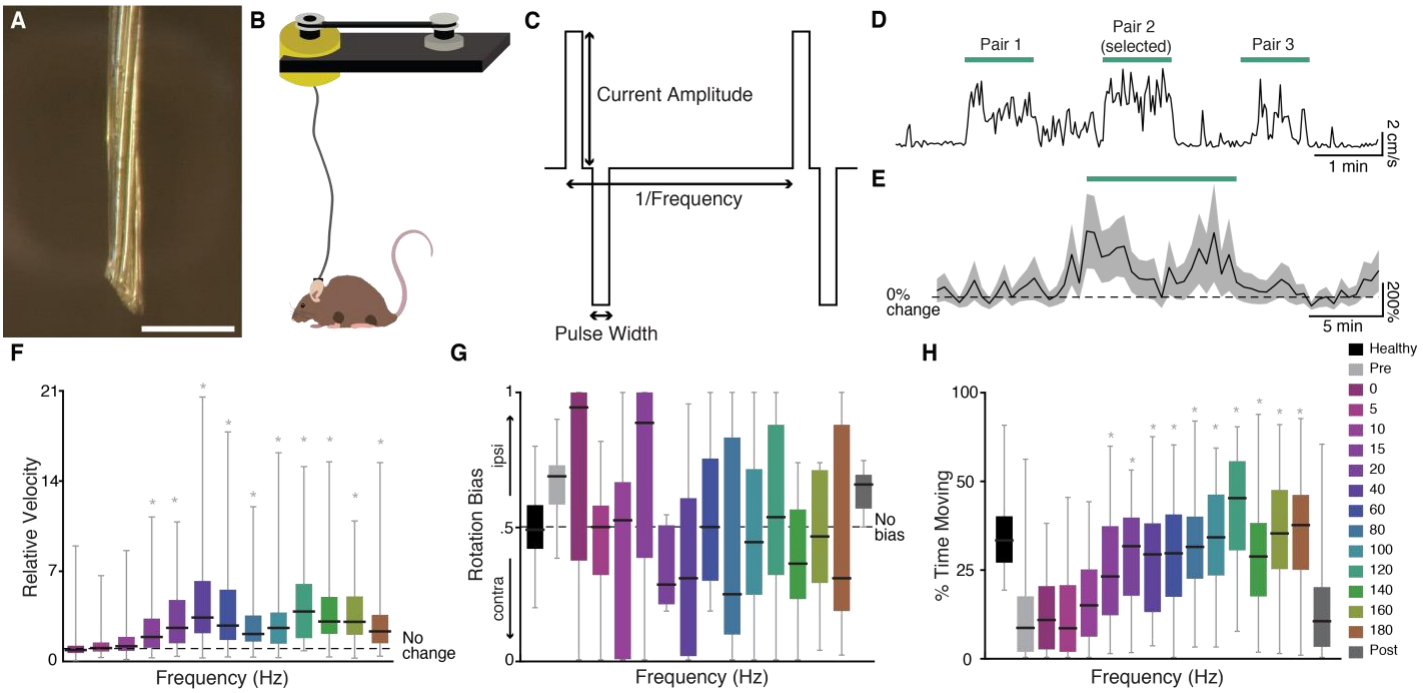
REFERENCES

1. Koss AM, Alterman RL, Tagliati M, Shils JL. Calculating total electrical energy delivered by deep brain stimulation systems. *Ann. Neurol.* 2005;58(1):168–168.

2. Cenci MA, Lundblad M. Ratings of L-DOPA-induced dyskinesia in the unilateral 6-OHDA lesion model of Parkinson's disease in rats and mice. *Curr. Protoc. Neurosci.* 2007;Chapter 9:Unit 9.25.

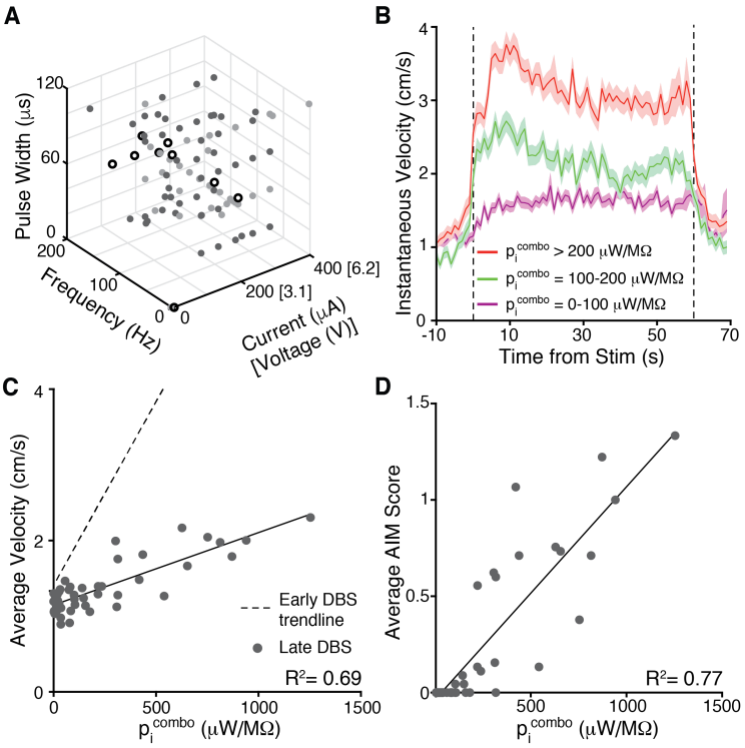
3. Moro E et al. The impact on Parkinson's disease of electrical parameter settings in STN stimulation. *Neurology* 2002;59(5):706–713.

SUPPLEMENTAL FIGURES



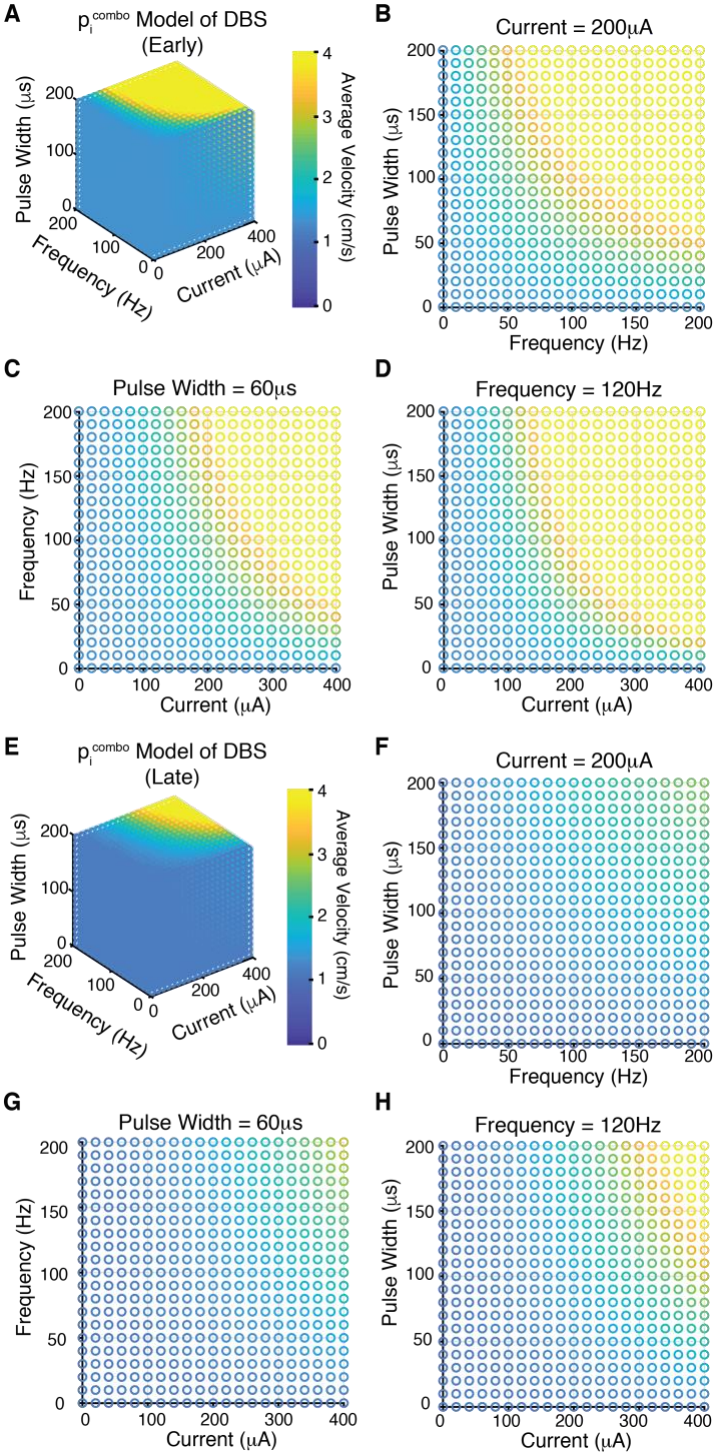
Supplemental Figure 1. STN DBS improves multiple movement metrics in hemiparkinsonian mice.

A) Photograph of DBS electrodes implanted in parkinsonian mice (scale bar = 1mm). **(B)** Schematic of experimental behavioral setup, including custom motorized commutator. **(C)** Diagram of bipolar, biphasic stimulation delivered during experimentation. **(D)** Sample raw velocity trace for optimal electrode pair determination. **(E)** Normalized velocity during 10 minute stimulation epochs (green bar). Dark line indicates averages, while lighter shading indicates +/- SEM (N=7). **(F-H)** Relative velocity (F), rotational bias (G), and percent time moving (H) of parkinsonian mice during stimulation (1min) at the denoted frequencies, with pulse width (60μs) and current (200μA) held constant. Pre and post refer to 30 seconds before and after stimulation. Box extends from 25th to 75th percentile, median is indicated by horizontal line. Whiskers represent max and min values. (N=9 for healthy, N=9 for parkinsonian). Significance determined by one-way repeated measures ANOVA followed by Tukey's Honest Significant Difference test, * p<.05 compared to pre-stim period. N=mice.



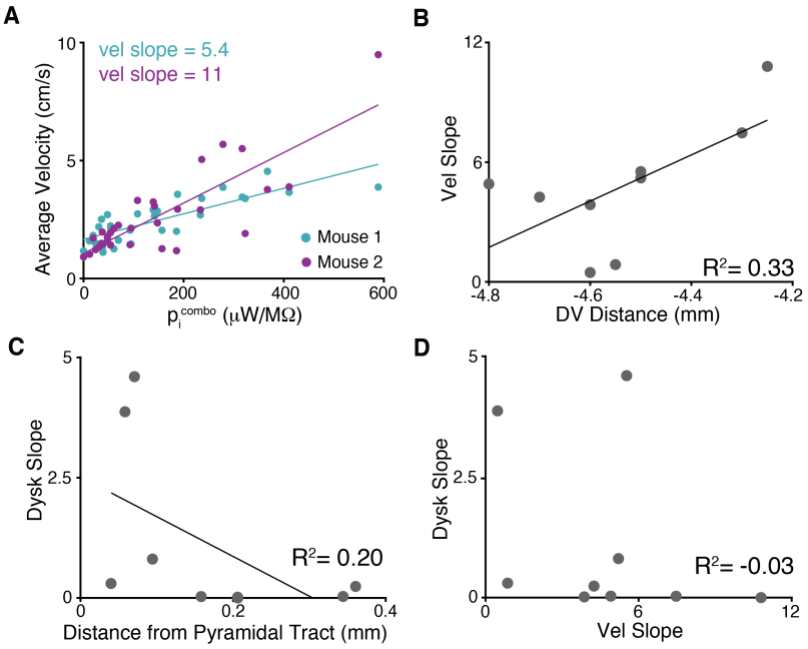
Supplemental Figure 2. Both dyskinesia and DBS efficacy scale with p_i^{combo}.

(A) Distribution of early (31 total, light grey dots) and late (50 total, dark grey dots) mouse and human (9 total, open circles) DBS parameters. **(B)** Instantaneous velocity during stimulation for parameters within the indicated p_i^{combo} ranges. Dark lines indicate averages, while lighter shadings indicate +/- SEM. **(C)** Correlation of velocity with p_i^{combo} (50 conditions, N=9) in late DBS (~2 months after early DBS). **(D)** Correlation of average AIM score with p_i^{combo} (50 conditions, N=9). N=mice. Each point represents an average across subjects and trials for a given condition.



Supplemental Figure 3. Modeling DBS using p_i^{combo} predicts the therapeutic parameter space.

(A) 3-D representation of STN DBS parameter space based on the early regression generated from data in Figure 3E. **(B-D)** Cross-sections of STN DBS parameter space from (A) at constant current (B), pulse width (C), and frequency (D). **(E)** 3-D representation of STN DBS parameter space based on the late regression generated from data in Supplemental Figure 2C. **(F-H)** Cross-sections of STN DBS parameter space from (E) at constant current(F), pulse width (G), and frequency (H).



Supplemental Figure 4. Movement velocity and dyskinesia relationships to p_i^{combo} by stimulation sites across individual mice.

(A) Correlations between velocity and p_i^{combo} in two sample mice (31 conditions) illustrating differences in the slope of the correlation (vel slope). **(B)** Correlation between dorsoventral (DV) stimulation location and vel slope across individual mice (N=9). **(C)** Correlation between the stimulation site-pyramidal tract distance and dysk slope across individual mice (N=9). **(D)** Correlation between vel slope and dysk slope across individual mice (N=9). N=mice.

Current (µA)	Frequency (Hz)	Pulse Width (µs)
100	100	40
100	120	60
100	120	120
150	120	60
175	20	50
175	40	50
200	5	60
200	10	60
200	10	120
200	15	60
200	20	60
200	40	60
200	60	60
200	60	100
200	80	60
200	100	60
200	120	20
200	120	40
200	120	60
200	140	60
200	160	60
200	180	60
225	80	80
250	10	60
250	60	40
250	140	70
300	5	120
300	10	60
300	10	120
400	1	120
0	0	0

Supplemental Table 1. Early DBS Parameters.

Table showing the parameters used for early DBS testing.

Current (µA)	Frequency (Hz)	Pulse Width (µs)
10	88	46
10	170	113
13	19	99
35	100	116
42	84	110
83	109	115
100	10	30
100	10	60
100	10	90
100	10	120
100	120	30
100	120	90
102	117	27
118	131	20
147	89	78
150	10	30
150	10	60
150	10	90
150	10	120
150	120	30
150	120	90
150	120	120
200	10	30
200	10	90
200	120	30
200	120	90
204	131	19
204	152	89
209	63	114
212	6	33
213	151	33
230	159	22
238	192	79
245	182	15
250	10	30
250	10	90
250	10	120
250	120	30
250	120	60
250	120	90
250	120	120

274	127	11
288	97	96
290	31	117
300	10	30
300	10	90
300	120	30
300	120	60
300	120	90
300	120	120

Supplemental Table 2. Late DBS Parameters.

Table showing the parameters used for late DBS testing.

Voltage (V)	Frequency (Hz)	Pulse Width (μs)
1	155	65
2	155	65
3.1	5	65
3.1	50	65
3.1	130	65
3.1	185	65
0	0	0
3.5	155	65
3.1	155	60

Supplemental Table 3. Human DBS Parameters.

Table showing the parameters used in human DBS testing.