The effect of striatal dopaminergic grafts on the neuronal activity in the substantia nigra pars reticulata and subthalamic nucleus in hemiparkinsonian rats

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The electrophysiological correlates of parkinsonism in the basal ganglia have been well studied in patients with Parkinson’s disease and animal models. Separately, striatal dopaminergic cell transplantation has shown promise in ameliorating parkinsonian motor symptoms. However, the effect of dopaminergic grafts on basal ganglia electrophysiology has not thoroughly been investigated. In this study, we transplanted murine foetal ventral mesencephalic cells into rats rendered hemiparkinsonian by 6-hydroxydopamine injection. Three months after transplantation, extracellular and local field potential recordings were taken under urethane anaesthesia from the substantia nigra pars reticulata and subthalamic nucleus along with cortical electroencephalograms and were compared to recordings from normal and hemiparkinsonian controls. Recordings from cortical slow-wave activity and global activation states were analysed separately. Rats with histologically confirmed xenografts showed behavioural improvement measured by counting apomorphine-induced rotations and with the extended body axis test. Firing rates in both nuclei were not significantly different between control and grafted groups. However, burst firing patterns in both nuclei in the slow-wave activity state were significantly reduced (P < 0.05) in rats with large surviving grafts, compared to hemiparkinsonian controls. The neuronal firing entropies and oscillations in both nuclei were restored to normal levels in the large-graft group. Electroencephalogram spike-triggered averages also showed normalization in the slow-wave activity state (P < 0.05). These results suggest that local continuous dopaminergic stimulation exerts a normalizing effect on the downstream parkinsonian basal ganglia firing patterns. This novel finding is relevant to future preclinical and clinical investigations of cell transplantation and the development of next-generation therapies for Parkinson’s disease that ameliorate pathophysiological neural activity and provide optimal recovery of function.

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Introduction

Parkinson’s disease is a neurodegenerative disorder characterized by the progressive death of dopaminergic neurons in the substantia nigra pars compacta (SNC), and the loss of the nigrostriatal tract, the principal source of dopamine in the striatum. The resulting striatal dopamine depletion leads to significant changes of neuronal activity in the basal ganglia output nuclei, the internal segment of globus pallidus and the substantia nigra pars reticulata (SNR), which are thought to underlie the development of parkinsonism.

Studies in animal models of Parkinson’s disease have shown that dopamine depletion leads to a replacement of the regular firing of SNR and subthalamic nucleus neurons with irregular, bursting activity with periodic oscillations (Bergman et al., 1994; Nini et al., 1995; Wichmann et al., 1999; Ni et al., 2001; Tseng et al., 2001a). These abnormalities are not fully normalized under pharmacological dopamine replacement therapy (PDRT; Heimer et al., 2006). The irregular, bursty and oscillatory firing patterns have been implicated in the pathophysiology of Parkinson’s disease (Bergman et al., 1994; Nini et al., 1995; Levy et al., 2000).

Among anti-parkinsonian treatments, transplantation of dopaminergic cells is a unique approach because of its ability to restore continuous dopamine release within a given area in the brain and its ability to form reciprocal connections with the host. Foetal dopaminergic cells have been transplanted successfully into the striatum of rats and monkeys, showing re-innervation within the host brain and providing behavioural improvement (Astradsson et al., 2008). In humans, although dopaminergic transplants have not yet reached the level of efficacy and safety needed to recommend transplantation as a widespread therapy for Parkinson’s disease (Freed et al., 2001; Olanow et al., 2003), development continues to advance transplant techniques. Several individuals with Parkinson’s disease who have undergone allogenic striatal foetal ventral mesencephalic grafts have shown long-term sustained improvement in parkinsonism without deleterious side-effects (Mendez et al., 2008). What distinguishes such patients from the majority of foetal ventral mesencephalic recipients, who either develop graft induced dyskinesias or are found to have neurodegenerative disease in the graft, remains unclear. Newer transplantation techniques including alternate dopaminergic cell sources and better understanding of graft-host interactions may potentially overcome the disadvantages of allogenic cell transplantation therapy.

Separately, systemic continuous dopaminergic stimulation has been shown to reduce drug induced dyskinesias in human patients (Quinn et al., 1984; Nyholm and Aquilonius, 2004; Splinter, 2007). A single case report of palidal recordings from patients with foetal ventral mesencephalic transplants has recently been published (Richardson et al., 2011) and one mouse study has looked at local electrophysiology immediately adjacent to nigral graft (Besnard et al., 2010). However, no studies to date have systematically examined the downstream electrophysiological correlates of either transplant-based or infusion-based continuous dopaminergic stimulation in human patients or in animal models of Parkinson’s disease. Instead, previous transplantation studies have been evaluated only on the basis of behavioural changes (improvements in parkinsonism), and histological evidence of cell survival and cell ability to secrete dopamine and form synaptic connections with the host. The observation of basal ganglia firing pattern abnormalities associated with Parkinson’s disease thus warrants investigation of the effects of heterotopic striatal dopaminergic grafts (the most commonly used experimental cell transplantation approach in Parkinson’s disease) on these aberrant neuronal firing patterns.

In this study, we coupled behavioural, histological and electrophysiological studies in a parkinsonian rat model performed at the optimal survival time of the grafts to investigate the functional effect of striatal dopaminergic transplants in the SNR and subthalamic nucleus of the host brain.

Materials and methods

Rats and lesioning surgery

Forty-four female Sprague-Dawley rats (CRL) were used in these experiments, weighing 220–250 g at the time of acquisition and housed on a 12:12 light:dark cycle with ad libitum access to food and water. All procedures were approved by the Pennsylvania State University Institutional Animal Care and Use Committee.

Hemiparkinsonism was induced in 34 rats by the animal vendor (CRL) by lesioning the left nigrostriatal pathway with a stereotactic injection of 6-hydroxydopamine using a modified version of the classic Ungerstedt paradigm (Ungerstedt and Arbuthnott, 1970; Lieu et al., 2010). Briefly, 6-hydroxydopamine-hydrogen bromide (12 µg in 4 µl) was injected into the medial forebrain bundle (AP −1.5 mm, ML +1.8, DV −7.5 from dura) at a rate of 0.67 µl/min, followed by leaving the needle in place for 5 min.

Thirty-four rats received unilateral 6-hydroxydopamine injection and the remaining 10 rats were not treated (normal controls). The hemiparkinsonian rats were randomly assigned to one of two groups: control (n = 8) and foetal ventral mesencephalic transplanted (n = 26).

Behavioural tests

Following a 3-week recovery period, lesioned rats were challenged with apomorphine HCl (0.2 mg/kg, s.c.) at 3 and 5 weeks after 6-hydroxydopamine exposure. Apomorphine-induced rotations were counted in an automated ‘rotometer’ (San Diego Instruments). Only rats with more than 245 rotations over 35 min were used in the study. Transplanted rats were retested approximately every 2 weeks, up to 12 weeks.
In the transplanted group, a modified version of the extended body axis bias test (Borlongan and Sanberg, 1995) was used to measure posturing activity, as detailed elsewhere (Lieu et al., 2010). Briefly, each rat was placed individually in a plastic testing cage. After attaining a neutral position with all four limbs touching the bottom of the cage, the rat was vertically lifted by the base of the tail so its head was \( \sim 1\) inch from the cage bottom. The first direction of body deviation away from the vertical axis of \( 10^\circ \) was recorded during a 5 s interval. An ipsilateral turn towards the lesioned side was scored with a \(+1\), a contralateral turn away from the lesioned side scored as \(-1\), and no bias as \( 0 \). Five trials were averaged for each test, and each test was repeated twice per time point. In contrast to the apomorphine-induced rotation, which is a forced measure of the dopaminergic striatal sensitivity due to nigrostriatal denervation, the extended body axis bias test is a spontaneous activity test that measures the tendency of the rat to swing to a particular side when held briefly upside-down by its tail.

A combined behavioural improvement index was created by adding the percentage of apomorphine-induced rotation improvement divided by the maximum possible percentage of apomorphine-induced rotation improvement to the percentage of extended body axis bias test improvement divided by the maximum possible percentage of extended body axis bias test improvement.

**Tissue preparation and transplantation**

Ventral mesencephalic tissue was harvested from embryonic Day 13.5 C57BL/6 mouse foetuses. Under pentobarbital anaesthesia, the fetuses were removed from the pregnant mother. The ventral mesencephalon was dissected under aseptic conditions and were placed in Dulbecco’s modified Eagle’s medium. The foetal ventral mesencephalic tissue was dissociated into a single cell suspension (Yurek et al., 2009) and kept on ice until transplantation. A final cell concentration of \( \sim 100,000 \text{cells}/\mu l \) was used, with 90–98% viability as confirmed with trypan blue staining. Cell suspension (1 \( \mu l \)) was injected at two depths into three striatal sites (AP 1.7, 0.7, \(-0.3\); ML 2.5, 2.5, 3.5; DV \(-6.0/\sim 5.0\), \(-6.5/\sim 5.5\), \(-6.5/\sim 5.5\)) at the rate of 1 \( \mu l/\text{min} \), for a total of 600,000 cells per rat. The stereotaxic frame’s incisor bar was set at \(-3.3\) mm for all rats. All transplanted rats were immunosuppressed for the duration of the experiment with daily injections of cyclosporine (10 mg/kg s.c.) to prevent graft rejection.

**Electrophysiology**

The electrophysiological experiments were conducted 12 weeks after the transplantation and after final behavioural tests. The rats were deeply anaesthetized with urethane, with a nominal initial intraperitoneal dose of 1.3 g/kg and additional doses given as needed to maintain surgical anaesthesia depth as checked by the foot pinch and corneal reflex. Tungsten microelectrodes (5–7 M\( \Omega \), FHC Inc.) were used for extracellular single unit recordings, and the signal was pre-amplified and filtered between 500 Hz–10 kHz (ISO-80, WPI) and digitized at 25,000 samples per second (Micro1401, CED). A stainless steel screw implanted into the left occipital cranial bone was used as the ground potential. The EEG signal allowed classification of the recording by brain activation state: either slow-wave activity, dominated by large amplitude waves at \( \sim 1\) Hz, or global activation, characterized by smaller amplitude waves with peak frequency between 2 and 4 Hz (Magill et al., 2000, 2001; Clement et al., 2008). Recordings were excluded if they were \(<\sim 120\) s in length, if they could not be classified as clearly either pure slow-wave activity or pure global activation, if they contained spontaneous transitions between brain states, or if their spike isolation or local field potential signal quality was contaminated by electrical artefacts.

**Analysis of electrophysiological data**

In addition to the firing rate, the coefficient of variation of the spike inter-spike interval sequence was computed for each record as a measure of the regularity of the spike firing. A burst index was computed as the mode inter-spike interval divided by the mean inter-spike interval, where higher burst indices indicate burst firing (Levy et al., 2001). Individual bursts were also detected using the Poisson surprise method, using surprise threshold of five or greater (Legendy and Salcman, 1985; Wichmann and Soares, 2006). The density discharge histogram of each neuronal recording was compared with that of a Poisson random process. If a \( \chi^2 \) goodness of fit test did not show a significant difference between the computed density discharge histogram and the Poisson density, the recording was classified as ‘Poisson’. Otherwise, the recording was classified as ‘regular’ if the variance of the density discharge histogram was \(<1 \text{ or ‘bursty’ if the variance was } >1 \) (Kaneoke and Vitek, 1996; Levy et al., 2001).

The sample entropy was computed as a measure of spike randomness, using an embedding dimension of 2 and a tolerance of 0.2 \( \times \) SD (Richman and Moorman, 2000). Oscillatory cells were detected by thresholding the spectral density of the inter-spike intervals, with the threshold set to 5 SD of 50 randomly reshuffled versions of the inter-spike interval sequence and a minimum of 10 consecutive points above the threshold (Soares et al., 2004).

The spectra of the EEG and local field potentials were computed using a Welch periodogram in a custom Matlab (Mathworks, Inc.) script. Prior to computation of the periodogram, signals were filtered offline at 1–99 Hz, pre-normalized by the root mean square power, and down-sampled to 200 samples per second. The Fourier transform length for the spectra and magnitude squared coherence was 256 samples, with periodogram overlap of 64 samples. The spectra were normalized by the total summed power in the 5–59 Hz and 61–95 Hz bands to avoid the 60 Hz line noise peak.

To assess the degree of synchrony between local field potentials and neuronal spikes, spike-triggered averages of the local field potential and EEG were computed for each spiketrain in a 6 s window around each spike using a custom Matlab script. Randomized spike triggered averages were also computed by shuffling the inter-spike intervals before computing the spike triggered average. Peak-to-peak spike...
triggered average magnitudes were computed excluding the 15 ms around the spike to avoid local field potential spike artefacts.

**Histology**

At the end of the recording session, rats were euthanized by transcardial perfusion as detailed elsewhere (Lieu et al., 2010, Subramanian et al., 2002). Each brain was frozen and sectioned coronally into 10 wells at a thickness of 40 or 60 µm. Sections in one or more wells were stained for cresyl violet to localize the electrophocogulation lesions and microelectrode tracks, and for tyrosine hydroxylase as previously described (Lieu et al., 2011) to confirm lesion-induced nigral cell loss and localize the striatal graft.

Graft derived tyrosine hydroxylase-positive neurons in each animal were estimated using design-based systematic random sampling and the optical fractionator method of unbiased stereology (Stereoinvestigator, Micro Bright Field) as detailed in Lieu et al. (2011).

**Statistical analyses**

The statistical significance of graft-induced changes in rotational behaviour was assessed with an ANOVA followed by post hoc t-tests with Dunnett’s Multiple Comparison Test. Extended body axis bias test results were compared using a Mann–Whitney rank-sum test because the data were non-Gaussian. The criteria for including data from a given rat in the final statistical analysis were: (i) tyrosine hydroxylase-stained sections showing definite surviving grafts in at least two separate grafted sites in the striatum (meeting the conditions for stereological estimation); and (ii) histological confirmation of electrocoagulation or electrode track in the subthalamic nucleus or SNR.

Electrophysiological values were first tested using the Jarque–Bera normality test, and subsequently compared using the Kruskal–Wallis non-parametric ANOVA when found to be significantly non-Gaussian. Group Kruskal–Wallis ANOVAs were followed by pairwise rank-sum tests with the Tukey–Kramer Honest Significant Difference correction (using P < 0.05) if the Kruskal–Wallis test was significant. Fisher’s exact 2 × 2 test was used to compare the oscillatory properties and the density discharge histogram burst pattern proportions (combining regular and Poisson categories together).

The 95% confidence interval for coherence plots was computed based on the framework of Halliday et al. (1995). The coherences in selected bands were also summed and compared using a Kruskal–Wallis ANOVA with post hoc pairwise rank-sum tests with the Honest Significant Difference correction.

The mean peak magnitudes of the spike triggered averages were compared between groups using a Kruskal–Wallis ANOVA and post hoc rank-sum tests with the Honest Significant Difference correction. The mean peak spike triggered average magnitudes of each group were also compared directly with the mean peak magnitudes of the corresponding randomized spike triggered averages using pairwise rank-sum tests.

**Results**

**Histology and behaviour**

As expected, the 6-hydroxydopamine lesions of the medial forebrain bundle produced a ~95–97% loss of tyrosine hydroxylase-positive dopamine neurons in the striatum and SNC (Fig. 1A and D). Electrode tracks were histologically confirmed in the SNR and subthalamic nucleus (Fig. 1H and I).

Nine transplanted rats showed definite surviving striatal grafts with tyrosine hydroxylase-positive neurons. Figure 1B and C shows an example striatal graft. Each identified graft site in each animal had healthy appearing tyrosine hydroxylase-positive neurons and graft derived tyrosine hydroxylase-positive neurites around the graft that appeared to innervate the surrounding denervated striatum. Graft-derived tyrosine hydroxylase-positive cells were also dopamine transporter-positive (Fig. 1E–G), confirming their dopaminergic status. Supplementary Fig. 2 shows additional views of the graft cells.

The nine rats with surviving grafts fell naturally into two categories: five showed large, robust grafts with many surviving cells, and four showed small grafts with few surviving cells. The discrimination between the large graft and small graft groups was based on stereological cell counts, using an arbitrary threshold of 4000 cells. The mean count of tyrosine hydroxylase-positive grafted neurons in the large graft group was 8975 ± 2386 (SEM), with coefficient of error 0.18 ± 0.01. The mean in the small graft group was 1471 ± 328 neurons with coefficient of error 0.29 ± 0.03. Although this range of surviving graft size was not the intended result of our transplantation paradigm, it gave us the opportunity to analyse the electrophysiology from these two groups separately to evaluate whether the size of the striatal graft had a differential effect on the downstream firing rates and patterns.

As shown in Fig. 2, both the ‘large graft’ and ‘small graft’ groups showed apomorphine-induced rotation improvement at months 1, 2 and 3 compared to pre-transplant (P < 0.05), but only the large graft group showed improvements in the extended body axis bias test (P < 0.05). The correlation of cell count against behavioural improvement index was significant (P < 0.01), with an R² value of 0.8055. We did not observe graft-induced dyskinesias in any of the rats.

The remaining 17 transplanted rats were excluded from the analysis for one of the following reasons: (i) the perfusion or tyrosine hydroxylase histology was problematic allowing no firm conclusion to be made about graft survival (10 rats); (ii) surviving cells were seen but were all misplaced outside the boundary of the striatum (one rat); or (iii) no surviving tyrosine hydroxylase-positive graft cells were seen but not enough total neurons were recorded to make a valid ‘no-graft-seen’ control group to compare with the other groups (six rats).

**Electrophysiology**

Figure 3 shows a sample neural recording, including a transition from slow-wave activity to global activation states. Table 1 lists the numbers of neuronal spike trains and local field potential waveforms recorded in the different groups and brain activation states. When possible, neurons were recorded from both slow-wave activity and global activation states, but these transitions were deliberately kept spontaneous rather than manipulated (e.g. by paw pinch or thermal stimuli, cf. Mallet et al., 2008b), causing some unevenness of sampling among the different activation states.

Firing rates in the slow-wave activity state were significantly different between groups in the SNR (P < 0.05) but not in the subthalamic nucleus (Fig. 4A). Post hoc testing showed that the
only significant difference in the SNR was that the large graft group had lower firing rates than the small graft group.

Firing patterns in the slow-wave activity state in both SNR and subthalamic nucleus showed increased burstiness in the hemiparkinsonian state and a restoration of burstiness towards more normal levels in the large graft group (Fig. 4B–G). In the SNR this trend was significant in the coefficient of variance \( (P < 0.001) \), proportion of spikes in bursts \( (P < 0.001) \), and density discharge histogram range \( (P < 0.001) \). In each of these measures, post hoc tests showed that the hemiparkinsonian group and the small graft group were burstier than the normal group, and the large graft group was restored to a level not statistically different from the normal group. Additionally, the sample entropy was reduced in the hemiparkinsonian and small graft groups \( (P < 0.001) \) restored to normal levels in the large graft group. The proportion of SNR cells classified as bursty by the Poisson density discharge histogram comparison increased in the hemiparkinsonian group, but the trend did not reach statistical significance (Fisher’s exact two-sided test, \( P = 0.068 \)).

In the subthalamic nucleus, the same pattern was seen of increased burstiness in the hemiparkinsonian group and restoration to normal levels in the large graft group. This trend was significant...
in the Poisson density discharge histogram comparison (Fisher’s exact two-sided test; \( P = 0.025 \) between normal and hemiparkinsonian groups; \( P = 0.0046 \) between hemiparkinsonian and large graft groups) and proportion of spikes in bursts \( (P < 0.05) \). Post hoc tests showed that the large graft group was significantly less bursty than the hemiparkinsonian control group in the coefficient of variance, burst index, density discharge histogram range, and sample entropy metrics.

The small graft group showed mixed results in both SNR and subthalamic nucleus, with burstiness levels closer to the normal group in some measures, and closer to the hemiparkinsonian group in other measures. The majority of measures showed the burstiness of the small graft group falling between that of the hemiparkinsonian group and the large graft group.

The percentage of oscillatory SNR neurons was significantly higher in the hemiparkinsonian group compared to the normal group \( (P = 0.022) \). In the small graft group, the percentage of oscillatory SNR neurons was even higher than in the hemiparkinsonian group \( (P = 0.0025) \), between hemiparkinsonian and small graft groups). The percentage of oscillatory SNR neurons in the large graft group was restored to normal levels. The subthalamic nucleus showed a similar trend. The hemiparkinsonian oscillatory percentage was not significantly increased from normal, but the small graft group had more oscillatory neurons than the normal group \( (P = 0.0023) \).

In the global activation state, firing rates did not significantly differ between groups in either the SNR or subthalamic nucleus (Fig. 5A). Firing patterns in the global activation state were less bursty overall than in the slow-wave activity state in both SNR and subthalamic nucleus, and smaller differences were seen between the groups than in the slow-wave activity state. In the SNR, the density discharge histogram range showed an increase in burstiness in the hemiparkinsonian group \( (P < 0.01) \). The sample entropy showed a significant reduction in the hemiparkinsonian group \( (P < 0.05) \) and the percentage of oscillatory neurons was
higher in the hemiparkinsonian group than in the normal group (Fisher’s exact two-sided test; \( P = 0.022 \)). In contrast, the small graft and large graft groups did not show any significant differences in firing pattern from the normal group.

In the slow-wave activity state, the mean EEG spectra showed a slight increase in power \( P < 30 \) Hz in the large graft group (Fig. 6). This increase was also seen in the SNR local field potentials and in the EEG-local field potential coherence, especially in the 30–55 Hz band. Quantification of this effect by summing the power in the 30–55 Hz coherence band showed a significant increase in the large graft group (\( P < 0.05 \)). However, this peak was seen primarily in only two out of the five rats, from which a large number of recordings were obtained (Supplementary Fig. 1). To visualize the coherence results in another way that de-emphasizes the variability of contributions from rats with greater or lesser numbers of recordings, the mean of each rat’s mean coherences was also computed (Fig. 6, inset). This mean showed smaller differences between the groups (statistics not performed because of small sample size). The subthalamic nucleus in the slow-wave activity state showed an increase in the 10–15 Hz band in the hemiparkinsonian and large graft groups (\( P < 0.05 \)). However, while the differences between groups were significant in the 10–15 Hz band in the SNR and subthalamic nucleus in the slow-wave activity state, these peaks did not cross the coherence significance level,
Figure 6 Spectral measures. (Top row) Mean EEG log-spectra (bold lines) and SEM (thin lines) across all recordings in each group. Power units are arbitrary. (Second row) Mean local field potential log-spectra (bold lines) and SEM (thin lines) across all recordings in each group (the spike at 60 Hz in the local field potential spectra is due to line noise). Power units are arbitrary. (Third row) Mean EEG-local field potential coherence (bold lines) and SEM (thin lines) across all recordings in each group. (Inset) Mean of each rat’s mean coherence (bold lines) and SEM (thin lines). The black dashed line shows the 95% significance level for the number of windows used. (Bottom row) Summed coherence in the 10–15 Hz band and the 30–55 Hz band to highlight the differences in these regions [*P < 0.05 compared to normal controls; #P < 0.05 compared to hemiparkinsonian (HP) controls].
and were thus not considered significant in summary Table 2. All rat groups showed a significant coherence peak in the 30–55 Hz band, with no significant differences seen between groups in this band.

In the global activation state, a significant peak was seen in the 10–15 Hz band in the hemiparkinsonian group in both the SNR and subthalamic nucleus. This peak was significantly reduced in the small graft group SNR and in the large graft group SNR and subthalamic nucleus. However, these peaks were seen primarily in one rat in each group, and the average of each rat’s mean coherences (Fig. 6, inset) showed smaller differences between the groups in this band. The hemiparkinsonian SNR and the small graft group subthalamic nucleus also showed significantly increased coherence in the 30–55 Hz band compared to the normal group.

In the slow-wave activity state, spike triggered averages showed a trend toward increased post-spike amplitude in the hemiparkinsonian and small graft groups from both the local field potential and EEG waveforms (Fig. 7). This trend was significant in the EEG \((P < 0.05, \text{post hoc rank-sum tests on peak-to-peak amplitudes})\). Additionally, the local field potential showed significant modulation in the small graft SNR and the hemiparkinsonian subthalamic nucleus, but no significant modulation in either SNR or subthalamic nucleus of the normal and large graft groups.

In the global activation state, the majority of groups showed significant spike triggered average modulation, but no significant differences between groups were observed.

**Discussion**

To our knowledge, this is the first study to systematically compare the electrophysiological properties of subthalamic nucleus and SNR neurons before and after striatal dopaminergic transplantation (local continuous dopaminergic stimulation restoration paradigm) in parkinsonian animals. In striatal-grafted rats that showed good behavioural recovery and xenograft survival at 3 months, we found that firing rates in the SNR and subthalamic nucleus were unchanged compared to normal controls, but bursting firing patterns in the subthalamic nucleus were normalized in the group with large surviving graft. This finding is similar to what has been reported in a single patient with a foetal ventral mesencephalic transplant who underwent pallidal recordings (Richardson et al., 2011).

**Firing rates**

The literature is not unanimous on typical firing rates in the urethane-anaesthetized SNR and subthalamic nucleus in normal and hemiparkinsonian conditions. Although many studies report SNR and subthalamic nucleus firing rate increases in the hemiparkinsonian condition (Breit et al., 2008), some studies report no change or even a rate decrease (Rohils et al., 1997; Ni et al., 2001; Tseng et al., 2001a; Walters et al., 2007). In our study, although we observed that the large graft group firing rate was lower than the small graft group firing rate, we did not see any significant firing rate changes between control and grafted groups, lending support to the idea that parkinsonian electrophysiology may be more strongly correlated with pathological patterns than with rate changes.

Our firing rate results from the grafted groups contrast with results from intermittent PDRT. During PDRT, SNR firing has been shown to slow while subthalamic nucleus rates are typically unchanged (Levy et al., 2001; Gilmour et al., 2010). The difference between the rate changes effected by transplants and PDRT could be due to the notion that grafts provide continuous dopaminergic stimulation, whereas PRDT is intermittent.

**Firing patterns**

There is broad agreement in the literature that basal ganglia firing patterns become more bursty in the parkinsonian condition (Rohils et al., 1997; Magill et al., 2001; Ni et al., 2001; Tseng et al., 2001a; Breit et al., 2008; Parr-Brownlie et al., 2009). A plausible partial explanation for this increased burstiness is that dopamine depletion increases the excitability of striatal medium spiny neurons, allowing cortical slow-waves to have a larger effect on downstream basal ganglia firing patterns (Tseng et al., 2001b; Murer et al., 2002). Our results corroborated this observation of

### Table 2 Summary of electrophysiological changes in the large graft group compared to hemiparkinsonian control group

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<th>SNR</th>
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<td>Slow-wave activity</td>
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<td>Firing rate</td>
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<td>Low beta EEG-local field potential coherence</td>
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<td>Gamma EEG-local field potential coherence</td>
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<td>Local field potential spike triggered average magnitude</td>
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<td>EEG spike triggered average magnitude</td>
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= no significant change; ▼ = increase; ▼ = decrease.

*Significant difference between groups but the peak did not cross the coherence significance level.

**The peak that was significantly increased but seen in only one of the five rats. Burstiness row shows the combined result of the five burstiness metrics.
increased burstiness in both the SNR and subthalamic nucleus. However, the effects were much more pronounced in the slow-wave activity state than the global activation state. This accords with previous reports that show reduction of bursting activity in the global activation state (Magill et al., 2000, 2001), suggesting that grafts may provide normalization in both brain activation states but the contrast is much more obvious in the slow-wave activity state due to its intrinsically higher burstiness.

In the transplanted groups, our results confirm the hypothesis that dopaminergic grafts exert a normalizing effect on the subthalamic nucleus firing patterns. Future studies should probe the mechanisms behind this normalization. It is possible that local

**Figure 7** Spike-triggered waveform averages. In each box, the top four rows show the mean spike triggered averages for each group (bold lines) with the SEM (thin lines). Dotted lines show the maximum and minimum of the mean randomized spike triggered averages. The bar graphs show the mean peak spike triggered averages (black) and the mean peak randomized spike triggered averages (white) for each group regions (*) $P < 0.05$, (***) $P < 0.01$, (****) $P < 0.001$ comparing the randomized spike triggered average to the corresponding un-randomized spike triggered average; (#) $P < 0.05$ compared to the normal control group spike triggered average. LFP = local field potential; STA = spike-triggered average.
Restoration of dopamine reverses the striatal polarization changes that have been shown to increase cortico-striatal transmission of the cortical rhythms (Tseng et al., 2001b). Besnard and colleagues (2010) recently performed a related experiment to ours using orthotopic dopaminergic transplantation into the SNC in the hemiparkinsonian mouse model. Their observation of fewer bursting neurons in their grafted mouse group corroborates our results.

The observation of differential effects of striatal dopaminergic transplants in slow-wave activity and global activation reinforces the importance of measuring brain state via EEG monitoring during basal ganglia electrophysiology experiments. While anesthetized recordings are not a substitute to recordings in awake animals, the reported results provide motivation for future studies in awake animals using an identical transplantation paradigm. This would allow comparison of the effects of natural sleep, wakefulness, and movement on electrophysiology in this rat model of Parkinson’s disease with striatal dopaminergic transplants. There is also some recent clinical evidence that continuous dopaminergic stimulation reduces sleep disturbances (Trenkwalder et al., 2011), fitting with the electrophysiological normalization we observed in this experiment.

More research should also be performed in the future to assess the effects of varying graft-host connectivity on electrophysiological normalization (Soderstrom et al., 2008). The experimental paradigm described here could serve as a model to test the downstream neurophysiological normalization capabilities of next generation pluripotent stem cells that have the potential to provide customized patient-specific therapeutic options for Parkinson’s disease.

Similar to our graft effect results, high frequency electrical stimulation of the subthalamic nucleus has been shown to reduce burstiness in the SNR (Mallete et al., 2007) and subthalamic nucleus (Shi et al., 2006), supporting the notion that a reduction in burstiness correlates with antiparkinsonian therapeutic improvement.

The observed effect of grafts in reducing burstiness contrasts with PDRT. Intermittent apomorphine and levodopa have been shown to increase bursting activity of SNR and subthalamic nucleus neurons (Murer et al., 1997; Levy et al., 2001; Gilmour et al., 2010), possibly because of the pulsatile nature of the stimulation as opposed to the putative restoration of continuous dopaminergic stimulation via dopaminergic cell transplantation. This hypothesis requires further testing, and such an experiment that allows direct comparison of PDRT to cell transplants is now readily feasible using the grafting paradigm described here.

The differential effect of small versus large dopaminergic grafts on downstream basal ganglia electrophysiology is another novel and interesting finding. This conclusion fits with previous literature suggesting that larger grafts provide better behavioural symptomatic amelioration (Mukhida et al., 2001). The stereological estimates in the large graft group suggested that approximately 50% of the number of tyrosine hydroxylase-positive SNC neurons in a healthy rat (12,000–15,000 cells) survived in the heterotopic striatal location. It has been shown that 50–70% loss of SNC dopaminergic neurons is required to cause clinically detectable Parkinson’s disease symptoms. Thus, it makes sense that the 50% cell number replenishment of the large graft group would provide substantial normalization of basal ganglia electrophysiological patterns while the ~10% replenishment of the small graft group would be unable to provide consistent normalization.

**Neural firing oscillations**

Previous studies have shown that synchronous spiking oscillations appear in the output nuclei of the basal ganglia in the parkinsonian state (Nini et al., 1995; Hurtado et al., 1999; Levy et al., 2000; Raz et al., 2000), and PDRT has been shown to reduce these oscillations in the globus pallidus (Heimer et al., 2006). In both the SNR and subthalamic nucleus in the slow-wave activity condition, our small graft group showed increased oscillatory activity compared with the hemiparkinsonian group, whereas our large graft group showed reduction and restoration to normal percentages. In the global activation condition, the subthalamic nucleus showed the same trend but the SNR showed the same amount of normalization in both small graft and large graft groups. The differences in the global activation condition were not as significant as in the slow-wave activity condition. These results support the conclusion that graft induced resupply of dopamine mediates a normalization of oscillatory firing patterns, with larger grafts providing better normalization.

**Sample entropy**

The large graft group showed a full restoration of the sample entropies in the SNR and subthalamic nucleus, especially in the slow-wave activity condition. The small graft group showed a much less pronounced effect, as would be expected. The information-theoretic randomness of a spike train is an independent metric to the burstiness and inter-spike interval variability, as demonstrated by (Kostal and Lansky, 2007), although increases in burstiness often reduce entropy because of the increased inter-spike interval predictability relative to the inter-spike interval standard deviation within bursts.

By comparison, PDRT has been shown to reduce SNR entropy even further than the parkinsonian condition (Lafrunei-Roula et al., 2010). Interestingly, while deep brain stimulation reduces burstiness and PDRT does not, both deep brain stimulation and PDRT cause similar reductions in entropy in basal ganglia output nuclei (Dorval et al., 2008; Gilmour et al., 2010). However, this is likely through different mechanisms: PDRT may simultaneously reduce entropy and increase burstiness by changing receptor and basal ganglia circuit properties due to the intermittency of dopamine availability, whereas DBS reduces entropy by phase-locking neuronal spikes to a multiple of the stimulus train.

In contrast, dopaminergic grafts may reduce the burstiness of the basal ganglia in a fundamentally different manner than deep-brain stimulation. Instead of forcing the basal ganglia output into a rigid synchronicity with the stimulus train, the continual release of dopamine from the striatal transplants may restore physiological firing of the striatum, subthalamic nucleus and downstream nuclei, allowing firing at higher entropy levels that are closer to normal patterns without the bursting activity that emerges in the dopamine-depleted state.
Local field potentials and local field potential coherences with EEG

Overall, we observed variability in the coherence peaks observed between rats and within the recording session from the same rat (Supplementary Fig. 1). We observed some small peaks in the low beta band (10–15 Hz) and in the low gamma band (30–55 Hz) in some hemiparkinsonian rats, but we did not see the robust pattern of all hemiparkinsonian rats showing pronounced mid-beta band (20 Hz) activity in the global activation state which some other laboratories have reported and which has been linked to human parkinsonism (Brown, 2003; Mallet et al., 2008a, b). Despite the variability between rats, it is plausible that the observed lack of a low beta peak in the grafted groups is due to the grafts’ chronic replenishment of dopamine. The increase in the large graft group in the gamma band has two potential interpretations. It may be a transient and variable phenomenon. We did not see any significant 60–100 Hz peaks in any groups, differing from some published studies in the awake rat (Brown et al., 2002), or it may be considered an outlier due to the fact that it was mainly seen in only one rat. We lean toward the latter hypothesis because even our anaesthetized rats from the normal group did not show the gamma peak which has been measured in awake healthy rats.

Some recent findings in other experiments and other species have also shown variability in the link between parkinsonism and beta and gamma local field potential activity. For example, Avila and colleagues (2010) showed that awake hemiparkinsonian rats showed beta activity primarily in particular activity paradigms, not at all times. In human patients, while most parkinsonian patients undergoing intraoperative recordings show beta oscillations while off medications, substantial variability may be involved. Giannicola and colleagues (2010) reported that three out of nine patients did not show beta peaks, and de Solages and colleagues (2010) reported coherence peaks occurring within a wide spectral range of 10–35 Hz. Thus, the presence and location of coherence peaks may be a transient and variable phenomenon. We did not see any significant 60–100 Hz peaks in any groups, differing from some published studies in the awake rat (Brown et al., 2002) similar to other studies (Avila et al., 2010).

In light of the beta and gamma local field potential oscillation variability in our results and in the literature, it is not yet fully clear how dopaminergic grafts affect this activity. Additional future investigation is warranted to elucidate the exact connection between local field potential activity and parkinsonism and to clarify the effect of transplant therapy on this activity.

Spike-triggered averages

The large graft group mean peak EEG spike triggered average and local field potential spike triggered average were not statistically different from the normal group in both SNR and subthalamic nucleus in both brain activation states. In contrast, the hemiparkinsonian and small graft groups tended to have higher spike triggered averages, and this trend was significant in the EEG in both slow-wave activity and global activation states. This result suggests that the synchronization of individual neuronal output spikes to their local input potentials and distant cortical circuit potentials was partially normalized by the large surviving grafts. This normalization was not complete, and in some cases (such as the EEG spike triggered average in the global activation state) the large graft group showed spike triggered average modulation whereas the normal group did not. But overall, the data suggest that the striatal grafts affected the downstream nuclei by partly decoupling the neurons’ outputs from their field potential inputs, making the parkinsonian basal ganglia more normal. This fits with other reports such as those from Bergman and colleagues (1994) that show that in the parkinsonian state there is increased crosstalk between neurons in the basal ganglia output nuclei (Heimer et al., 2002, 2006; Goldberg et al., 2004).

Implications and clinical relevance

Several models of parkinsonian pathophysiology have been described that emphasize various basal ganglia changes, such as firing rate changes in the striatal direct and indirect pathways (Albin et al., 1989; DeLong, 1990), local field potential anti-kinetic and pro-kinetic oscillations (Brown et al., 2001; Brown and Williams, 2005; Hammond et al., 2007), increased synchrony between neighbouring neurons (Heimer et al., 2002), and excessive bursting activity (Sanderson et al., 1986; Bergman et al., 1994). Our results emphasize the firing patterns (bursts, oscillations and entropies) as being the most unambiguous correlate of parkinsonism in the anaesthetized 6-hydroxydopamine rat model (Table 2). We did not see significant rate changes in the hemiparkinsonian state. The local field potential changes we saw were variable between rats, although the reduction in the low beta EEG-local field potential coherence seen in one rat fit the oscillations model of Brown and colleagues. The spike triggered average changes we observed were consistent with, but did not directly measure, the synchrony changes between neighbouring neurons reported in the literature. While the 6-hydroxydopamine rat model is a well-established paradigm which mimics some of the symptoms of PD related to nigrostriatal system damage, it is not a progressive disease model. Future transplant studies should also investigate transgenic models and other models which involve aggregate accumulations of proteins such as α-synuclein.

The firing patterns were robustly altered in the hemiparkinsonian state and restored by the large grafts. This normalization suggests that continuous dopaminergic stimulation is an important therapeutic paradigm that is able to normalize electrophysiological parameters un-normalized by PDRT. Due to this and because of the proven therapeutic effectiveness of continuous dopaminergic stimulation, it is worthy of further investigation in the ongoing search for Parkinson’s disease therapies which avoid the eventual dyskinesia side effects of PDRT.

Conclusion

We report that dopaminergic cell grafts into the striatum have a normalizing effect on the bursty firing patterns, neuronal oscillations, low beta EEG-local field potential coherence, spike triggered averages, and firing entropies in the SNR and subthalamic nucleus. These electrophysiological results were combined with behavioural improvement and tyrosine hydroxylase positive graft derived...
partial reinervation of the host striatum in this xenotransplant paradigm. This suggests that with future advances in cell transplantation techniques that lead to improved cell survival and optimized host–graft integration, better electrophysiological normalization may be achieved than currently possible with intermittent PDRT, potentially providing therapeutic benefits with fewer side effects.

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Supplementary material

Supplementary material is available at Brain online.

References


Avila I, Parr-Brownlie LC, Brazhnik E, Castaneda E, Bergstrom DA, Walters JR. Beta frequency synchronization in basal ganglia output during rest and walk in a hemiparkinsonian rat. Exp Neurol 2010; 221: 307–19.


