Effects of subthalamic deep brain stimulation on blink abnormalities of 6-OHDA lesioned rats

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PARKINSON’S DISEASE (PD) patients display obvious voluntary motor deficits, but disruptions of voluntary movement in rodent models of PD are not as apparent as those of PD patients (Bove et al. 2005; Cenci et al. 2002). Nevertheless, the blink and stretch reflex abnormalities of PD patients (Agostino et al. 1987; Berardelli et al. 2001; Cody et al. 1986; Duysens et al. 2010; Kimura 1973; Rothwell et al. 1983; Sunohara et al. 1985; Tatton and Lee 1975) and rodent models of PD (Basso et al. 1993; Wolfarth et al. 1996) are nearly identical. For example, both PD patients and the 6-hydroxydopamine (6-OHDA) rat model exhibit strong reflex blink hyperexcitability. These data suggest that blink abnormalities of the rat 6-OHDA lesion model of PD are ideal for testing treatment options and identifying the neural circuits and modifications that create PD reflex movement abnormalities.

High-frequency subthalamic nucleus deep brain stimulation (STN DBS) is an accepted treatment for advanced PD (Benabid et al. 2009; Bronstein et al. 2011) and restores some aspects of blinking in PD patients (Bologna et al. 2012). One effect of 130-Hz STN DBS is disruption of the basal ganglia’s characteristic hypersynchronized beta band activity seen in both PD patients (Dorval et al. 2010; Ray et al. 2008; Wingeier et al. 2006) and in the 6-OHDA rat model of PD (Lehmkuhle et al. 2009; Li et al. 2007; McConnell et al. 2012). The therapeutic effectiveness of high-frequency STN DBS may be a result of reducing this 10- to 30-Hz beta-oscillation. Consistent with this interpretation, beta frequency STN DBS of normal rats causes the same reflex blink abnormalities as occur in PD patients (Kaminer et al. 2014). If 130-Hz STN DBS reduces beta band oscillations in 6-OHDA lesioned rats, then we predict that this frequency will eliminate blink abnormalities in 6-OHDA lesioned rats, whereas STN DBS in the beta frequency range will not improve blinking.

PD also disrupts reflex plasticity (Battaglia et al. 2006; Chong et al. 2000; Horak et al. 1992; MacAskill et al. 2002; White et al. 1983). The trigeminal blink reflex provides a model for this type of plasticity. Depending upon its timing relative to reflex blink occurrence, high-frequency stimulation of the supraorbital branch of the trigeminal nerve can increase or decrease the amplitude of subsequent reflex blinks for a period of at least 1 h (Mao and Evinger 2001; Ryan et al. 2014). Both PD in humans and 6-OHDA lesions in rats impair this form of trigeminal blink reflex plasticity (Battaglia et al. 2006; Kaminer et al. 2014). We predict that 130-Hz STN DBS will restore normal reflex blink plasticity in 6-OHDA lesioned rats. The abnormal basal ganglia activity of PD could impair reflex plasticity by preventing expression of the plasticity, blocking induction of the plasticity, or a combination of the two processes. We propose to investigate the mechanism involved by presenting 130-Hz STN DBS only during the induction of blink reflex plasticity.

In addition to its effects on trigeminal reflex blinks, PD reduces the rate of spontaneous blinking (Bologna et al. 2012; Karson et al. 1982; Korosoc et al. 2006). Consistent with the loss of dopaminergic neurons with PD, dopamine receptor blockade in normal primates and rodents reduces the spontaneous blink rate (Kaminer et al. 2011; Lawrence and Redmond 1991; Taylor et al. 1999) and disrupts the temporal organiza-
tion of spontaneous blinking (Kaminer et al. 2011). It is unknown whether 6-OHDA lesion rats exhibit a similar reduction in spontaneous blink rate and disruption of the temporal organization of blinking. We measure spontaneous blinking in 6-OHDA lesioned rats and test whether 130-Hz STN DBS restores normal spontaneous blinking.

MATERIALS AND METHODS

Experiments were performed on 13 male Sprague-Dawley rats (350–550 g) maintained on a reversed 12:12-h light/dark cycle and fed ad libitum. All experiments received approval by the Stony Brook University Institutional Animal Care and Use Committee and complied with all Federal, State, and University regulations and guidelines regarding the use of animals in research.

Surgery. Under general anesthesia (ketamine, 90 mg/kg, and xylazine, 10 mg/kg), rats received a pair of unilateral 6-OHDA injections of 7.5 and 6 μg into the right substantia nigra pars compacta and medial forebrain bundle (Basso et al. 1993). They were then implanted unilaterally with laboratory-designed stimulating electrodes in the STN. The stimulation electrodes were 4 twisted stainless steel Teflon-coated wires (0.003-in. diameter bare, 0.0055-in. coated; A-M Systems, Everett, WA). Electrodes were implanted stereotaxically into the right STN (AP: –3.8 mm, ML: 2.5 mm) (Paxinos and Watson 1998). Final electrode position was determined by recording through one of the DBS leads as the stimulus moved through the zona incerta into the STN. After STN implantation, rats were prepared for chronic recording of the left orbicularis oculi EMG (OOemg) and stimulation of the left supraorbital (SO) branch of the trigeminal nerve (Basso et al. 1993; Daugvergne and Evinger 2007; Evinger et al. 1993). Wires were led subcutaneously to a connector embedded in a dental acrylic platform on the skull. The platform was attached to the skull by four stainless-steel screws. A silver wire connected to one of the stainless-steel screws served as the ground. Rats received analgesic (ketorolac, 7 mg/kg) for at least 24 h after the surgery. Rats were alert and eating within 24 h of the surgery. The experiments began 10 days after surgery. After completion of experiments, rats were anesthetized with ketamine, 90 mg/kg, and xylazine, 10 mg/kg, and perfused intracardially with 6% dextran in 0.1 M phosphate buffer followed by 4% paraformaldehyde in 0.1 M phosphate buffer. The brains were cryoprotected and sectioned at 40 μm, and every other section was stained with cresyl violet to identify DBS electrode location or immunohistochemically for tyrosine hydroxylase to assess lesion magnitude (Basso et al. 1993). Tyrosine hydroxylase staining revealed full chemically for tyrosine hydroxylase to assess lesion magnitude (Basso et al. 1993). Tyrosine hydroxylase staining revealed full unilateral lesions of the substantia nigra pars compacta in all thirteen 6-OHDA injected rats. All but one rat had correct STN electrode placement. This rat’s data were not included in the results.

Procedures. In all experiments, the SO stimulus current was twice the threshold (T) current at which a 100-μs stimulus reliably elicited the R1 component of the reflex blink. The threshold current was determined at the beginning of each day for each rat. Threshold varied little across days (Ryan et al. 2014). We attempted to maximize the effect of STN DBS on blinking without causing any abnormal motor behavior. We found that biphasic 100-μA, 100-μs STN DBS delivered at either 130 Hz or 16 Hz did not cause any irregular motor behaviors or STN tissue damage and that 130-Hz STN DBS modified blinking.

Blink reflex excitability. We evaluated the effects of a beta frequency, 16-Hz, and 130-Hz STN DBS on trigeminal blink excitability using the paired stimulus paradigm. Rats underwent three blocks of twenty trials comprised of pairs of 2T SO with a 100-ms interstimulus interval. Trials occurred pseudorandomly with a 20 ± 5 s intertrial interval. During each block, the rat received 16- or 130-Hz STN DBS or No DBS. They experienced all three conditions each day. STN DBS began 5 min before a block and terminated immediately after completing the block of 20 trials. There were 5 min between blocks. Because our previous study (Basso et al. 1993) demonstrated that the side contralateral to the 6-OHDA lesion exhibited the largest changes in blink excitability, we only examined OOemg activity contralateral to the 6-OHDA lesion and the STN stimulating electrode.

Blink reflex plasticity. To investigate the effects of 130- or 16-Hz STN DBS on blink reflex plasticity, we utilized a protocol previously designed to depress blink reflex gain (Mao and Evinger 2001), adapted for use in rodents (Ryan et al. 2014). A 2T SO high-frequency stimulus (HFS, 400 Hz) was delivered before the R2 component of the SO-evoked blink reflex (HFS-B). Each day’s data collection consisted of five blocks: 1) pre-HFS-B (Fig. 1, Pre); 2) HFS-B treatment (Fig. 1, HFS-B); 3) immediately post-HFS-B (Fig. 1, T0); 4) 30 min post-HFS-B (Fig. 1, T30); and 5) 60 min post-HFS-B (Fig. 1, T60). HFS-B trials induced blink plasticity, and the three post-HFS-B blocks revealed expression of the plasticity relative to the blinks before HFS-B treatment. Pre- and post-HFS blocks were the same for all experiments. In these blocks, rats received 20 trials of a pair of 2T SO stimuli separated by 100 ms with an intertrial interval of 20 ± 5 s. Each HFS treatment trial consisted of a single SO stimulus at 2T to evoke a reflex blink followed by five, 400-Hz 2T SO stimuli delivered before the onset of the R2 component of the OOemg activity. The sixty HFS trials also occurred with a 20 ± 5 s intertrial interval. During the plasticity paradigm, rats received either No STN DBS, 16-Hz, or 130-Hz STN DBS in a counterbalanced design. Rats were

Fig. 1. Diagram of the blink reflex plasticity paradigms. Triangles show a twice-threshold 100-μs supraorbital nerve stimulus. In the primary paradigm, deep brain stimulation (DBS) of the subthalamic nucleus (STN) began 5 min before data collection and continued throughout the experiment. In the 130-D paradigm, 130-Hz subthalamic nucleus DBS only occurred while the rat received HFS-B.
in the same DBS stimulus condition for at least 10 days of experiments before switching to a different DBS condition. In these experiments, STN DBS began before testing blink threshold and continued throughout the entire day’s experiment. Three rats also underwent a protocol in which 130-Hz STN DBS was only turned on 5 min prior to HFS-B treatment and turned off immediately after HFS-B treatment concluded (130-D). Post-HFS data collection began 5 min after 130-Hz STN DBS was turned off in this paradigm.

**Spontaneous blinking.** We monitored spontaneous blinking in three rats with and without 130-Hz STN DBS. Using a counterbalanced design, we collected 35 min of spontaneous blinking for both conditions each day. This duration of data collection was critical because our previous study demonstrated that short periods of data collection overestimated the blink rate (Kaminer et al. 2011). The first 5 min of data were not analyzed to allow the rats to habituate to the experimental setup. In addition to using the Ooemg to monitor spontaneous blinks, we also recorded lid movements with an infrared detector (Weiss and Disterhoft 2008).

**Data collection and analysis.** All data were collected as rats moved freely in their home cage in a darkened room during their subjective night. Ooemg signals were amplified, filtered at 0.3–5 kHz, collected at 4 kHz per channel, and stored for later offline analysis on laboratory-developed software (Dauvergne and Evinger 2007). Blink amplitude was determined by integrating the rectified Ooemg activity of each blink component.

As with clinical analysis of trigeminal blink reflex excitability in PD patients (Agostino et al. 1987), we quantified trigeminal reflex blink excitability by dividing blink amplitude evoked by the second SO (test response) by the amplitude of the blink elicited by the first SO (condition response). We calculated R1 and R2 blink component excitability separately.

In humans, HFS-B treatment depressed the R2 component of subsequent blinks (Mao and Evinger 2001). We, however, investigated the effects of HFS-B on the R1 component of the blink reflex, as it is the largest component of the blink reflex in nonprimate mammals and exhibits the same changes as R2 in the HFS-B paradigm (Ryan et al. 2014). We normalized all within-day blink amplitudes by dividing each Ooemg amplitude by the median pre-HFS Ooemg amplitude. As there was no difference between post-HFS blocks (Ryan et al. 2014), post-HFS blink amplitude was averaged over all three posttreatment blocks. The normalized mean pre-HFS blink amplitude was subtracted from the normalized mean postblink amplitude. As the blink-evoking stimulus remained constant throughout the day (Ryan et al. 2014), this short-term change in blink amplitude following HFS-B was a gain change.

We calculated two measures of long-term modifications induced by HFS-B treatment. For each day of treatment, we determined the mean amplitude of 2T SO evoked blinks pre-HFS relative to the median pre-HFS amplitude on the first day of HFS-B treatment. If HFS-B treatment produced a long-term reduction in the ability of trigeminal circuits to drive blinks, then pre-HFS blink amplitude should decrease across days of HFS treatment. Second, to determine if repeated HFS-B treatment became more effective at reducing within-day blink gain with repetition, we plotted the gain change each day as a function of the number of days of treatment. If the treatment became more effective at modifying trigeminal reflex blink gain across repeated treatments, then the gain changes should be larger in later days of treatment than in the early days of HFS-B.

We employed a variety of mathematical measures to identify and characterize temporal patterns of spontaneous blinking (Kaminer et al. 2011) in addition to the typical measures of blink rate and interblink interval (IBI). For the temporal analysis, we treated each spontaneous blink as a point process, a unitary event that occurred at the start of a spontaneous blink. To identify temporal organization in the pattern of spontaneous blinking, we divided the period of data collection into 1-s bins and determined the blink rate (blinks/min) for each bin spanning the analysis period to create a blink frequency histogram. Using MATLAB scripts, we calculated the autocorrelation of the frequency histogram to identify cyclical patterns of spontaneous blinking. A fast Fourier transform (FFT) was then performed on the autocorrelation to quantify the dominant frequency of cyclical components of the spontaneous blink pattern.

We used the Fano factor, normalized variance, to characterize the periodicity of the spontaneous blink pattern further. The Fano factor was calculated using counting intervals that increased in 1-s increments. For example, the analysis period was divided into 1-s bins and the number of blinks occurring in each bin determined. The Fano factor for this counting interval was the variance divided by the mean number of blinks averaged over all bins for this counting interval. This calculation was repeated for each counting interval, e.g., 2 s, 3 s, 4 s. We plotted the Fano factor as a function of the counting intervals on a log-log plot (see Fig. 5). If the occurrence of spontaneous blinks followed a Poisson distribution, then the Fano factor would be 1 at all counting intervals. If occurrence of spontaneous blinks was not independent, however, the Fano factor increases with the counting interval (Eden and Kramer 2010).

To test whether the temporal organization of spontaneous blinks was critical in these measurements, we randomly shuffled the IBI order and recalculated the autocorrelation, FFT, and Fano factor for these randomized blink sequences. Shuffling did not change the mean blink rate or IBI distribution. For comparison with the 6-OHDA rat data in Fig. 5, we utilized data from normal rats collected in a previous study (Kaminer et al. 2011).

Statistical tests of significance (P < 0.05) were performed with SPSS software (SPSS, Chicago, IL) using an ANOVA with post hoc Tukey tests, paired or independent t-tests. Data were presented as means ± SE. Regressions were performed using linear, power, logarithmic, and exponential curves. The function generating the highest correlation was employed to describe the data.

**RESULTS**

**Blink reflex excitability.** Parkinsonian patients and the rat 6-OHDA lesion model of PD exhibit hyperexcitability of the R2, but not the R1, component of the trigeminal reflex blink circuit (Fig. 2A, top traces) (Agostino et al. 1987; Basso et al. 1993). The 130-Hz STN DBS significantly reduced R2 excitability by 50 ± 10% relative to the No DBS condition (t6 = 5.19, P < 0.01; Fig. 2, A and B) but did not alter R1 excitability significantly (t6 = −0.61, P > 0.05; Fig. 2, A, middle traces, and B). In contrast, 16-Hz STN DBS did not significantly alter either R1 (t6 = −0.52, P > 0.05; Fig. 2, A and B) or R2 (t6 = −0.17, P > 0.05; Fig. 2, A, bottom traces, and B) excitability. In reducing blink excitability, 130-Hz STN DBS could increase condition blink amplitude or decrease test blink amplitude. As with PD patients receiving 130-Hz STN DBS (Bolognini et al. 2012), DBS did not affect condition R2 amplitude in 6-OHDA lesioned rats (Fig. 2A, Condition, compare middle and top traces) (t7 = 0.16, P > 0.05). Instead, 130-Hz STN DBS reduced R2 excitability by decreasing test blink R2 amplitude (Fig. 2A, Test, compare middle and top traces) (t7 = 1.87, P = 0.052).

**Blink reflex plasticity.** Like the impaired blink reflex plasticity with PD (Battaglia et al. 2006), the 6-OHDA rat model of PD exhibited the same disruption of plasticity (t6 = 2.08, P > 0.05; Fig. 3, No DBS) (Kaminer et al. 2014). For 10 consecutive days, we tested the effects of 16-Hz STN DBS and 130-Hz STN DBS delivered throughout the blink plasticity paradigm in 6-OHDA lesioned rats. During 16-Hz STN DBS, HFS-B treatment showed insignificant reductions in blink amplitude (13.7 ± 6.3%, t5 = 2.26, P > 0.05, Fig. 3, 16 Hz).
Thus the responses of rats not receiving DBS at the 3 time points (Fig. 3A, No DBS, T0, T30, and T60) were nearly identical to those of rats receiving 16-Hz stimulation (Fig. 3A, 16 Hz, T0, T30, T60). Furthermore, no plasticity was evident as the responses at these SO stimulation times did not differ from those seen before stimulation (Fig. 3A, Pre for No DBS and 16 Hz). When rats received 130-Hz STN DBS, however, HFS-B treatment significantly decreased blink amplitude over all time points (28.4 ± 6.4%; \( t_8 = 4.73, P < 0.001 \); Fig. 3, 130 Hz) similar to the degree of depression observed in normal rats (Ryan et al. 2014). A repeated-measures ANOVA performed on five rats that received all three DBS conditions (No DBS, 130 Hz, and 16 Hz) revealed a significant main effect of treatment condition (\( F_{2,8} = 10.60, P < 0.01 \); Fig. 3B). Post hoc analyses demonstrated that the 130-Hz STN DBS condition differed significantly from the No DBS condition (\( P < 0.05 \)) and the 16-Hz STN DBS condition (\( P < 0.05 \)), but there were no significant differences between the No DBS and the 16-Hz STN DBS conditions (\( P > 0.05 \)). Therefore, 130-Hz STN DBS restored blink reflex plasticity in this rat model of PD.

The 130 Hz STN DBS may enable induction of the HFS-induced gain change, permit expression of the gain change, or affect both aspects of this blink plasticity. We investigate this issue by presenting 130-Hz STN DBS only during the HFS-B treatment (130-D). If 130-D restores normal plasticity, then abnormal basal ganglia activity with PD primarily impairs the induction of blink plasticity and does not significantly affect expression of the plasticity.

Presentation of 130-Hz STN DBS during only the HFS-B allowed HFS-B treatment to reduce blink amplitude significantly (29.6 ± 8.0%; \( t_2 = 4.52, P < 0.05 \); Fig. 3, 130-D). A repeated-measures ANOVA performed on three rats that received both 130-Hz conditions (130 and 130-D) and the No DBS condition revealed a significant main effect of treatment condition (\( F_{2,4} = 14.68, P < 0.01 \); Fig. 3). Post hoc analyses demonstrated a significant difference between the 130-D and No DBS conditions (\( P < 0.01 \)). As there were no significant differences between 130 and 130-D on gain change (\( P > 0.05 \)),

**Fig. 2.** Effects of DBS of the subthalamic nucleus at different frequencies on trigeminal reflex blink excitability. A: from the same 6-OHDA lesioned rat, individual records of condition and test blinks evoked by supraorbital nerve stimulation (▲) without DBS and while receiving 130- and 16-Hz DBS. B: excitability of trigeminal reflex blinks (test/condition) for the R1 and R2 components relative to the No DBS condition with 130- or 16-Hz DBS averaged across all rats and days. Error bars are SE.

Thus the responses of rats not receiving DBS at the 3 time points (Fig. 3A, No DBS, T0, T30, and T60) were nearly identical to those of rats receiving 16-Hz stimulation (Fig. 3A, 16 Hz, T0, T30, T60). Furthermore, no plasticity was evident as the responses at these SO stimulation times did not differ from those seen before stimulation (Fig. 3A, Pre for No DBS and 16 Hz). When rats received 130-Hz STN DBS, however, HFS-B treatment significantly decreased blink amplitude over all time points (28.4 ± 6.4%; \( t_8 = 4.73, P < 0.001 \); Fig. 3, 130 Hz) similar to the degree of depression observed in normal rats (Ryan et al. 2014). A repeated-measures ANOVA performed on five rats that received all three DBS conditions (No DBS, 130 Hz, and 16 Hz) revealed a significant main effect of treatment condition (\( F_{2,8} = 10.60, P < 0.01 \); Fig. 3B). Post hoc analyses demonstrated that the 130-Hz STN DBS condition differed significantly from the No DBS condition (\( P < 0.05 \)) and the 16-Hz STN DBS condition (\( P < 0.05 \)), but there were no significant differences between the No DBS and the 16-Hz STN DBS conditions (\( P > 0.05 \)). Therefore, 130-Hz STN DBS restored blink reflex plasticity in this rat model of PD.

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**Fig. 3.** A: individual records from one 6-OHDA lesioned rat in the plasticity paradigm designed to decrease blink amplitude. Records are taken before (Pre), immediately after (T0), 30 min after (T30), and 60 min (T60) after HFS-B treatment while the rat received either no deep brain stimulation of the subthalamic nucleus (No DBS), 130-Hz, or 16-Hz DBS throughout the entire experiment or 130-Hz STN DBS only during the HFS-B treatment (130-D). Error bars are SE.
we collapsed the groups for subsequent analyses of long-term modifications.

In normal rats, averaging gain changes across days obscured the long-term enhanced effectiveness of HFS-B treatment in depressing reflex blink gain and the progressive weakening of the trigeminal system’s drive on reflex blinks (Ryan et al. 2014). Given that 6-OHDA lesioned rats without 130-Hz STN DBS did not show short-term gain changes (Fig. 3, No DBS), we predicted that rats in this condition would not exhibit any long-term modifications. When rats did not undergo STN DBS, there was no significant difference in the gain change between the first three (6.8 ± 4.3%) and last three (13.3 ± 3.3%) days of HFS-B treatment ($t_2 = 0.01, P > 0.05$) (Fig. 4A, No DBS). The 16-Hz STN DBS that also failed to allow HFS-B short-term gain modification (Fig. 3, 16 Hz) had no significant effect on long-term gain changes ($t_2 = 1.40, P > 0.05$, Fig. 4A, 16 Hz). Although normal short-term blink plasticity occurred when rats received 130-Hz STN DBS throughout the HFS-B paradigm (130 Hz) or just during HFS-B treatment (130-D), long-term modifications might not occur because of the abnormal basal ganglia activity transpiring throughout the period between experiments. Nevertheless, the gain decrease caused by HFS-B treatment in rats receiving 130-Hz and 130-D STN DBS was significantly larger during the last three days (52.8 ± 10.9%), than during the first three days (22.4 ± 12.6%; $t_2 = 4.23, P > 0.05$) of HFS-B treatment (Fig. 4A, 130 Hz). Unlike the absence of a significant increase in gain modification for rats receiving 16-Hz DBS ($r^2 = 0.34, P > 0.05$; Fig. 4B, ▲) or No DBS ($r^2 = 0.005, P > 0.05$; Fig. 4B, ◌), there was a significant, negatively sloped line relating the amount of blink depression produced by HFS-B treatment each day, as a function of days of HFS-B treatment when receiving 130-Hz STN DBS ($r^2 = 0.42, P < 0.05$; Fig. 4B, ●).

Our study in normal rats demonstrated that the trigeminal system became less effective in evoking reflex blinks across days of HFS-B treatment, because blink amplitude before HFS-B treatment decreased across days, while blink threshold remained constant (Ryan et al. 2014). The 6-OHDA lesioned rats in the present study also did not exhibit a change in threshold across days of HFS-B treatment, while receiving either No DBS ($r^2 = 0.12, P > 0.05$, not illustrated), 130-Hz STN DBS ($r^2 = 0.01, P > 0.05$, not illustrated), or 16-Hz STN DBS ($r^2 = 0.05, P > 0.05$, not illustrated). As with normal rats (Ryan et al. 2014), 6-OHDA lesioned rats undergoing 130-Hz STN DBS exhibited a logarithmic decrease in Pre HFS R1 amplitude across days of HFS-B treatment ($r^2 = 0.56, P < 0.01$, Fig. 4C, ●). This long-term decrease did not occur in rats receiving 16-Hz STN DBS ($r^2 = 0.09, P > 0.05$; Fig. 4C, ▲). Rats that did not receive DBS exhibited a small but significant increase in pre HFS R1 blink amplitude across days ($r^2 = 0.65, P < 0.01$; Fig. 4C, ◌).

Spontaneous blinking. As with PD (Agostino et al. 2008; Bologna et al. 2012; Deuschl and Goldmeier 1998; Korosc et al. 2006), the 6-OHDA rat lesion model of PD exhibited a significantly lower spontaneous blink rate (3.2 ± 0.3 blinks/min) than normal (Kaminer et al. 2011) (5.30 ± 0.02 blinks/min; $t_0 = 2.46, P < 0.02$; Fig. 5A). Also similar to PD patients (Bologna et al. 2012), 130-Hz STN DBS, did not restore a normal blink rate in 6-OHDA lesioned rats. The blink rate with 130-Hz STN DBS, 2.9 ± 0.3 blinks/min, was not significantly different from the No DBS condition ($t_4 = 1.8, P > 0.05$; Fig. 5A).

As with dopamine receptor blockade (Kaminer et al. 2011), the 6-OHDA lesioned rat model of PD exhibited increased variability in the temporal organization of spontaneous blinking relative to normal rats. The positive slope of the counting interval-Fano factor relationship revealed underlying periodicity of spontaneous blinking (Middleton et al. 2003) and demonstrated that occurrences of spontaneous blinks were not independent processes. Although the spontaneous blinks of both control (Kaminer et al. 2011) (Fig. 5, B and C) and 6-OHDA lesioned rats (Fig. 5, C and D) exhibited a positive slope of the counting interval-Fano factor relationship, the mean slope of the Fano factor for control rats (0.42 ± 0.03) was significantly larger than that of 6-OHDA lesioned rats (0.29 ± 0.04; $t_11 = 2.2, P < 0.05$; Fig. 5B). The steeper slope indicated that the periodicity was stronger in control than in

\[ \text{Fig. 4. Long-term changes in reflex blink plasticity with 16-Hz, 130-Hz, or no STN DBS. A: mean gain change over the first three and last three days for 6-OHDA lesioned rats receiving consecutive days of No DBS, 130, or 16 Hz STN DBS. B: mean change for 6-OHDA lesioned rats receiving consecutive days of No DBS (○), 130-Hz STN DBS (●), or 16 Hz (▲) as a function of days of HFS-B treatment. Lines are linear best fits. C: average pre-HFS blink amplitude relative to median pre-HFS blink amplitude on day 1 for 6-OHDA lesioned rats receiving consecutive days of No DBS (○), 130-Hz STN DBS (●), or 16 Hz (▲) as a function of days of HFS-B treatment. Lines are logarithmic best fits. Error bars are SE.} \]
6-OHDA lesioned rats. If the periodicity was real, then shuffling the order of the interblink intervals to disrupt temporal patterns should diminish the slope of counting interval-Fano factor relationship. Shuffling significantly reduced the slope of the Fano factor for control rats ($t_{27} = 6.7, P < 0.001, 5, B$ and $C$), but not for 6-OHDA lesioned rats ($t_{4} = 0.22, P > 0.05$) or without 130-Hz STN DBS ($t_{4} = 0.36, P > 0.05$; Fig. 5, B and D).

An autocorrelation of the occurrence of spontaneous blinks provided a second measure of spontaneous blink periodicity (Fig. 5, E and F). For control rats, the autocorrelation revealed a repeating pattern of frequent blinks interspersed with a period of few blinks. Shuffling interblink interval order significantly reduced the autocorrelation periodicity (Fig. 5E). Consistent with the shallow slope of the counting interval-Fano factor relationship for 6-OHDA lesioned rats, autocorrelation demonstrated weak periodicity, and shuffling the order of interblink intervals did not appear to affect this periodicity significantly (Fig. 5F). We quantified autocorrelation periodicity by performing a fast Fourier transform on normal and shuffled data. For control rats, shuffling the order of interblink intervals significantly reduced the power at the peak frequency relative to the normal data ($t_{27} = 5.25 P < 0.001$; Fig. 5G, Control). In addition, shuffling significantly shifted the dominant frequency relative to normal data ($t_{27} = -3.15, P < 0.01$; Fig. 5H). In contrast, there was no significant difference in the peak frequency power between normal and shuffled data for 6-OHDA rats without DBS ($t_{4} = 1.26, P > 0.05$) or when receiving 130-Hz STN DBS ($t_{4} = 0.18, P > 0.05$; Fig. 5G). Moreover, there was no significant difference in the dominant frequency between normal and shuffled data with ($t_{4} = 0.51, P > 0.05$) or without 130-Hz STN DBS ($t_{4} = -0.24, P > 0.05$; Fig. 5H). Therefore, 6-OHDA lesions disrupted the normal periodicity of spontaneous blinking and 130-Hz STN DBS failed to restore a normal blink rate or temporal organization to spontaneous blinking.

**DISCUSSION**

The shared blink abnormalities in PD and the 6-OHDA lesion rat model of PD indicate that it is possible to identify circuits in rodents through which the aberrant basal ganglia
activity of PD disrupts blinking. For example, neurons in the substantia nigra pars reticulata, superior colliculus, nucleus raphe magnus, and spinal trigeminal complex comprise a trisynaptic circuit through which the basal ganglia can modulate trigeminal reflex blink excitability (Basso and Evinger 1996; Basso et al. 1996; Gnadt et al. 1997). In the paired stimulus paradigm, the condition stimulus activates trigeminal blink circuits to evoke the condition blink, and trigeminal complex neurons send an excitatory drive to intermediate/deep layer collicular neurons (Huerta et al. 1983; Nagata and Kruger 1979; Ndiaye et al. 2000; Redgrave et al. 1996; Rhoades et al. 1989). Trigeminal activation of these collicular neurons provides a transient excitatory drive onto nucleus raphe magnus neurons, which inhibit spinal trigeminal blink circuits (Basso and Evinger 1996; de Tommaso et al. 2000). Reduction in test blink amplitude takes place because the test stimulus occurs during nucleus raphe magnus inhibition of trigeminal blink circuits. The hyperexcitability of the R2 component of the trigeminal reflex blink in both PD and the rat 6-OHDA model of PD (Agostino et al. 1987; Basso et al. 1993; Deuschl and Goddeheimer 1998; Kimura 1973; Peshori et al. 2001) could result from changes in the substantia nigra input that reduce the collicular response to trigeminal input from the condition stimulus. Our demonstration that 16-Hz STN DBS creates R2 trigeminal reflex blink hyperexcitability in normal rats (Kaminer et al. 2014) supports the hypothesis that hypersynchronized beta oscillations associated with PD (Brown and Eusebio 2008; Gatev et al. 2006; Jenkinson and Brown 2011) and the 6-OHDA rat model (Lehmkuhle et al. 2009; Li et al. 2007; Mallet et al. 2008; McConnell et al. 2012; Sharott et al. 2005) can reduce the responsiveness of collicular neurons to trigeminal inputs in PD. The suppression of beta oscillations by 130-Hz STN DBS (Dorval et al. 2010; McConnell et al. 2012; Ray et al. 2008; Wingeier et al. 2006) and its restoration of normal blink reflex excitability (Fig. 2) further support the interpretation that exaggerated beta oscillations reduce collicular excitation by trigeminal inputs from the condition stimulus. This circuit organization also explains why 130-Hz STN DBS affects the test, but not the condition blink.

Although our data (Figs. 3 and 4) (Kaminer et al. 2014) and patient studies (Battaglia et al. 2006; Crupi et al. 2008; Suppa et al. 2011) highlight a critical role of the basal ganglia in normal blink reflex plasticity, the circuits through which the basal ganglia modulate this behavior are uncertain. The cerebellum plays an essential role in HFS-induced blink reflex plasticity (Ryan et al. 2014). It is likely that the disruption of normal basal ganglia-cerebellar interactions (Bostan et al. 2010; Bostan and Strick 2010; Hoshi et al. 2005; Jinnah and Hess 2006) by PD’s pathological basal ganglia activity impairs blink reflex plasticity. Given that 16-Hz STN DBS impairs blink reflex plasticity in normal rats (Kaminer et al. 2014), the hypersynchronized beta oscillations found in PD (Brown et al. 2001; Kuhn et al. 2005) and 6-OHDA lesioned rats (Lehmkuhle et al. 2009; Li et al. 2007; Mallet et al. 2008; McConnell et al. 2012; Sharott et al. 2005) are probably responsible for impairing blink plasticity in PD. The frequency of hypersynchronized basal ganglia oscillatory activity may be critical in how basal ganglia disorders affect plasticity. Dystonia, a basal ganglia disorder characterized by a lower frequency of oscillatory activity than PD (Chen et al. 2006; Liu et al. 2008; Silberstein et al. 2003; Starr et al. 2005; Weinberger et al. 2012) exaggerates blink plasticity (Quartarone et al. 2006). Consistent with this interpretation, 7-Hz STN DBS in normal rats exaggerates blink reflex plasticity (Kaminer et al. 2014).

At 130 Hz, but not 16 Hz, STN DBS restores normal blink reflex plasticity in 6-OHDA lesioned rats (Figs. 3 and 4). Given the apparent importance of hypersynchronized beta oscillations in impairing blink plasticity (Kaminer et al. 2014), 130-Hz STN DBS may restore plasticity by suppressing exaggerated beta oscillations in 6-OHDA lesioned rats (Lehmkuhle et al. 2009; Li et al. 2007; McConnell et al. 2012). Our experiment showing that delivering 130-Hz STN DBS only during the HFS-B treatment (130-D) restores blink reflex plasticity (Fig. 3) indicates that the hypersynchronized beta oscillations of PD only affect the induction of plasticity. Indeed, 130-Hz STN DBS only during the HFS-B treatment (130-D) was sufficient to restore long- as well as short-term plasticity of the blink reflex in 6-OHDA lesioned rats. Thus exaggerated beta oscillations present in the absence of 130-Hz STN DBS in 6-OHDA lesioned rats do not affect the long-term enhanced effectiveness of HFS-B treatment in depressing reflex blink gain and the progressive weakening of the trigeminal system’s drive on reflex blinks caused by repeated HFS-B treatments (Fig. 4).

Although 130-Hz STN DBS restores normal blink reflex excitability (Fig. 2) and plasticity (Figs. 3 and 4), it fails to reinstate a normal blink rate or temporal organization to spontaneous blinking (Fig. 5). Spontaneous blinking is remarkably insensitive to PD treatments. With dopamine therapy, the spontaneous blink rate of PD patients is between 50 and 60% of normal (Bologna et al. 2012; Deuschl and Goddeheimer 1998; Karson et al. 1982; Kimber and Thompson 2000), and combining STN DBS and dopamine therapy does not significantly improve the blink rate over drug treatment alone (Bologna et al. 2012). The limited effects of STN DBS on restoring normal behavior of the spontaneous blink central pattern generator are consistent with the unreliable effects of STN DBS on restoring normal gait in PD patients (Lozano and Snyder 2008; Moreau et al. 2008; St George et al. 2010).

The study of the blink reflex makes the relationship between exaggerated beta oscillations in PD and reflex movement clearer. Although PD increases the average power of beta oscillations, moment-to-moment oscillation strength varies with voluntary movement and movement cues (Brucke et al. 2012; Doyle et al. 2005; Kuhn et al. 2004; Kuhn et al. 2006; Leventhal et al. 2012; Levy et al. 2002; Liu et al. 2008; Priori et al. 2002; Williams et al. 2003). Over the long term, however, reflex blinks are uncorrelated with the temporal variations in oscillation strength associated with voluntary movement. Because of the hypersynchronized beta oscillations of PD, reflex blink circuits experience higher than normal beta oscillations on average. Our previous data demonstrate that beta frequency STN DBS is sufficient to induce Parkinsonian-like blink reflex abnormalities in normal rats (Kaminer et al. 2014), and the current data show that 130-Hz STN DBS, which suppresses hypersynchronous beta oscillations (Lehmkuhle et al. 2009; Li et al. 2007; McConnell et al. 2012), restores normal behavior of this brain stem reflex in the 6-OHDA lesion model of PD. Nevertheless, 130-Hz STN DBS does not affect the rate or temporal organization of spontaneous blinking in 6-OHDA lesion animals. This result highlights the complexity of Parkinson’s disease, as dopamine depletion does not affect all blink systems via the same neural mechanisms.

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REFERENCES
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REFERENCES
Agostino R, Berardelli A, Crucig C, Stocchi F, Manfredi M. Corneal and
blink reflexes in Parkinson’s disease with “on-off” fluctuations. Mov Disord
Agostino R, Bologna M, Dinapoli L, Gregori B, Fabbriini G, Accornero N,
Berardelli A. Voluntary, spontaneous, and reflex blinking in Parkinson’s
Basso MA, Evinger C. An explanation for reflex blink hyperexcitability in
Parkinson’s disease. II. Nucleus raphe magnus. J Neurosci 16: 7318–7330,
1996.
Basso MA, Powers AS, Evinger C. An explanation for reflex blink hyper-
excitability in Parkinson’s disease. I. Superior colliculus. J Neurosci 16:
7308–7317, 1996.
Basso MA, Strecker RE, Evinger C. Midbrain 6-hydroxydopamine lesions
Bataglia F, Ghilardi MF, Quararone A, Bagnato S, Girlanda P, Halliet
M. Impaired long-term potentiation-like plasticity of the trigeminal blink
Benabid AL, Ghilardi MF, Modugno N, Fabbriini G, Berardelli A. Effects of
subthalamic nucleus on deep brain stimulation and L-DOPA on blinking in
Bostan AC, Dum RP, Strick PL. The basal ganglia communicate with the
Bostan AC, Strick PL. The cerebellum and basal ganglia are interconnected.
Bove J, Prou D, Perier C, Przedborski S. Toxin-induced models of Parkinson’s
Bronstein JM, Tagliati M, Alterman RL, Lozano AM, Volkman J,
Stefani A, Horak FB, Okun MS, Foote KD, Krack P, Pahwa R,
Henderson J, Horak MA, Bakay RA, Rezaei A, Marks WJ Jr, Moro E,
Vitek JL, Weaver FM, Gross RE, Delong MR. Deep brain stimulation for
Parkinson disease: an expert consensus and review of key issues. Arch
Brown P, Eusebio A. Paradoxes of functional neurosurgery: clues from basal
Dopamine dependency of oscillations between subthalamic nucleus and
Brucke C, Hueli J, Schonecker T, Neumann WJ, Yarrow K, Kupsch A,
Scaling of movement is related to pallidal gamma oscillations in patients
Cenci MA, Whishaw IQ, Schallert T. Animal models of neurological
Chen CC, Kuhn AA, Trottenberg T, Kupsch A, Schneider GH, Brown P.
Neuronal activity in globus pallidus interna can be localized to specific
fields of cell activity over 3–12 Hz in patients with dystonia. Exp Neurol
Chong RK, Horak FB, Woollacott MH. Parkinson’s disease impairs the
Cody FW, MacDermott N, Matthews PB, Richardson HC. Observations on
the genetics of the stretch reflex in Parkinson’s disease. Brain 109: 229–249,
1986.
Crupi D, Ghilardi MF, Mosiello C, Di Rocco A, Quararone A, Battaglia
F, Cortical and brainstem LTP-like plasticity in Huntington’s disease. Brain
Dauvergne C, Evinger C. Experiential modification of the trigeminal reflex
de Tommaso M, Guido M, Libro G, Sciruicchio V, Puca F. Zolmitriptan
reverses blink reflex changes induced during the migraine attack in humans.
Deuschl G, Goedemeier C. Spontaneous and reflex activity of facial muscles
in dystonia, Parkinson’s disease, and in normal subjects. J Neurol Neurosurg
Dorval AD, Kuncel AM, Birdno MJ, Turner DA, Grill WM. Deep brain
stimulation alleviates parkinsonian bradykinesia by regularizing pallidal
Doyle LM, Kuhn AA, Hariz M, Kupsch A, Schneider GH, Brown P.
Levodopa-induced modulation of subthalamic beta oscillations during self-
paced movements in patients with Parkinson’s disease. Eur J Neurosci 21:
Duy sens J, Van Wezel BM, Smits-Engelsman B. Modulation of cutaneous
reflexes from the foot during gait in Parkinson’s disease. J Neurophysiol
Eden UT, Kramer MA. Drawing inferences from Fano factor calculations. J
Evinger C, Basso MA, Manning KA, Sibony PA, Pellegrini JJ, Horn AK.
A role for the basal ganglia in nicotinic modulation of the blink reflex. Exp
Gatev P, Darbin O, Wichmann T. Oscillations in the basal ganglia under
Gnaadt JW, Lu SM, Brezenen B, Basso MA, Henriquez VM, Evinger C.
Influence of the superior colliculus on the primate blink reflex. Exp Brain
Horak FB, Nutt JG, Nashner LM. Postural inflexibility in parkinsonian
Hoshi E, Tremblay L, Feger J, Carras PL, Strick PL. The cerebellum
communicates with the basal ganglia. Nat Neurosci 8: 1491–1493, 2005.
Huerta MF, Frantartur A, Harting JK. Studies of the principal sensory and
spinal trigeminal nuclei of the rat: projections to the superior colliculus,
Jenkinson N, Brown P. New insights into the relationship between dopamine,
Jinnah HA, Hess EJ. A new twist on the anatomy of dystonia: the basal
Kaminer J, Powers AS, Horn KG, Hui C, Evinger C. Characterizing the
11267, 2011.
Kaminer J, Thakur P, Evinger C. Frequency matters: beta-band subthalamic
circuit induces Parkinsonian-like blink abnormalities in normal rats. Exp
Korosec M, Zidar I, Reits D, Evinger C, Vanderwerf F. Eyelid movements
during blinks in patients with Parkinson’s disease. Mov Disord 21:
Kuhn AA, Brandt SA, Kupsch A, Trottenberg T, Brocke J, Ihrlieker C,
Schneider GH, Meyer BU. Comparison of motor effects following sub-
cortical electrical stimulation through electrodes in the globus pallidus
internus and cortical transcranial magnetic stimulation. Exp Brain Res 155:

J Neurophysiol • doi:10.1152/jn.01072.2014 • www.jn.org