**Population-Specific Neuromodulation Prolongs Therapeutic Benefits of Deep Brain Stimulation**

**One-Sentence Summary:** Electrical stimulation protocols, tailored for cell-type specificity, improve the therapeutic efficacy of deep brain stimulation.

Teresa A. Spix1,3, Shruti Nanivadekar3,4,5, Noelle Toong2, Irene M. Kaplow2,3,Brian R. Isett1,3, Yazel Goksen1, Andreas R. Pfenning1,2,3, Aryn H. Gittis1,3,\*

1Biological Sciences, 2Computational Biology, and 3Neuroscience Institute Departments, Carnegie Mellon University; Pittsburgh, Pennsylvania, USA.

4Center for the Neural Basic of Cognition, Carnegie Mellon University; Pittsburgh, Pennsylvania, USA.

5University of Pittsburgh School of Medicine; Pittsburgh, Pennsylvania, USA.

\*Corresponding author. Email: agittis@cmu.edu

Symptoms of neurological diseases emerge through the dysfunction of neural circuits whose diffuse and intertwined architectures pose significant challenges for delivering therapies. Deep brain stimulation (DBS) improves Parkinsonian symptoms acutely but does not differentiate between neuronal circuits, and its effects decay rapidly if stimulation is turned off. Recent findings suggest that optogenetically manipulating distinct neuronal subpopulations in external globus pallidus (GPe) provides long-lasting therapeutic effects in dopamine depleted mice. We used synaptic differences to excite parvalbumin-GPe and inhibit lim-homeobox-6-GPe neurons simultaneously using brief bursts of electrical stimulation. In dopamine depleted mice, circuit-inspired DBS provided long-lasting therapeutic benefits that far exceeded those induced by conventional DBS, extending for hours beyond stimulation. These results establish the feasibility of transforming knowledge about circuit architecture into translatable therapeutic approaches.

Identification of distinct neuronal subpopulations has been essential for understanding brain function. Interventions that target specific neuronal subpopulations, such as optogenetics, can alleviate disease symptoms with few side effects in animal models, but clinical approaches in humans are rarely tailored to achieve population-specific neuromodulation. Electrical deep brain stimulation (DBS) is increasingly used to treat an expanding list of human conditions. While theoretical work has long hypothesized that patterned electrical stimulation can improve its precision (*1*–*5*), these approaches have not yet achieved the desired cell-type specificity. Motor symptoms of Parkinson’s disease (PD) are acutely responsive to DBS, but symptoms return quickly when stimulation is turned off (*6*). The need for constant stimulation increases the risk of side effects and drains battery power. Using optogenetics in mice, we identified cellular nodes in the external globus pallidus (GPe) of the basal ganglia where targeted interventions ameliorate circuit dysfunction and restore movement for hours beyond stimulation (*7*). This optogenetic approach requires genetically targeted tools to drive opposite firing responses of spatially intermixed GPe subpopulations: parvalbumin-expressing (PV-GPe) neurons must be excited while lim-homeobox-6 (Lhx6-GPe) neurons must be suppressed. Refining DBS protocols to achieve the same degree of population-specific neuromodulation would provide a human-translatable treatment approach that could extend the therapeutic duration of stimulation, representing a major therapeutic advance.

To gain insights into the cellular principles that dictate the responses of PV-GPe and Lhx6-GPe neurons to electrical stimulation, we recorded from these populations in acute brain slices from Lhx6-GFP+/-; PV-tdTmt+/-  mice (Fig. 1A, S1A). Both populations were similarly excited during continuous electrical stimulation (100 Hz/30 s) (Fig. 1B-C). However, synaptic recordings showed that Lhx6-GPe neurons receive proportionately more inhibition than PV-GPe neurons (Fig. 1D, S1B-C). This synaptic asymmetry was even more pronounced at high stimulation frequencies, where rapid synaptic depression was observed (Fig. 1E-F). Notably, Lhx6-GPe neurons experienced significantly greater inhibitory charge than PV-GPe neurons during the 1st second of stimulation (Fig. 1G).

To determine whether these synaptic asymmetries could be leveraged to drive population-specific neuromodulation, we performed sequential extracellular/intracellular recordings. Firing responses of PV-GPe and Lhx6-GPe neurons were heavily influenced by the dynamics of their underlying synaptic inputs (Fig. 1H). Large inhibitory currents in Lhx6-GPe neurons were well-correlated with transient suppressions in extracellular firing rates that diminished as inhibitory synapses depressed. In contrast, PV-GPe neurons, which lacked large inhibitory currents, were instantly excited by stimulation.

Extracellular recordings revealed additional differences between Lhx6-GPe and PV-GPe neurons after stimulation initiation. Lhx6-GPe neurons were routinely inhibited during the first 1 s of stimulation but less so in subsequent time bins. Some neurons even switched from being inhibited to being excited within 3 seconds of continuous stimulation (Fig. 1I, S1D). In contrast, PV-GPe neurons were typically excited throughout (Fig. 1I, S1D). The degree to which firing responses of PV-GPe and Lhx6-GPe neurons could be separated during stimulation was calculated as a ‘population separation index’ (PSI) (Fig. 1I, *see methods*). PSI was maximal during the first 1 s of stimulation and decayed over time, which was largely due to lessening suppression of Lhx6-GPe neurons (Fig. 1I).

These results led to the hypothesis that delivering stimulation in short bursts, rather than continuously, could achieve population-specific neuromodulation in the GPe. We therefore delivered repeated bursts (100 Hz/1 s) to pairs of Lhx6-GPe and PV-GPe neurons. This stimulus pattern drove robust and reproducible suppression of Lhx6-GPe neurons and simultaneous excitation of PV-GPe neurons (Fig 1J-K).

To identify the range of parameter combinations that separate the responses of PV-GPe and Lhx6-GPe neurons, we used an iterative machine learning approach (*Methods*). Experimental data (Fig. 2A) were used to train models to predict the potentially non-linear relationships between parameter combinations and firing responses of PV-GPe and Lhx6-GPe populations. In the first iteration, a data set of 208 unique burst combinations (Table S1), collected across 19 pairs of Lhx6-GPe and PV-GPe neurons, was used to train Gaussian process regressions with quadratic polynomial kernels for each population. This approach yielded better leave-one-out cross validation performance than other approaches (Table S2). Parameters were then sampled (Table S3) and evaluated to identify ranges of parameters predicted to yield a large PSI. This range was then used to collect a second iteration of data (Table S4), which further refined the models (Fig. S3A-B). Because there was strong convergence of the predictions between the first and second iterations of the models, no additional iterations were run. Surface plots with modulation factor predictions for PV-GPe and Lhx6-GPe populations across many thousands of artificially generated parameters, as well as the predicted PSI across the parameter space, are shown in Fig. 2B & S2A.

As predicted by our model, 100 Hz/1 s bursts, delivered in brain slices, were highly effective at segregating the responses of PV-GPe and Lhx6-GPe neurons at a stimulus intensity of 2.5x, but less effective at weaker stimulus intensities (Fig. 2C-E). Furthermore, we improved our experimentally-measured PSI by using shorter, higher frequency bursts (175 Hz/200 ms, Fig. 2B, C, F). The model also accurately predicted the boundaries of parameter combinations that could dissociate the responses of PV-GPe and Lhx6-GPe neurons. For example, reducing intraburst frequency to 50 Hz (50 Hz/200 ms) weakened the PSI but did not eliminate it entirely (Fig. 2B, C, G), whereas increasing the burst duration (175 Hz/10 s) prevented stimulation from driving population-specific responses (Fig. 2B, C, H). Deviations of experimental outcomes from model predictions were quantified with root mean squared error (Fig. S3C-D, Table S5).

Given the critical role of inhibitory synaptic transmission in differentiating the responses of PV-GPe and Lhx6-GPe neurons we sought to identify the mechanism underlying its cell-type specificity. To test the hypothesis that Lhx6-GPe inhibition was driven by the local GPe collateral network, engaged by excitation from the subthalamic nucleus (STN) (*8*, *9*), we selectively stimulated STN afferents using optogenetics. The fast channelrhodopsin variant, Chronos, enabled stimulation of STN fibers at high frequencies (*10*). However, 1 s bursts of STN stimulation (100 Hz) excited both populations of GPe neurons to a similar degree (Fig. 3A,E).

Next, we tested the hypothesis that inhibition from the striatum (*11*), was weighted more heavily towards Lhx6-GPe neurons than PV-GPe neurons. Chronos was globally expressed in the striatum (Fig. 3B-C). Optogenetic stimulation (100 Hz/1 s) of striatal afferents in the GPe similarly inhibited both PV-GPe and Lhx6-GPe neurons (*12*–*14*) (Fig. 3B,E).

These results seemed to suggest that striatal inputs lack the cell-type specificity needed for population-specific neuromodulation. However, considering the placement of our stimulating electrode (Fig. 3C), electrical stimulation was not likely to engage all striatal cell types equally. Instead, electrical stimulation was likely biased towards antidromic activation of D1-type dopamine receptor SPNs (D1-SPNs), which are not a canonical source of inhibitory input to the GPe, but do make some synaptic contacts (*12*, *15*–*18*). To test the hypothesis that striatal afferents from D1-SPNs preferentially inhibit Lhx6-GPe neurons, Chronos expression was restricted to D1-SPNs (Fig. 3D) in the striatum. This time, optogenetic stimulation of D1-SPN afferents differentiated the responses of GPe neurons, driving robust inhibition of Lhx6-GPe neurons but not PV-GPe neurons (Fig. 3D,E).

These results suggested that the circuit mechanism through which electrical stimulation drives population-specific neuromodulation was through the converging effects of

excitatory inputs from the STN and inhibitory inputs from D1-SPNs. We therefore assessed the effects of blocking each input, systematically, during electrical stimulation. Blocking excitatory inputs eliminated the excitation of PV-GPe neurons during stimulation and unmasked an even stronger inhibition of Lhx6-GPe neurons (Fig. 3F,H). Conversely, chemogenetic suppression of D1-SPN fibers during electrical stimulation blocked the inhibitory response of Lhx6-GPe neurons, unmasking an excitatory effect (Fig. 3G,H).

Finally, to test whether an electrical DBS protocol, tailored to drive population-specific neuromodulation, would mimic the persistent therapeutic effects induced by cell-type specific optogenetic manipulations *(7)*, we compared the therapeutic efficacy of ‘conventional’ vs. ‘burst DBS’ (*Methods)* in mice rendered parkinsonian by bilateral injections of the toxin 6-hydroxydopamine (6-OHDA) into the medial forebrain bundle (MFB) (Fig. 4A, *Methods*). Burst DBS consisted of short bursts, delivered once a second, with a duration of 200 ms and an intraburst frequency of 175 Hz. Recordings in brain slices confirmed that the population-specificity of this protocol was retained when delivered with a biphasic stimulating electrode, as required *in vivo,* both for naïve tissue and in tissue from dopamine-depleted (DD) mice (Fig. S4A-B).

The therapeutic effects of ‘burst’ vs. ‘conventional’ DBS were then compared using a cross-over study design with a one-day washout period in between treatments in DD mice (Fig. 4C), using an approach shown to recapitulate a number of features of DBS in humans (19). Six-lead DBS electrodes were implanted bilaterally near the entopeduncular nucleus (EPN) (Fig. 4B, S5), an electrode placement often traversed in human patients receiving DBS in the internal globus pallidus (GPi) (*20*, *21*) and an area where fibers from the STN and D1-SPNs can be co-activated.

Before stimulation, DD mice were highly immobile. Both ‘conventional’ and ‘burst’ DBS reduced this immobility, enabling mice to move around the arena during stimulation (Fig. 4D-F, *pre/DBS*, Video S1-2). After receiving conventional DBS, mice quickly drifted back to the immobile state (Fig. 4D-H). Conversely, after receiving burst DBS, mice continued to move around the arena (Fig. 4D-H), with 5 out of the 8 mice still moving at the end of the trial, ~2.5 hours after stimulation (Fig. 4I-J, *90’+*, Video S1-2). On average, the therapeutic effects of burst DBS persisted >4.5-fold longer than those of conventional DBS (Fig. 4I), a conservative estimate as only 37% of mice had returned to the immobile state at the end of the trial (Fig 4J). DBS protocols predicted to be less effective at dissociating the responses of PV-GPe and Lhx6-GPe neurons were less effective at inducing persistent rescue (Fig. S5).

These results demonstrate how fundamental knowledge about the organization and function of basal ganglia circuitry can be used to refine the population specificity of electrical stimulation, ultimately prolonging the therapeutic benefits of DBS beyond that achieved with conventional methods. Other alternative forms of DBS, including ‘adaptive’ and ‘coordinated reset’ DBS (*22*–*25*), can induce persistent therapeutic effects with the use of specialized implants, and it is plausible that the long-lasting effect observed in our study shares a common underlying mechanism with these approaches. However, our ‘burst’ DBS protocol can be delivered through commonly used DBS implants and falls within FDA-approved stimulus frequencies, enabling immediate testing in PD models across species, including human patients. This work joins the growing field of opto-inspired DBS (*26-28*), where fundamental discoveries about the organizing principles of neural circuits, gained through the use of optogenetics, guide the development of more robust electrical DBS approaches that can be rapidly translated to humans.

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Visualization: AHG, TAS, BRI, SN

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Project administration: AHG

Writing: AHG, TAS, SN, IMK, NT

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**Supplementary Materials:**

Materials and Methods

Figs. S1 to S6

Tables S1 to S6

Captions for Movies S1 to S2

References (*29*–*33*)

Movies S1 to S2

**Fig. 1: Bursts of stimulation segregate the firing responses of PV-GPe and Lhx6-GPe neurons. (A)** Recording configuration and fluorescent identification of GPe subpopulations. **(B)** Extracellular traces during 100 Hz/30 s stimulation after artifact removal**. (C)** Stimulation at 100 Hz for 30 s modestly excited both populations, quantified as ‘Modulation Factor’ (MF) for each neuron: (FRstim- FRbaseline)/(FRstim + FRbaseline) (*Bars*: MFLhx6= 0.16 ± 0.11; MFPV=0.22 ± 0.12; Mann-Whitney U, MWU p = 0.29; 12 pairs of neurons; 6 mice, error bars: sem). Throughout, *colored symbols* *=* neurons significantly modulated during stimulation (paired t-test, p < 0.05); *gray symbols* = neurons not significantly modified. **(D)** Inhibition-excitation ratios, using peak current amplitudes ([IPSC] / [IPSC + EPSC]), showed Lhx6-GPe neurons receive more inhibition than PV-GPe neurons (KW, χ2(7) = 57.4, p < 0.00001, post-hoc pairwise MWU, \*p < 0.05, \*\*p < 0.001; 15 pairs; 9 mice). Insets show currents from representative neurons. **(E-F)** Rapid depression of synaptic currents during 100 Hz stimulation (1 mA), from representative neurons (E) and across the population (F) (10 pairs; 4 mice; medians, error bars: sem). **(G)** Synaptic charges (IPSQ and EPSQ) transferred during the 1st, 2nd, and 3rd seconds of stimulation. IPSQ was larger in Lhx6-GPe neurons than PV-GPe neurons in the first second of stimulation (IPSQLhx6 = 284 ± 300 pC, IPSQPV = 98 ± 103 pC, MWU, \*p = 0.023) (10 pairs; 4 mice). **(H)** Three-part recordings with 3 s of 100 Hz stimulation. *Bottom*: Extracellular recording traces. *Middle-Top,* Whole-cell recordings of EPSCs *(*'Exc', *middle*, Vhold = -70 mV), andIPSCs ('Inh', *top,* Vhold = 0 mV) **(I)** *Top*: MF by cell type in 1 s time bins. *Bottom*: Modulation was most segregated in the 1st second of stimulation [‘Population Separation Index’ (‘PSI’=MFPV - MFLhx6, *see methods*)] (PSI0-1s = 1.27 ± 0.49; PSI1-2s = 0.77 ± 0.54; PSI2-3s = 0.55 ± 0.49; KW, χ2(2) = 12.0, \*p = 0.003; 14 pairs; 3 mice). **(J)** Raster plots of extracellular modulation by 1 s bursts of stimulation (100 Hz, 1 mA, 30 s inter-trial interval). **(K)** PV-GPe neurons are excited while Lhx6-GPe neurons are inhibited (MFPV = 0.5 ± 0.1; MFLhx6 = -0.7 ± 0.3; ; 14 pairs; 3 mice).

**Fig. 2: Stimulus optimization using a machine learning approach accurately predicts burst designs that achieve population-specific neuromodulation in the GPe using electrical stimulation. (A)** Samples of neural response data used to train models (*Methods*). **(B)** Surface plots (2.5x intensity) showing predicted FR responses of Lhx6-GPe (MFLhx6, *left*) and PV-GPe (MFPV, *middle*) populations across a range of stimulus parameters. Predicted increases in FR are shown in red and predicted decreases in FR are shown in blue. (*Right*) Surface plot showing the degree to which stimulus parameters are predicted to segregate the responses of PV-GPe and Lhx6-GPe populations (PSI, red = more segregation; blue = less segregation). Lettered points indicate burst combinations selected for experimental validation (D-G). **(C)** Experimentally measured responses of PV-GPe and Lhx6-GPe populations to burst protocols developed using our model (B). The degree to which burst designs segregated the responses of PV-GPe and Lhx6-GPe neurons (PSI) is shown to the right, with letters on right referencing matching panel letter. Error bars, sem. (**D-H)** Experimental validation of model predictions. *Left*: Rasters of extracellularly recorded FR from Lhx6-GPe (*blue*) and PV-GPe (*red*) neurons responding to burst designs drawn from regions of parameter space indicted in B. Shaded boxes denote time bin used to calculate modulation factors. *Middle*: Modulation factors for each neuron (averaged across 5 trials), plotted against its baseline firing rate (14 pairs of neurons; 4 mice). *Gray symbols:* Neurons in which firing rate was not significantly modified from baseline (p > 0.05, paired t-test FRbaseline vs. FRstim, Trials 1-5). Vertical markers show average MFLhx6 and MFPV, error bars: sem. *Right*: Bar graphs indicating the percentage of Lhx6-GPe and PV-GPe neurons that were excited (‘*Exc’*), inhibited (‘*Inh’*), or had no significant change in firing rate (‘*Insig’*) during stimulation.

**Fig. 3: Population-specific neuromodulation in the GPe is driven by convergent excitation from the STN and inhibition from D1-SPNs. (A)** *Left:*Schematic and rasters from two representative GPe neurons during optogenetic stimulation of STN fibers (100 Hz/1 s). *Right****:***Modulation factors (MFs) for individual neurons (*symbols*) and populations (*vertical bars*) show PV-GPe and Lhx6-GPe neurons are similarly excited (MFLhx6 = 0.36 ± 0.27, MFPV = 0.34 ± 0.17; Mann Whitney U, MWU p = 0.976; 17 pairs of neurons; 3 mice). **(B)** *Left:*Schematic and rasters from two neurons during striatal fiber stimulation (100 Hz/1 s). *Right****:***MFs show both PV-GPe and Lhx6-GPe neurons are similarly inhibited by striatal fiber stimulation (MFLhx6= -0.77 ± 0.29, MFPV: -0.59 ± 0.41; MWU p = 0.25; 16 pairs; 2 mice). **(C)** *Top:* Fluorescent image of striatopallidal pathway. *Bottom*: Fluorescent image of striatonigral pathway. Typical placement of the stimulating electrode is shown for reference. (**D)** *Left:*Schematic and rasters from two neurons during D1-SPN striatal fiber stimulation. *Right****:***MFs show Lhx6-GPe neurons are preferentially inhibited (MFLhx6 = -0.68 ± 0.34; MFPV = -0.1 ± 0.23; MWU \*p<0.00001; 27 pairs; 4 mice). **(E)** Summary ofMFLhx6 (*blue*) and MFPV (*red*) for experiments A-D, error bars: sem. **(F)** MFs in response to electrical stimulation before (*left*) and after (*right*) application of 10 µM NBQX/50 µM APV. Excitation of PV-GPe neurons was blocked (MFCtrl: 0.36 ± 0.05, MFNBQX/APV: 0.01 ± 0.07, paired t test, p = 0.0001), but not Lhx6-GPe inhibition (MFCtrl: -0.47 ± 0.66, MFNBQX/APV: -0.96 ± 0.05, paired t test, p = 0.18) (5 Lhx6-GPe neurons, 4 PV-GPe neurons; 2 mice). **(G)** MFs in response to electrical stimulation before (*left*) and after (*right*) chemogenetic inhibition of D1-SPN fibers (AAV2-hsyn-DIO-hM4D(Gi)-mCherry + CNO, *see methods*). Inhibition of Lhx6-GPe neurons was blocked (MFpre = -0.54 ± 0.39, MFCNO = 0.28 ± 0.33, paired t-test, p = 0.003), but excitation of PV-GPe neurons was not (MFpre = 0.57 ± 0.15, MFCNO = 0.59 ± 0.13, paired t-test, p = 0.7)(n = 10 Lhx6-GPe neurons, n = 8 PV-GPe neurons; N = 5 mice). **(H)** Summary ofMFLhx6 (*blue*) and MFPV (*red*) for experiments F-G. Error bars: sem.

**Fig. 4: Burst stimulation restores movement persistently in parkinsonian mice. (A**) Schematic showing bilateral dopamine depletion (DD, 6.43 ± 0.09% TH remaining compared to littermate controls). **(B)** Schematic showing DBS electrode placement near the EPN. **(C)** Crossover design illustrating pseudorandom experimental protocol where DD mice receive conventional or burst DBS on alternate days. **(D)** Average velocity of DD mice increased during conventional DBS *(gray)* but did not persist after stimulation (Pre: 0.54 ± 0.07, Stim: 0.94 ± 0.13, Post: 0.40 ± 0.06 cm/s)(Wilcoxon Signed Rank WSR: pre vs stim, +p = 0.055; stim vs 90’ \*p = 0.008; pre vs. 90’ p = 0.195, n = 8 mice). Average velocity increased during Burst DBS *(green)* and was still elevated 90 min after stimulation(Pre: 0.47 ± 0.09, Stim: 1.03 ± 0.19, Post: 1.77 ± 0.54 cm/s)(WSR: pre vs stim \*p =0.016; stim vs 90’ p = 0.195; pre vs. 90’ \*p = 0.008). Data from individual mice are shown and colors and symbols are consistent throughout. **(E)** Immobility of DD mice decreased during conventional DBS *(gray),* but did not persist (Pre: 75 ± 6 Stim: 51 ± 7, Post: 87 ± 4% time immobile)(WSR: pre vs stim, +p = 0.056 stim vs 90’ \*p = 0.008, pre vs 90’ = 0.195). Immobility decreased during burst DBS *(green)* and was still low 90 min after stimulation(Pre: 82 ± 6, Stim: 48 ± 7, Post: 45 ± 9 % time immobile)(WSR: pre vs stim, \*p = 0.016; stim vs 90’; p = 0.641; pre vs. 90’ \*p = 0.016). **(F)** Movement paths over 8 min intervals throughout a trial. Data are from the same mouse treated with conventional DBS (grey) or burst DBS (green). **(G-H)** Average movement velocities (G) and immobility (H) plotted for the duration of behavioral trials. Movement velocities were averaged over 30 s time bins, % immobility was averaged over 30 min time bins. *Grey*, Conventional DBS; *Green*, Burst DBS (n = 8 mice). **(I-J)** Amount of time after DBS was turned off before mice returned to pre-stim levels of immobility (‘therapeutic duration’). Therapeutic duration was significantly longer following burst DBS (123.8 ± 37.4 min, *green*) than following conventional DBS (37.5 ± 25.5 min, *grey*)(WSR, \*p = 0.012, n = 8 mice), shown for individual mice (I) and cumulatively across the population (J).