Medial frontal ~4 Hz activity in humans and rodents is attenuated in PD patients and in rodents with cortical dopamine depletion

Krystal L. Parker¹*, Kuan-Hua Chen¹*, Johnathan R. Kingyon¹, James F. Cavanagh³, Nandakumar S. Narayanan¹,²

¹- Department of Neurology, 2- Aging Mind and Brain Initiative, Carver College of Medicine, University of Iowa, Iowa City, IA, and 3- Department of Psychology, University of New Mexico

Running title: MFC ~4 Hz rhythms and PD

Keywords: Medial frontal cortex; dopamine; Parkinson’s disease; interval timing

Abbreviations: MFC: Medial frontal Cortex; PD: Parkinson’s disease

*-These authors contributed equally to this work

Pages: 36 Figures: 7 Tables: 3

Word Count: Abstract: 170 Introduction: 634 Discussion: 1375

Conflict of Interest: The authors declare no competing financial interests.

Acknowledgements: We would like to acknowledge Ergun Uc for advice of subject recruitment, Steven W. Anderson for advice on cognitive testing, Deanne Tadlock for technical consult advice, and Tracy Lukasiewicz for data entry.

CONTACT
Nandakumar Narayanan
319-353-5698
Nandakumar-narayanan@uiowa.edu
169 Newton Road
Pappajohn Biomedical Discovery Building – 1336
University of Iowa, Iowa City, 52242
ABSTRACT

The temporal control of action is a highly conserved and critical mammalian behavior. Here, we investigate the neuronal basis of this process using an interval timing task. In rats and humans, instructional timing cues triggered spectral power across delta and theta bands (2-6 Hz) from the medial frontal cortex (MFC). Humans and rodents with dysfunctional dopamine have impaired interval timing, and we found that both humans with Parkinson’s disease (PD) and rodents with local MFC dopamine depletion had attenuated delta and theta activity. In rodents, spectral activity in this range could functionally couple single MFC neurons involved in temporal processing. Without MFC dopamine, these neurons had less functional coupling with delta/theta activity and less temporal processing. Finally, in humans this 2-6 Hz activity was correlated with executive function in matched controls but not in PD patients. Collectively these findings suggest that cue-evoked low-frequency rhythms could be a clinically important biomarker of PD that is translatable to rodent models, facilitating mechanistic inquiry and the development of neurophysiological biomarkers for human disease.
INTRODUCTION

Temporal control of action, or guiding movements in time to achieve behavioral goals, is a crucial function of mammalian nervous systems. This process depends on the integrated activity of corticostriatal systems (Matell et al., 2003; Buhusi and Meck, 2005; Jahanshahi et al., 2010), and requires intact dopaminergic signaling (Drew et al., 2003). Patients with Parkinson’s disease (PD) with depleted dopamine have dramatically impaired temporal control (Malapani et al., 1998). Despite these data, the neural circuitry influenced by dopamine during temporal computations is not understood.

Here we study this issue in PD patients and in animal models by investigating the neural basis of an elementary cognitive task: interval timing. In this task, participants estimate an interval of several seconds as instructed by a cue. In the range of seconds, interval timing requires executive resources such as working memory and attention to time (Brown, 2006; Parker et al., 2013) and is consistently impaired in patients with PD (Malapani et al., 1998; Buhusi and Meck, 2005; Merchant et al., 2008). Because this task is highly conserved across mammalian species (Buhusi and Meck, 2005; Merchant et al., 2013) it can be rapidly trained in rodent models, facilitating mechanistic hypothesis testing (Drew et al., 2003; Narayanan et al., 2012).

Controlling the timing of action requires the integrated activity of corticostriatal circuits (Hinton and Meck, 2004; Matell and Meck, 2004, 2004) that are dysfunctional in PD patients (Jahanshahi et al., 2010). Recent work has demonstrated that the medial frontal cortex (MFC) is necessary for the temporal control of action. Inactivation of MFC in rodents profoundly impairs interval timing (Narayanan et al., 2012; Kim et al., 2013). Blocking D1-type dopamine receptors impairs both interval timing performance and neural activity correlated with temporal control.
(Narayanan et al., 2012; Parker et al., 2014). Sustained activity of single MFC neurons appears to be involved in temporal processing across species including humans, primates, and rodents (Niki and Watanabe, 1979; Narayanan and Laubach, 2009; Sheth et al., 2012; Kim et al., 2013). These single MFC neurons are strongly functionally coupled with delta (1-4 Hz) and theta (4-8 Hz) rhythms, which can be attenuated by focal D1 dopamine blockade in the MFC (Parker et al., 2014). Collectively, these findings suggest that functional coupling of D1 neurons underlies the ability to estimate temporal durations.

In humans, EEG events in theta and delta frequencies from medial frontal cortex can index time-based decision making processes (van Rijn et al., 2011). Mid-frontal low-frequency oscillations are suggested to be a candidate mechanism for cognitive control, underlying a variety of flexible behaviors (Cavanagh et al., 2012; Cavanagh and Frank, 2014). These findings motivate the specific hypothesis that low-frequency activity in mid-frontal cortex is involved in temporal processing, and may be attenuated in PD patients as a consequence of diminished dopamine signaling in MFC neurons. This idea has implications for many basic cognitive operations.

To test this hypothesis, we recorded EEG activity from non-demented PD patients and age- and education-matched controls during performance of an interval timing task. We also recorded local field potentials (LFPs) and single MFC neurons from rodents performing an interval timing task before and after focal dopamine depletion, allowing us to investigate how dopamine influences temporal processing by single MFC neurons. We report four main results: a) humans and rodents have cue-triggered delta and theta spectral activity during interval timing, b) this activity was attenuated in PD and in rodent models involving focal MFC dopamine depletion, c) single MFC neurons were functionally coupled with field potentials at ~2-6 Hz, and
this coupling as well as temporal processing by these neurons was attenuated with dopamine
depletion, and d) in humans, delta/theta activity was correlated with executive function in
controls but not PD patients. These data implicate low-frequency activity in MFC as a
mechanism of cue-related temporal control requiring intact cortical dopamine.

EXPERIMENTAL PROCEDURES

Human Participants: Informed consent was obtained and all procedures complied with the
Institutional Review Board at the University of Iowa. In an initial pilot experiment, data from 13
young undergraduates were collected. In the main study, data from 13 humans with PD and 13
age- and education-matched control participants were enrolled in this study (Table 1). PD
patients were recruited from the movement-disorders clinic or a PD patient registry administered
by Dr. Ergun Uc at the University of Iowa as part of a study on exercise (Uc et al., 2014). All
participants had normal or corrected-to-normal vision and were not demented at the time of
evaluation (Montreal Cognitive Assessment or MOCA score ≥ 26). Control and piloting
participants had no history of neurological conditions and were free from current psychoactive
medication use. Patients with PD did not have other confounding neurological or psychiatric
disorders.

Procedure: Eleven PD patients were currently taking levodopa. Two patients were not on
levodopa but their levodopa dose equivalencies for the dopamine agonist pramipexole are
included in Table 1 (Tomlinson et al., 2010). PD patients were asked to take medication as usual.
Upon arrival, participants provided informed consent in accordance with the Institutional Review Board at the University of Iowa. Electrodes for EEG (described below) and vertical electrooculogram (VEOG) recording were then attached. Participants performed the interval timing task for approximately one hour. In addition, a battery of neuropsychological tests and a neurological test were administered. All examinations were conducted in the morning. All participants were debriefed at the end of their research participation.

Cognitive & Neurological Assessment: Cognitive function was assessed using a battery of neuropsychological tests targeting the executive function domain. Motor status was evaluated by part III of the Unified Parkinson’s Disease Rating Score (UPDRS III). All patients were less than Hoehn and Yahr Stage 3 when under medication.

The Trail Making Test (TMT) parts A and B examined the cognitive flexibility in switching attention between two competing tasks, with higher scores indicating worse performance. Difference in time to complete part A and B was scored (Tombaugh, 2004). The Stroop task examined the ability to inhibit dominant responses. Interference scores from the original version (Stroop 1) and revised version (Stroop 2) were used. Higher scores corresponded to better performance (Baldo et al., 2001). Verbal Fluency (VF) examines cognitive flexibility. The task consists of three 1-minute trials. Participants were asked to generate words that began with F, A, and S in each trial, respectively. The total number of words generated was scored (Baldo et al., 2001). Digit Span Forward and Backward (DFB) examined working memory, where the participants repeat two to nine digits in either the forward or backward orders. The number of correct repetitions was scored (Anderson et al., 1991). The Wisconsin Card Sort Test
(WCST) examined the ability to "learn new rules" and "shift from old to new rules" based on
dynamic feedbacks provided by the experimenter. Perseverative error was scored (Robinson et
al., 1980).

Human interval timing task: The interval timing task consisted of 4 blocks of 40 trials (160
trials in total). In each trial, participants were asked to estimate a time period of 3 or 12 s (Fig
1A). Trials were presented in pseudorandom order. All trials began when a numerical cue
stimulus appeared on the center of the screen indicating the temporal interval the participants
were instructed to estimate (3 or 12 s). Participants made responses by pressing the space bar on
a keyboard using their dominant hand when they estimated the temporal interval had elapsed.
Participants received feedback about their response time at the end of each trial. There was a 3-6
s interval between response and feedback. After feedback, participants moved to the next trial by
pressing the space bar again. The task was self-paced and the participants were asked not to
count in their head during the task. Participants performed 4 practice trials prior to the real task.
For pilot experiments in young subjects, there was a 1 second pause between pressing space bar
(to start a trial) and the appearance of the next numerical cue stimulus (3 or 12 s).

EEG Recording and Preprocessing: EEG was recorded on a Nihon Kohden system with a
sampling rate of 500 Hz. EEG was recorded from 21 channels based on the 10-20 system (Fz,
Cz, Pz, FP1/2, F3/4, C3/4, P3/4, F7/8, T3/4, T5/6, O1/2, M1/2), as well as left-eye VEOG and
ground (forehead). Impedance of all electrodes was below 5 kΩ. Continuous data were parsed in
to 16 s epochs (-2 to 14 seconds following the cue) and re-referenced to the mathematical
average of the two mastoid channels, yielding a total of 19 scalp EEG channels. Bad channels were interpolated (11 participants had one interpolated electrode, one had two interpolated, 14 had none; mid-frontal leads were never interpolated) and bad epochs were rejected (median number of rejected epochs were eight for patients and seven for controls). Eye blinks and horizontal eye movements were removed following independent component analysis from EEGLab (Delorme and Makeig, 2004). For cue-related activity, our exploratory analyses suggested that cue-related activity on Int3 and Int12 trials were not significantly different for human or rodents, all subsequent analyses of EEG and field potentials collapsed across Int3 and Int12 trials. For boxplots, outliers were defined as those 1.5 interquartile ranges outside of Q1 and Q3 (Tukey, 1977).

**Rodent Operant Behavior:** Eleven Long-Evans rats (aged two months; 200-225 g) were trained to perform interval timing tasks using standard operant procedures and by motivation through regulated access to water, while food was available *ad libitum*. The Animal Care and Use Committee at the University of Iowa approved all procedures. Rats were motivated by regulated access to water. Rats consumed 10-15 ml of water during each behavioral session, and additional water (5-10 ml) was provided 1-3 hours after each behavioral session in the home cage. Single housing and a 12 hour light/dark cycle was used; all experiments took place during the light cycle. Rats were maintained at ~90% of their free-access body weight during the course of these experiments and received one day of free access to water per week.

To learn interval timing tasks, animals first learned to make operant lever presses to receive liquid rewards (Narayanan et al., 2006). After fixed-ratio training, animals were trained
in a 12 s fixed-interval timing task in which rewards were delivered for responses after a 12 s interval (Fig 1A). Rewarded presses were signaled by a click and an ‘off’ houselight. Each rewarded trial was followed by a 6, 8, 10 or 12 s pseudorandom intertrial interval which concluded with an ‘on’ houselight signaling the beginning of the next trial. All behavioral devices were extinguished during the intertrial interval. For some animals in some sessions, the intertrial interval began 6 seconds after interval end if the animal did not respond. Early responses occurring before interval end were not reinforced. After animals learned the 12 s interval as indicated by a peak in their time-response histograms, a secondary delay of 3 s was added (either before/after implantation). The short interval was signaled with an additional light on the left side of the drinking tube. All behavior took place in operant chambers (MedAssociates, St Albans, VT) were equipped with a lever and a drinking tube. Behavioral arenas were housed in sound-attenuating chambers (MedAssociates). Water rewards were delivered via a pump (MedAssociates) connected to a standard metal drinking tube (AnCare) via Tygon tubing.

Rodent Surgical Procedures: Surgical, Infusion, and Perfusion Procedures: Rats trained in the interval timing procedure were implanted with a 33-gauge infusion cannula (Plastics One; n=11) in the MFC according to procedures described previously (Narayanan et al., 2006; Parker et al., 2014). Eight of these animals also had microwire arrays implanted into MFC. Briefly, animals were anesthetized using Ketamine (100 mg/kg) and Xylazine (10 mg/kg). A surgical level of anesthesia was maintained with hourly (or as needed) ketamine supplements (10 mg/kg). Under aseptic surgical conditions, the scalp was retracted, and the skull was leveled between bregma and lambda. A single craniotomy over the area above the left MFC and four holes for
skull screws were drilled. A microelectrode array configured in 4x4 (n=1) or 2x8 (n=7) arrays of 50 μm stainless steel wires (250 μm between wires and rows; impedance measured in vitro at 400-600 kΩ; Plexon: Dallas, TX) were implanted in eight animals (coordinates from bregma: AP +3.2, ML ±1.2, DV -3.5 @ 12° in the lateral plane). Electrode ground wire was wrapped around the skull screws. The electrode array was inserted while concurrently recording neuronal activity to verify implantation in layer II/III of the MFC. The infusion cannula was then lowered at an angle to target the neurons being recorded (coordinates from bregma: AP +0.6, ML ±1.0, DV -4.6 @ 40° in the lateral plane). The craniotomy was sealed with cyanoacrylate ('SloZap', Pacer Technologies, Rancho Cucamonga, CA) accelerated by 'ZipKicker' (Pacer Technologies), and methyl methacrylate (i.e., dental cement; AM Systems, Port Angeles, WA). Following implantation, animals recovered for one week before being reacclimatized to behavioral and recording procedures. Animals were lightly anesthetized with isoflorane via a nosecone for 5 min, recording cables were attached, and the animal was allowed to recover for 30 minutes before being tested in the interval timing task. After full recovery, neuronal activity was recorded during a normal pre-injection period followed by medial frontal dopamine depletion using a focal infusion of 3 µL of 1 µg/µL 6-hydroxydopamine (6-OHDA) into MFC according to procedures described previously (Narayanan et al., 2006).

Following completion of recording experiments, rats were anesthetized, sacrificed by injections of 100 mg/kg sodium pentobarbital, and transcardially perfused with 4% formalin. Brains were postfixed in a solution of 4% formalin and 20% sucrose before being sectioned on a freezing microtome. Brain slices were mounted on gelatin-subbed slides and stained for immunofluorescence using tyrosine hydroxylase (TH; Rabbit anti-TH; Millipore; 1:500), with secondary antibody (Alexa Flour 568 goat anti-rabbit IgG, 1:400, Invitrogen, Grand Island, NY,
USA), and stained for cell bodies using DAPI. Histological reconstruction was completed by analyzing electrode and cannula placements in the MFC (Fig 2A). Fluorescent microscopy revealed the intensity of TH staining in dopaminergic axons in the MFC between the injected (left) and non-injected (right) control side (Fig 2B). Mean intensity was measured using the Photoshop histogram feature (Fig 2C). Higher intensity indicated the presence of more TH immunofluorescent axons.

Neurophysiological Recordings: Neuronal activity was recorded immediately before, and 5-7 days after MFC 6-OHDA infusion. Neuronal ensemble recordings in the MFC were made using a multi-electrode recording system (Plexon, Dallas, TX). Putative single neuronal units were identified on-line using an oscilloscope and audio monitor. The Plexon Off-Line Sorter program was used to analyze the signals after the experiment completion to remove artifacts. Spike activity was analyzed for all cells that fired at rates above 0.1 Hz. Statistical summaries were based on all recorded neurons. No subpopulations were selected or filtered out of the neuron database. Local field potential was recorded using wide-band boards with bandpass filters between 0.07 and 8000 Hz. Principal component analysis (PCA) and waveform shape were used for spike sorting. Single units were identified as having 1) consistent waveform shape, 2) separable clusters in PCA space, 3) average amplitude estimated at least three times larger than background activity and 4) a consistent refractory period of at least 0.002 s in interspike interval histograms. All local field potentials were averaged over the recording array and compared to single neuron activity on each electrode. Analysis of neuronal activity and quantitative analysis of basic firing properties were carried out using NeuroExplorer (Nex Technologies, Littleton,
MA), and with custom routines for MATLAB. Peri-event rasters and average histograms were constructed around light on, lever release, lever press, and lick.

**Time-frequency and statistical analysis**: Time-frequency measures were computed by multiplying the fast Fourier transformed (FFT) power spectrum of single trial EEG data with the FFT power spectrum of a set of complex Morlet wavelets (defined as a Gaussian-windowed complex sine wave: \( e^{i2\pi tf} e^{-t^2/(2 \times \sigma^2)} \), where \( t \) is time, \( f \) is frequency (which increased from 1 to 50 Hz in 50 logarithmically-spaced steps), and \( \sigma \) defines the width (or “cycles”) of each frequency band, set according to \( 4/(2\pi f) \), and taking the inverse FFT. The end result of this process is identical to time-domain signal convolution, and it resulted in estimates of instantaneous power (the magnitude of the analytic signal). Each epoch was then cut in length (-0.5 to +2 s). Power was normalized by conversion to a decibel (dB) scale \( (10\times\log_{10}(\text{power}/\text{power}_{\text{baseline}})) \), allowing a direct comparison of effects across frequency bands (Cohen, 2014). The baseline for each frequency consisted of the average power from -0.5 to -0.3 s prior to the onset of the cues.

Human time-frequency plots and ERPs are displayed from the Cz electrode. Statistical significance against the baseline was computed via a paired t-test and is indicated by contours in the time-frequency plots, with a minimum threshold of a 500-pixel cluster size. Correction for multiple comparisons were not run because the low N does not facilitate effective permutation of participants between groups, thus inflating the range of critical values to a degree that precludes data-driven correction in a special population. To ameliorate this problem, a similarly powered pilot study was run to identify Regions of Interest (ROI), obviating the need for data-driven
correction since most of the time-frequency activity was not identified to be of interest. This pilot study identified a low-frequency (~2-6 Hz) burst of activity immediately following the cue that was similar to ROIs previously studied in humans and rats (Narayanan et al., 2013a).

To examine the time-frequency component of interactions between individual spikes and the field potential, we applied spike-field coherence analysis using the Neurospec toolbox (Rosenberg et al., 1989), in which multivariate Fourier analysis was used to extract phase-locking among spike trains and local field potentials. Coherence and phase between spikes and fields was calculated using type ‘2’ analysis with the Neurospec function sp2a_m.m. Theta coherence was calculated with sliding windows 1.024 s (1024 points at 1 kHz), shifting by increments of 100 ms, resulting in ~0.9 Hz frequency resolution. A hanning window with full cosine taper and line frequency suppression was used. Phase-locking coherence values varied from 0 to 1, where 0 indicates no coherence, and 1 indicates perfect coherence. For coherence, an average of LFP across the array was compared to individual neuronal activity. Spike-field coherence was normalized to 95% confidence intervals of coherence to compare across neurons, animals, and sessions. Confidence intervals were verified by bootstrapping time-shuffled data.

RESULTS

Interval timing and dopamine in humans and rodents.

We collected EEG data from 13 PD patients and 13 age- and education-matched controls. There were no significant differences of age, education, or MOCA scores between groups (MOCA mean±SEM: 28.6±0.4 vs. 27.9±0.4; Table 1). PD patients had 633 mg±93 mg dose of levodopa including the levodopa equivalencies of Pramipexole for two of the patients (Table 1).
Consistent with previous reports (Malapani et al., 1998; Buhusi and Meck, 2005; Merchant et al., 2008), patients with PD were impaired on the interval timing task with 3 and 12 s intervals (Int3 and Int12, respectively; Fig 1B; Table 2). A mixed ANOVA revealed that across participants, there was a main effect of both PD and interval, as well as a significant interaction term (F=100.8, p<<0.05). We measured interval timing performance using a curvature index that increases as responses are guided by time during interval timing tasks (Fry et al., 1960; Narayanan et al., 2012; Parker et al., 2014). Around 12 s peaks, humans with PD response-time distributions were less curved compared to controls (curvature index 0.17±0.6 vs. 0.0±0.04; t(1,24)=2.16, p<0.04), while around 3 s peaks, curvature was not significantly different (curvature index -0.47±0.08 vs. -0.41±0.04; t(1,24)=1.5, p<0.14). These data are consistent with prior work suggesting that patients with PD have impaired interval timing due to ‘temporal migration’, responding faster at long delays (Malapani et al., 1998).

Rodent behavior was significantly more variable than humans, possibly as a result of distinct behavioral strategies (Greater SEMs for humans compared to rats: t(1,81)=9.1, p<<0.05; Table 2; Fig 1C). The variability of rodent behavior observed in this study was in the range of previous studies of interval timing in rodents (Meck, 2006; Narayanan et al., 2012; Kim et al., 2013; Parker et al., 2014; Xu et al., 2014). Nonetheless, a repeated-measures ANOVA revealed that across rodents, there was a main effect of both MFC 6-OHDA and interval, as well as a significant interaction term (Table 2; F = 7.7, p<0.005). In sessions after unilateral MFC dopamine depletion (Fig 1C; Fig 2), animals pressed the lever less often overall (147±49 vs. 49±7; t(1,8) = 2.4, p<0.05). Rodent dopamine depletion also decreased curvature indices for 12 s trials only (Int3: -0.36±0.04 vs. -0.32±0.06; Int12 0.0±0.05 vs. -0.32±0.08; t(1,8) = 2.9, p <0.02; Fig 1C). Consistent with our prior work, these data suggest that unilateral rodent MFC dopamine...
depletion modeled aspects of PD in humans (Narayanan et al., 2012; Parker et al., 2014). Despite this, rodents still had different response times on Int3 and Int12 trials in control and dopamine depleted session (control: t(1,8)=11.3, p <<0.05; 6-OHDA: t(1,8)=3.2, p <0.01). These data suggest that disrupting dopamine impaired interval timing performance in both species by attenuating response-timing curves on Int12 trials.

*Interval timing involves MFC delta and theta activity in humans and rodents.*

To explore the neurophysiological basis of interval timing, we recorded EEG data from 11 young participants during an interval timing task with 3 and 12 s delays (see Table 2 for behavioral data). We found consistent cue-related potentials (Fig 3A) and delta/theta spectral activity occurring ~0-0.5 s after instructional cue (~4-7 Hz; Fig 3B). Our previous work have suggested that humans and rodents had similar patterns of activity in this range at critical moments during behavior (Narayanan et al., 2013a). To test this idea, we recorded LFPs from the MFC of 5 rodents. This signal represents the integrated synaptic activity of a brain region and has some similarities to EEG in humans (Murthy and Fetz, 1996). We found that in these rodents, event-related potentials and 2-6 Hz bursts of activity triggered by the cue during interval timing tasks (Fig 3C-D). Activity in this band is a well-described mechanism of cognitive control (Harmony, 2013; Cavanagh and Frank, 2014). For the remainder of the manuscript, we focus on and test hypotheses about these cue-related delta/theta oscillations.

*MFC delta/theta activity in humans and rodents requires intact dopamine circuits.*

We hypothesized that delta/theta activity in MFC would be attenuated in patients with PD and in PD animal models with dysfunctional dopamine signaling (allowing for some temporal
slowing and frequency decline in these ROI boundaries due to the advanced age of the PD and control participants, cf: Kok, 2000; Polich, 1997; Picton et al., 1984). To directly test this idea, we recorded EEG data from 13 patients with PD and 13 age- and education-matched controls using a standard clinical 19-lead montage. We found obligatory cue-locked ERPs in PD and in matched controls from mid-frontal lead Cz, with peaks approximately 0.2 s from the onset of the cue (Fig 4A, left; data from 3 s and 12 s trials were combined). Voltage topography was focused over MFC in matched controls as determined within the limits of a 19-lead clinical montage (0.1-0.2 s after cue; Fig 4A, right). Both matched control and PD groups had a vertex/mid-frontal orientation of event-related activity to the cue.

Consistent with previous findings and with our initial experiments in young humans (Fig 3), significant activity in delta and theta was observed in matched controls (Fig 4B; compared to the -0.5 to -0.3 s baseline). This oscillation was attenuated in humans with PD, who had significant de-activation in this range (Fig 4C). A direct comparison of these signals revealed significantly less ~2-5 Hz activity 0.3-0.5 s after the cue in PD patients compared to age-matched controls (Fig 4D). These data support the hypothesis that MFC delta/theta rhythms are diminished in PD.

Our recent work established that in rodents, MFC delta/theta rhythms are decreased with D1 dopamine receptor blockade (Parker et al., 2014). These data predict that depletion of MFC dopamine will attenuate delta/theta rhythms. Consistent with our prior work, we observed spectral activity from ~2-5 Hz in MFC LFPs (Fig 4E) (Narayanan et al., 2013a; Parker et al., 2014). This activity significantly attenuated in rodents after focal MFC dopamine depletion using the toxin 6-OHDA (Fig 4F-G).
We examined the distribution of delta/theta activity in PD and in animal models. Both human and rodents had normal distributions of cue-triggered delta/theta activity (0.3-0.5 s after cue onset, 2-5 Hz; Jarque-Bera test for normality; p <0.42 for humans; p <0.13 for rats). Both control humans and rats had similar strengths of cue-triggered delta/theta activity (t(1,30)=0.1, p < 0.92). PD patients had significantly less cue-triggered delta/theta activity compared to matched controls (t(1,24)=2.4, p < 0.03; Fig 5A). A similar effect was also observed in rodents (paired t(1,18) = 2.7, p < 0.02; Fig 5B). Taken together, these data indicate that in both human PD and in animal models, delta/theta activity depends on intact dopaminergic circuitry. While activation and deactivation in other bands were observed, we restricted our interpretation to cue-related delta/theta activity as predicted by our pilot experiment and past work. These data provide evidence that delta/theta activity triggered by the cue requires medial frontal dopamine in humans and rodents.

MFC neurons are functionally coupled to delta/theta activity.

Next, we examined MFC single neuron activity in detail. These neurons are intimately involved in temporal processing (Niki and Watanabe, 1979; Ito et al., 2003; Sheth et al., 2012; Kim et al., 2013; Parker et al., 2014; Xu et al., 2014). Here, we recorded MFC neurons from the same neurons with which we recorded MFC LFPs, facilitating analysis of how cue-related MFC LFP delta/theta activity was related to single MFC neurons during interval timing.

We recorded MFC neurons 1 week after MFC dopamine depletion (109 vs. 101 in MFC 6-OHDA sessions). Among these neurons, dopamine depletion did not change overall firing rate (2.6±0.2 vs. 2.2±0.3; t(1,208) = 0.7, p>0.5) or modulation around behavioral events such as house
light (7 vs. 6 neurons in MFC 6-OHDA sessions) or lever press (12 vs. 13 neurons in MFC 6-
OHDA sessions).

To formally quantify relationships between MFC neural activity and LFPs, we used
spike-field coherence (Rosenberg et al., 1989). Across 109 neurons, we found significant average
spike-field coherence (Fig 6A top; spike-field coherence plotted relative to 95% confidence
interval for coherence) in delta/theta ranges around the time of the imperative stimuli. This
coherence was also observed in our prior work (Parker et al., 2014). With MFC 6-OHDA, this
pattern of average coherence across 101 MFC neurons appeared to be distinct (Figure 6A
middle). A direct comparison of coherence between control and MFC revealed that there was
significantly more coherence in control compared to MFC 6-OHDA sessions (spike-field
coherence 2-6 Hz 0-2 s after cue; $t_{(1,208)} = 2.6, p<0.001$; Fig 6A bottom).

To investigate how MFC neurons involved in temporal processing were influenced by
dopamine, we examined neuronal activity in MFC after dopamine depletion. While there are
diverse patterns of temporal processing within MFC, one pattern is ‘ramping’ activity that
integrates temporal evidence during timing tasks consistently changes over the temporal interval
(Durstewitz, 2003; Parker et al., 2014). Single neurons could decrease firing over the interval on
Int12 trials, with an inflection point after 3 s (the timing that reward would be available on Int3
trials; Fig 6B). On average, the firing rate in MFC 6-OHDA sessions was significantly less
immediately after the cue (0-2 s after cue; $t_{(1,208)} = 2.2, p<0.03$; Fig 6C-D). Taken together, these
data indicate that after MFC 6-OHDA, single MFC neurons tended to be less coherent with MFC
fields at delta/theta bands and less active immediately after the cue during interval timing tasks.
Delta/theta activity correlates with executive function.

The preceding sections establish that low-frequency spectral activity in MFC is attenuated in PD patients and attenuates temporal processing by single MFC neurons in rodent models. If this activity was related to cognitive performance during interval timing, then it should correlate with executive functions. In our patient populations, we performed a battery of standard neuropsychological tests focusing on this domain, including Stroop tests, Trail-Making A and B (TMT), Digit Forward and Backwards (DFB), Verbal Fluency (VF), and Wisconsin Card-sorting (WCST). We focused only on tests in which PD patients and controls did not differ (Stroop, TMT, WCST). A composite cognitive index was generated by averaging test scores using rectified z-score where higher z-scores correspond to better executive functions. We correlated cognitive performance with spectral activity that was significantly distinct between control and PD patients (2-4 Hz; 0.3-0.5 s after the cue; Fig 4B-C).

In controls, cue-related spectral activity was significantly correlated with composite executive indices (R=0.75, p<0.004). This was not true in PD patients. This finding was replicated in both the Int3 and Int12 trials (Int3: R=0.61, p <0.03; Int12 R=0.70, p<0.01). A Fisher’s R-to-Z transformation indicated that this relationship was significantly different between PD patients and controls (T=2.6, p<0.007; Fig 7). These data indicate that MFC low-frequency spectral activity in medial frontal cortex correlates with executive function during interval timing, and this relationship is aberrant in PD.
DISCUSSION

We studied the neurophysiological basis of interval timing in humans and rodents, as well as how neural activity is affected by disruption in dopamine signaling in PD and in animal models of PD. We found four major results. First, humans and rodents had cue-triggered delta/theta band MFC activity during interval timing. Second, this activity was attenuated in patients with PD and in rodent models with focal MFC dopamine depletion. Third, single MFC neurons involved in temporal processing were functionally coupled with delta/theta rhythms and attenuated by MFC dopamine depletion. Finally cue-related delta/theta activity correlated with executive performance in controls but not PD, suggesting a disease-specific (and not treatment-specific) aberration in basic mechanisms that contribute to elementary cognitive processes.

Neuronal oscillations in low frequencies are a consistent correlate of cognitive control in adaptation, error, uncertainty, conflict, and surprise (Cavanagh et al., 2009; Harmony, 2013; Cavanagh and Shackman, 2014). Low-frequencies have been observed in rodent MFC (Narayanan et al., 2013a; Parker et al., 2014; Warren et al., 2014) and are observed again here triggered by the cue. It is likely that these signals are not unique to timing tasks, as they may represent an alerting or orienting response signifying a generic need for cognitive control (Cavanagh and Frank, 2014). Our data do not indicate that these signals are directly predictive of timing behavior. Rather, they are present at the time of cue, altered in humans and rodents with dysfunctional medial frontal dopamine, and can be coherent with medial frontal neurons that encode temporal signals (Niki and Watanabe, 1979; Durstewitz, 2003; Matell and Meck, 2004; Bekolay et al., 2014). This manuscript and prior work indicates that delta/theta oscillations could be involved in synchronizing neurons involved in temporal processing at the time of cue (Parker et al., 2014). Directly testing this hypothesis would involve manipulating these oscillations
independent of medial frontal neurons, which would require understanding the source of
delta/theta activity in the cortex.

Although it is clear that delta and theta activity is generated in the MFC networks, the
exact origin is still unclear. MCC has been shown to generate theta in humans and non-human
primates (Wang et al., 2005; Tsujimoto et al., 2010; Womelsdorf et al., 2010), but it is not known
how much of the scalp-recorded signal is comprised of MCC-generated activity. Our prior work
(Parker et al., 2014) and data in this manuscript demonstrate that disrupting dopamine signaling
in the MFC attenuates interval timing behavior, cue-related delta/theta activity and theta/delta
coupling with single neurons. However, with our current approach we cannot infer causal
relationships between cortical dopamine, temporal processing, and spectral activity. Our methods
also don’t indicate if our spikes are coherent with delta/theta activity, or if both phenomenon are
coherent with some other process, such as behavior or ascending neuromodulatory activity (Aru
et al., 2015). We observe decreased cue-related field potential activity in the delta/theta range,
spike-field coherence in delta/theta range as well as decreased temporal modulation by MFC
neurons with dopamine depletion. This decrease in spike-field coherence may not be
independent from the field-potential results. We cannot determine how these phenomena are
related, as decreased spike-field coupling that we observe in MFC 6-OHDA sessions could
simply be a related correlate of decreased field potential power in this range, and the
physiological significance of this loss of coupling is difficult to determine. Optogenetic

techniques that can independently manipulate spiking activity may provide insight into the
relationship between these phenomena.

Our data are among the first to report alterations in these frequencies in patients and
animal models of PD in MFC. In previous studies, it was found that temporal processing in PD
appears to markedly affect beta and alpha rhythms in PD in motor cortex, although previous
investigators did not focus on lower frequencies (Praamstra and Pope, 2007). Recent work has
implicated decreased theta in addition to alpha and beta frequencies in diagnosis of PD, in
distinct brain regions (Praamstra and Pope, 2007; Han et al., 2013; Benz et al., 2014; Gu et al.,
2014). Our work extends this line of research by focusing on medial frontal cortex and linking
spectral activity in PD patients with mechanistic theories (Cavanagh and Frank, 2014) and single
neuron activity that is consistently involved in temporal processing (Niki and Watanabe, 1979;
Matell and Meck, 2004; Narayanan and Laubach, 2009; Kim et al., 2013; Bekolay et al., 2014;
Parker et al., 2014; Xu et al., 2014).

The findings reported here suggest that cortical dopamine is a significant contributor to
temporal processing by medial frontal brain networks (Narayanan et al., 2013b). In MFC,
cortical dopamine appears to act via D1 dopamine receptors to achieve temporal control. Focal
MFC administration of D1 but not D2 antagonists selectively impairs interval timing (Narayanan
et al., 2012) and temporal processing in reaction-time tasks (Parker et al., 2013). Moreover,
optogenetic inhibition of MFC neurons expressing D1 dopamine receptors impairs interval
timing (Narayanan et al., 2012). Finally, MFC D1 blockade selectively attenuated 4 Hz activity
and ramping activity of MFC neuronal ensembles (Parker et al., 2014). D1-type dopamine
receptors in prefrontal regions are intimately involved in a range of executive processes
(Goldman-Rakic et al., 2000; Abi-Dargham et al., 2002). Our data indicate that patients with PD
no longer had correlations between low-frequency spectral activity and executive functions,
suggesting that activity ~2-6 Hz may contribute to this cognitive processing.

Of note, PD is a heterogeneous disease with massive loss of dopaminergic signaling in
corticostriatal circuits, and with broad changes in neurotransmitters such as acetylcholine,
norepinephrine, serotonin as well as corresponding changes in cortical networks (Narayanan et al., 2013b). Our data imply that in rodents, unilateral MFC 6-OHDA is sufficient to attenuate delta/theta cue-related activity in MFC, modeling deficits in PD patients. 6-OHDA depletes not only dopamine but related catecholamines, therefore we could not rule out the possibility that the observed effect in rodent is caused by disruption of catecholamines. However, in PD patients, the unique laterality and complexity of human cortex may be relevant. Because PD often begins unilaterally, future studies could study how delta/theta rhythms are influenced by laterality in PD and study disease progression (Djaldetti et al., 2006). These studies could also investigate the contribution of the diverse neurotransmitter systems involved in PD as well as synuclein overexpression on elementary cognitive function (Eberling et al., 2013).

These findings are limited by low-density clinical recording, obviating any ability to estimate generative sources. In addition, our ERPs were low-amplitude, in part due to the age of our population. Time-frequency findings were robust even with this highly limited clinical montage, suggesting that potential future biomarker assays could utilize clinical setups that are common in most major hospitals and clinics. However a more near-term goal remains to address these definitive limitations using high-density research arrays, magnetoencephalography, or by using intraoperative recordings. We also find significant activations in other frequencies, such as beta rhythms, particularly in rodents. We are attempting to compare rodent intracranial field-potentials with human extrascalp EEG. Despite vast in methods, species, and behavioral strategies, delta/theta activity was observed after the instructional stimulus from medial frontal regions of humans and rodents. This activity was attenuated with dopamine disruption. Future work will explore this issue with human intracranial recordings from medial frontal cortex as well as in non-human primates may help explore the generalizability of our work. Finally,
rodents and humans may adopt vastly distinct behavioral strategies during this elementary task. Both human and rodent impairments could be due to a similar degradation in a low-level mechanism that contributes to a wealth of cognitive states, which in turn may be differentially utilized on this task (e.g. working memory in humans yet sustained attention in rats). Future work could explore this issue by comparing the performance of PD patients and animal models on tasks that test these possibilities (Donnelly et al., 2015).

In summary, our data show that both humans and rodents have ~4 Hz MFC activity triggered by the instructional cue during timing tasks, and that this activity is attenuated when dopamine signaling is dysfunctional. Our data contribute to the neurophysiological basis of interval timing and provide insight into how medial frontal brain networks are dysfunctional during elementary executive processing in PD. These findings could be helpful in developing novel EEG-based biomarkers for PD and other diseases involving MFC dysfunction.
CONTRIBUTIONS

KLP, KHC, JFC, and NSN designed these experiments, analyzed data, and wrote the paper;

KHC, JRK and KLP collected data from these experiments.

FUNDING

This work was supported by NIH Grants R01 NS089470 and K08 NS078100, NARSAD Young Investigator Awards to K.L.P and N.S.N., and Nellie Ball Research Trust to K.L.P.
REFERENCES


<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>63.4±2.9</td>
<td>64.8±3.4</td>
</tr>
<tr>
<td>R handed (%)</td>
<td>92</td>
<td>92</td>
</tr>
<tr>
<td>Female (%)</td>
<td>58</td>
<td>66</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.8±0.6</td>
<td>16±0.5</td>
</tr>
<tr>
<td>MOCA</td>
<td>28.7±0.4</td>
<td>27.9±0.4</td>
</tr>
<tr>
<td>UPDRS Part III (pts)</td>
<td>n/a</td>
<td>9.7±1.8</td>
</tr>
<tr>
<td>Levodopa-Equivalent Dose</td>
<td>n/a</td>
<td>633±93 mg</td>
</tr>
</tbody>
</table>

**Table 1:** Demographics and MOCA scores.
<table>
<thead>
<tr>
<th>Species</th>
<th>Condition</th>
<th>Interval Length</th>
<th>Median</th>
<th>Mean</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Human</strong></td>
<td>Young</td>
<td>3</td>
<td>3.0</td>
<td>3.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>12.4</td>
<td>12.4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3</td>
<td>3.1</td>
<td>3.1</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>12.3</td>
<td>12.5</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>PD</td>
<td>3</td>
<td>3.4</td>
<td>3.4</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>12.1</td>
<td>12.2</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Rodent</strong></td>
<td>Control</td>
<td>3</td>
<td>3.7</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>9.1</td>
<td>9.1</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>MFC 6-OHDA</td>
<td>3</td>
<td>3.9</td>
<td>4.2</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12</td>
<td>7.1</td>
<td>7.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

**Table 2:** Response time medians and means. All values in seconds. Statistics in main text.
<table>
<thead>
<tr>
<th>Task</th>
<th>Matched</th>
<th>Parkinson's Disease</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMT</td>
<td>32.6 ± 6.1</td>
<td>44.7 ± 10.0</td>
<td>0.16</td>
</tr>
<tr>
<td>Stroop1</td>
<td>-0.2 ± 3.3</td>
<td>0.0 ± 1.7</td>
<td>0.48</td>
</tr>
<tr>
<td>Stroop2</td>
<td>30.1 ± 3.1</td>
<td>27.5 ± 2.5</td>
<td>0.26</td>
</tr>
<tr>
<td>Verbal fluency</td>
<td>50.0 ± 4.1</td>
<td>38.4 ± 2.4</td>
<td>0.01</td>
</tr>
<tr>
<td>Digit Total</td>
<td>19.5 ± 1.0</td>
<td>17.0 ± 0.9</td>
<td>0.03</td>
</tr>
<tr>
<td>WCST</td>
<td>12.0 ± 3.1</td>
<td>10.0 ± 2.1</td>
<td>0.30</td>
</tr>
</tbody>
</table>

**Table 3:** Neuropsychological tests.
**FIGURE LEGENDS**

**Figure 1:** Interval timing task. A) Participants estimate 3 s (Int3) and 12 s (Int12) intervals starting with the onset of an instructional cue by making a motor response. Rodents received liquid rewards for the first response after interval end, whereas humans received visual feedback of their response time. B) Average response-timing curves indicate that patients with PD (red) had flatter response curves at Int12 than at age-matched controls (blue). C) Although rodents were earlier and much more variable (control, prior to dopamine depletion, blue), medial frontal dopamine depletion (MFC 6-OHDA, red), unilateral medial frontal dopamine depletion flattened response-timing curves, as in humans with PD. Asterisk indicates a significant interaction between interval and dopamine depletion via ANOVA.

**Figure 2:** Infusions of 6-OHDA in rodent medial frontal cortex (MFC) causes dopamine depletion. A) Histology from brain slices from 8 animals revealed approximate electrode placement in the MFC (red dots). Cannula from all 11 animals located within MFC and within recording distance of the electrode tips (blue dots). Light gray patch – approximate maximum region of dopamine depletion, dark gray path – approximate minimum region of dopamine depletion. B) Low and medium power coronal sections stained with TH (red); MFC 6-OHDA was injected on the left side. M - midline; R - right; L - left. C) There was less TH+ staining in sections ipsilateral to MFC 6-OHDA infusion (left) compared to control, contralateral sides without infusion (right). Asterisk indicates significance at p<0.05 via paired t-test.
**Figure 3:** Cue-related delta/theta activity (2-6 Hz) during timing tasks in humans and rodents.

A) In 11 young participants, normalized EEG event-related potential (ERP) revealed peaks ~0.2 s from the cue over mid-frontal lead Cz on 12 s trials. Time-frequency analysis of signals from lead Cz revealed B) a prominent burst of theta and delta activity (~3-6 Hz). C) In 5 rodents, 20 local field potentials (LFPs) were recorded from medial frontal cortex and also revealed an ERP. D) A burst of delta and theta activity was triggered by the cue during interval timing tasks in rodents (panel B) from ~3-6 Hz. This band was subsequently studied in PD patients, age-matched controls, and rodents with and without MFC dopamine.

**Figure 4:** Medial frontal delta and theta rhythms depend on dopamine. A) Normalized EEG event-related potential (ERP) from 13 matched control participants (blue) and in PD patients (red) revealed peaks ~0.2 s from the onset of the cue on mid-frontal lead Cz. At right, the topographic distribution of ERPs over MFC are plotted (0.1-0.2 s after cue) in controls (left) and in PD patients (right). B) Time-frequency analysis of signals from lead Cz revealed a prominent burst of delta and theta activity (~2-6 Hz) in matched controls while C) patients with PD had deactivations 0.5-1.0 s following the cue. D) The between-group subtraction revealed that PD patients had significantly less low-frequency activity (~2-4 Hz) than the matched controls. E) Rodents had delta and theta cue-triggered activity (~2-4 Hz; note that these data are similar to Fig 3D in a different set of rodents) over MFC. F) With MFC dopamine depletion, deactivation was seen in this band. G) The between-group subtraction revealed that depleting dopamine in the MFC significantly minimizes the low-frequency activity (~2-5 Hz) when compared to control...
animals. Black lines indicate increases in power relative to baseline or matched controls in the subtraction condition at p < 0.05 via a t-test.

Figure 5: Boxplots of cue-triggered delta/theta bursts in PD and in animal models. A) In humans, 2-5 Hz activity 0.3 to 0.5 s after cue onset was attenuated in PD patients compared to age-/education-matched controls. B) In rodents, 2-5 Hz activity 0.3 to 0.5 s after cue onset was attenuated in rodents with unilateral MFC dopamine depletion. The top of the box is the third quartile, the white line is the second quartile, and bottom of the box is the first quartile, and the whiskers range from the smallest to the largest non-outlier. Asterisk indicates significance via a t-test.

Figure 6: Focal MFC dopamine depletion impairs delta/theta coupling of single MFC neurons with MFC field potentials. A) Spike-field coherence from control sessions revealed marked coherence in delta and theta range around the cue. Spike-field coherence was normalized to 95% confidence intervals of coherence to compare across neurons, animals, and sessions. Middle row: In sessions with MFC 6-OHDA, a markedly different pattern was observed in the same rodents from the same MFC neuronal ensembles. Bottom row: subtraction of control and MFC 6-OHDA sessions revealed significantly more spike-field coherence between 2-6 Hz around the cue during timing tasks. Black lines indicate significance via a t-test. B) A peri-event raster plot from a single MFC neuron that had increased firing earlier in the interval on Int12 trials only. C) On average, firing rates were significantly higher immediately after the cue in control sessions compared to sessions with MFC 6-OHDA (109 vs. 101 neurons; 0-2 s after the cue). Asterisk indicates p < 0.05 via a t-test. D) All neuronal activity sorted by principal component analysis in
control (left) and MFC 6-OHDA (right) sessions; much less modulation was observed in MFC 6-OHDA sessions.

Figure 7: Low-frequency activity was correlated with cognitive function in control but not PD patients. In matched controls, cue-related spectral activity (2-4 Hz, 0.3-0.5 s after cue) was strongly related to cognitive function (R=0.75, p <0.004; indicated by the asterisk). This was not observed in PD patients. Executive function was indexed by neuropsychological tests that did not differ in PD vs. controls (TMT+Stroop+WCST; Table S2). A Fisher’s R-to-Z transformation indicated that this relationship was significantly stronger for controls compared to PD patients.
A

Correct Response

Interval (3 or 12 s)

Reward / Feedback

Cue

Interval End

B

HUMAN

Int3

Matched
Parkinson's disease
n=26

Int12

0 3 12

Time from cue (s)

C

RODENT

Int3

Control
Post MFC 6-OHDA
n=9

Int12

0 3 12

Time from cue (s)
**Figure A**

Diagram showing the anatomical structure with the label "DV -3.5 @ 12°".

**Figure B**

Images showing the cannula insertion site labeled "CANNULA". Images labeled "M" and "R" indicate different views of the structure.

**Figure C**

Bar graph comparing Mean TH Intensity between 6-OHDA Infusion and Non-Infusion conditions. The graph shows a significant difference (*p < 0.05*) between the two conditions.
A  HUMANS  

B  

C  RODENTS  

D
HUMANS

Matched Parkinson’s disease
n=26

Control
MFC
n=8

RODENTS

6-OHDA
n=8

**dB**

**dB**

A

B

ΔdB

ΔdB

0

0

-1

0

-1

*
Cognitive Index (Stroop+TMT+WCST) vs. ∆dB

Matched
Parkinson's disease