

Prefrontal D1 dopamine signaling is required for temporal control

Nandakumar S. Narayanan^{a,b,1}, Benjamin B. Land^{b,1}, John E. Solder^b, Karl Deisseroth^{c,d}, and Ralph J. DiLeone^{b,2}

^aDepartment of Neurology, Carver College of Medicine, University of Iowa, Iowa City, IA 52242; ^bDepartment of Psychiatry and Ribicoff Research Facilities, Yale University School of Medicine, New Haven, CT 06511; and Departments of ^cBioengineering and ^dPsychiatry and Behavioral Sciences, Stanford University, Stanford, CA 94305

Edited by Ranulfo Romo, Universidad Nacional Autónoma de México, Mexico City, Mexico, and approved October 25, 2012 (received for review July 10, 2012)

Temporal control, or how organisms guide movements in time to achieve behavioral goals, depends on dopamine signaling. The medial prefrontal cortex controls many goal-directed behaviors and receives dopaminergic input primarily from the midbrain ventral tegmental area. However, this system has never been linked with temporal control. Here, we test the hypothesis that dopaminergic projections from the ventral tegmental area to the prefrontal cortex influence temporal control. Rodents were trained to perform a fixed-interval timing task with an interval of 20 s. We report several results: first, that decreasing dopaminergic neurotransmission using virally mediated RNA interference of tyrosine hydroxylase impaired temporal control, and second that pharmacological disruption of prefrontal D1 dopamine receptors, but not D2 dopamine receptors, impaired temporal control. We then used optogenetics to specifically and selectively manipulate prefrontal neurons expressing D1 dopamine receptors during fixed-interval timing performance. Selective inhibition of D1-expressing prefrontal neurons impaired fixed-interval timing, whereas stimulation made animals more efficient during task performance. These data provide evidence that ventral tegmental dopaminergic projections to the prefrontal cortex influence temporal control via D1 receptors. The results identify a critical circuit for temporal control of behavior that could serve as a target for the treatment of dopaminergic diseases.

Parkinson disease | schizophrenia | cingulate | top-down control | mesocortical

Temporal control, 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 (1–3) and requires intact dopaminergic signaling (4). Patients with Parkinson disease with depleted dopamine have dramatically impaired temporal control (5). Despite these data, the neural circuitry influenced by dopamine during temporal computations is not understood.

Temporal control can be carefully studied using an interval-timing task (Fig. 1) (6), in which participants estimate a discrete interval of time. In rodents, disrupting nigrostriatal dopamine (7) in the dorsal, but not the ventral, striatum (8) impairs temporal control. Overexpression of D2 receptors in the striatum also diminished temporal control of behavior (9).

A brain region that organizes goal-directed behavior is the medial prefrontal cortex (10, 11). In metabolic imaging studies, hypoactivity in this area is correlated with impaired executive function in Parkinson disease (12). Medial prefrontal regions are activated during human brain imaging of fixed-interval timing tasks (13). In rodents, the medial prefrontal cortex has single neurons that encode the passage of time (14) and exerts top-down control over other brain areas to control movements in time (11). Medial prefrontal regions receive prominent dopaminergic input from the midbrain ventral tegmental region (8), which loses dopamine neurons in Parkinson disease (15–17). Dysfunction of this system may contribute to cognitive symptoms of Parkinson disease (18). However, mesocortical projections

from the ventral tegmental area (VTA) to the medial prefrontal cortex have never been studied in interval timing and have never been linked to temporal control.

In the present study, we investigated the hypothesis that prefrontal dopamine from the VTA influences temporal control by selectively manipulating this circuit in rodents trained to perform a fixed-interval timing task. We find that (i) reducing VTA dopaminergic transmission by local virally mediated RNA interference impairs temporal control, (ii) blocking D1, but not D2, dopamine receptors within medial prefrontal cortex impairs temporal control, (iii) optogenetic inhibition of prefrontal neurons expressing D1 dopamine receptor impairs temporal control, and (iv) optogenetic stimulation of prefrontal neurons expressing D1 dopamine receptor enhances animals' efficiency during fixed-interval timing tasks. These results reveal how mesocortical dopaminergic projections control the timing of movement via D1 receptors within the prefrontal cortex.

Results

To test the hypothesis that VTA dopamine influences temporal control, we selectively silenced tyrosine hydroxylase, an enzyme required for dopamine synthesis, using virally mediated RNA interference locally within the VTA (Fig. 2*A* and *B*). This approach specifically decreases dopamine production and neurotransmission without affecting other signaling systems or cell survival (19). We trained 13 rats in fixed-interval timing tasks (20) and stereotaxically injected them with virus bilaterally within the VTA with either short hairpin targeting tyrosine hydroxylase (shTH) (six rats) or control virus expressing EGFP under an identical promoter (seven rats). There were no preoperative differences between groups, and after 4 wk to allow for viral expression, animals were tested in fixed-interval timing tasks.

Rats with reduced VTA dopaminergic transmission had fewer TH+ cells in the VTA ($P < 0.001$; Fig. 2*B*) and correspondingly fewer TH+ axons in the prefrontal cortex ($P < 0.001$; Fig. 2*C*). These rats had profoundly impaired performance in the fixed-interval timing task. Compared with control animals, they had fewer responses in anticipating interval end (17–20 s of the interval; $P < 0.02$; Fig. 2*D* and *E*). Animals also had fewer overall responses (24.1 ± 13.2 vs. 34.4 ± 4.2 ; $P < 0.002$). These data show that VTA dopaminergic projections influence performance of fixed-interval timing tasks. Silencing VTA dopaminergic transmission did not change locomotion (i.e., time between the operant responses and reward collection on the opposite chamber wall; 3.4 ± 1.3 s in shTH animals vs. 2.4 ± 0.4 s in controls; $P = 0.14$).

Author contributions: N.S.N., B.B.L., and R.J.D. designed research; N.S.N., B.B.L., and J.E.S. performed research; K.D. contributed new reagents/analytic tools; N.S.N. and B.B.L. analyzed data; and N.S.N., B.B.L., and R.J.D. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

¹N.S.N. and B.B.L. contributed equally to this work.

²To whom correspondence should be addressed. E-mail: ralph.dileone@yale.edu.

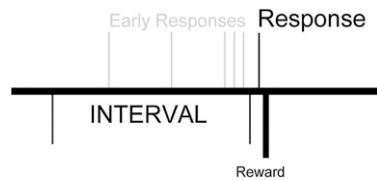


Fig. 1. Fixed-interval timing task, in which rewards are available for the first response 20 s after the last reward; early responses are unreinforced.

Next, we investigated dopamine within prefrontal targets of VTA projections. Rats were trained to perform the fixed-interval timing task and then implanted bilaterally with prefrontal cannulas (Fig. 3A) to allow drug delivery immediately before behavior. Inactivation using muscimol (11) decreased responses anticipating interval end (Fig. 3B; $P < 0.05$) relative to control sessions, suggesting that prefrontal regions are necessary for temporal control during interval timing. To specifically evaluate the contribution of prefrontal dopamine, we infused selective dopamine receptor antagonists into the prefrontal cortex immediately before behavior. The D2 antagonist sulpiride (0.5 μ g in 0.5 μ L) did not influence fixed-interval performance (Fig. 3C). However, the D1 antagonist SCH23390 (0.5 μ g in 0.5 μ L) impaired performance by decreasing responses anticipating interval end (Fig. 3D) without significantly altering locomotion (5.2 ± 0.9 s in SCH23390 sessions vs. 3.6 ± 0.3 s in control sessions; $P = 0.13$). These data suggest that prefrontal D1 dopamine receptors are necessary for temporal control. Of note, locomotion was not affected by D2 blockade ($P = 0.22$) or prefrontal inactivation ($P = 0.23$), and neither muscimol (79.8 ± 50.7 ; $P = 0.22$), D1 blockade (45.9 ± 14.6 ; $P = 0.13$), nor D2 blockade (111.2 ± 37.1 ; $P = 0.77$) significantly decreased overall responses relative to control sessions (91 ± 30.4).

Thus far, we have demonstrated that disruption of VTA dopamine and its targets in the medial prefrontal cortex impairs fixed-interval timing performance. To investigate whether this impairment is specifically related to timing, we used two measures. First, we explored whether dopamine disruption in the VTA and prefrontal cortex affected the curvature index of animals' time-response histograms. This index ranges between -1 and 1 and measures the deviation from the cumulative response record of a straight line, with 0 indicating a constant response rate throughout the interval. The curvature index has been used as a measure of timing during fixed-interval timing (20, 21) that is independent of overall response rate, because animals' curvature indices are close to zero (meaning they respond equally through the interval) before they learn to time but curvature indices increase (meaning they respond more at the end of the interval) as responses are controlled in time (21). Second, we measured postreinforcement pauses (20), which examined the delay between the last reinforcer and the next response, which also have been used extensively as a measure of timing independent of overall response rate.

Selective VTA dopamine disruption flattened the response curves (Fig. 4A; $P < 0.03$) and decreased postreinforcer pauses (Fig. 4B; $P < 0.02$). In the prefrontal cortex, both muscimol and D1 antagonism also significantly flattened response curves ($P < 0.01$ for D1; $P < 0.01$ for muscimol; Bonferroni-corrected P threshold < 0.017) and decreased postreinforcer delay ($P < 0.01$ for D1 and $P < 0.03$ for muscimol; Bonferroni-corrected P threshold < 0.017 ; Fig. 4A and B). However, D2 antagonism produced no significant changes in either of these metrics. Taken together, these data demonstrate that VTA dopaminergic disruption, prefrontal D1 blockade, and prefrontal inactivation influence timing during fixed-interval task performance and establish that prefrontal D1 receptors are required for temporal control during interval timing.

To further investigate this idea with both high temporal resolution and cell-type specificity, we combined optogenetics with a transgenic approach. We selectively expressed halorhodopsin (NpHR 3.0), a light-sensitive ion channel that inhibits neural activity, in mice (22) trained to perform a fixed-interval task (23). Prefrontal D1 neurons (i.e., neurons expressing the D1 dopamine receptor) were targeted by local injection of adeno-associated virus (AAV) expressing Cre-dependent halorhodopsin in D1-Cre mice (22) (Fig. 5A). Photoinhibition of prefrontal D1 neurons with 590-nm light decreased neural activity as measured by expression of the immediately early gene c-Fos (24) (Fig. 5B and C; $P < 0.01$). Unilateral medial prefrontal D1 photoinhibition during fixed-interval timing (Fig. 5D) reduced responses anticipating interval end relative to control trials (Fig. 5E; $P < 0.03$) and trended toward disrupting curvature ($P < 0.06$) and decreasing postreinforcer pause ($P < 0.09$). Prefrontal D1 photoinhibition did not change locomotion (3.8 ± 0.6 s in photo-inhibited vs. 3.8 ± 0.6 s in nonphotoinhibited trials; $P = 0.78$). D1 photoinhibition did not decrease the overall response rate (41 ± 7.5 vs. 46 ± 7.4 in control trials; $P = 0.97$). These data demonstrate that disrupting prefrontal D1 neurons via multiple techniques impairs fixed-interval timing performance in rats (pharmacology) and mice (optogenetics) and demonstrate that prefrontal D1 signaling is required for temporal control.

If prefrontal D1 neurons simply encoded the passage of time akin to an internal clock (6, 14), then stimulating these neurons should alter this clock function and shift animals' time-response

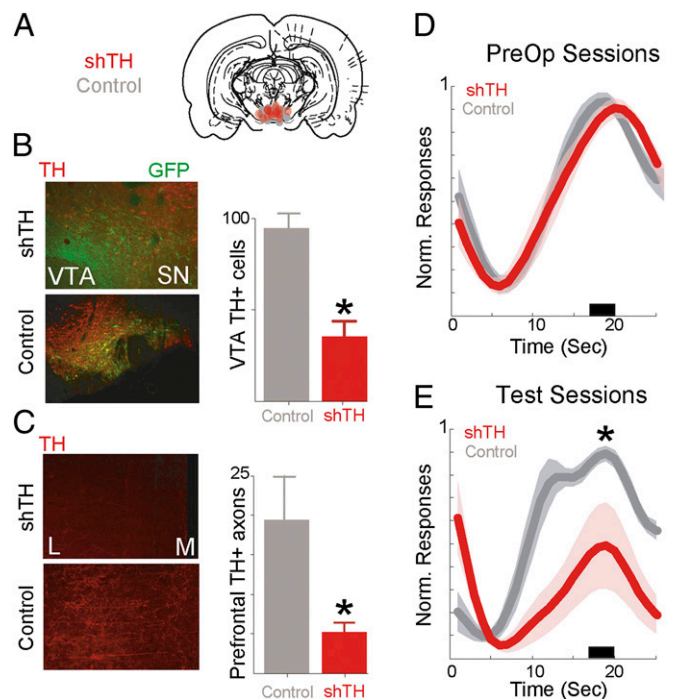


Fig. 2. Decreasing VTA dopamine and temporal control. (A) VTA targeting and placements for shRNA injection. (B) (Upper) Infected VTA neurons expressing GFP with little TH indicating knockdown. (Lower) With control virus, GFP colocalizes with TH+ neurons. (Right) VTA TH disruption decreases the number of TH+ cells in the VTA. (C) (Upper) In the prefrontal cortex, there are few TH+ axons in animals with VTA TH disrupted, compared with (Lower) control animals. L, lateral; M, medial. (Right) VTA TH disruption decreases the number of TH+ axons in the prefrontal cortex. (D) Normalized time-response histograms showing no differences between groups preoperatively. (E) Normalized time-response histograms demonstrating that VTA TH disruption ($n = 6$) impairs fixed-interval timing performance compared with controls ($n = 7$). Shaded areas represent SEM. Small black bar indicates interval used to compare response histograms.

approach with exploration of clock and memory subsystems, particularly when combined with recording large populations of neurons in multiple areas (36), may prove informative.

Our data are qualified by two notable caveats. We do not report direct measures of dopamine decrease in the prefrontal cortex after VTA shRNA injection, and it is possible that virally mediated reduction of tyrosine hydroxylase does not reliably decrease dopamine content. Likewise, the pharmacological manipulations we report may have off-target effects at the doses used. These limitations motivated our use of diverse approaches and complementary techniques (selective virally mediated RNA interference, pharmacology, and optogenetics) to advance the argument that prefrontal D1 dopamine receptor neurons are important for interval timing.

Prefrontal dopamine systems are involved in a number of neuropsychiatric diseases. One such disease, Parkinson disease, involves cognitive dysfunction (37) that can manifest as a dysexecutive syndrome (38) with impaired temporal control (9). Although temporal processing and executive function in Parkinson disease can be heterogeneous (37, 39), impairment in these processes in Parkinson disease is likely related to dysfunctional mesocortical networks (12, 18, 40). The present study provides data demonstrating that prefrontal dopamine is crucial to timing, and that stimulation of D1 systems in prefrontal cortex can facilitate temporal control. These data could inspire therapies that take advantage of emerging pharmacological, brain stimulation, or gene therapeutic strategies (41) to address clinical problems such as cognitive symptoms of Parkinson disease.

Materials and Methods

Animals. For viral and pharmacological manipulations, 31 adult male rats (*Rattus norvegicus*) weighing 250–300 g were used. For optogenetic manipulations, 10 mice (*Mus musculus*) weighing 25–30 g were used. All mice had Cre recombinase alleles driven by the D1 receptor promoter (D1-Cre+; derived from Gensat strain EY262) (22) bred and verified by genotype using primers for Cre recombinase. All animal procedures were performed in accordance with the protocol approved by the Yale Institutional Animal Care and Use Committee.

Interval-Timing Task. Rats and mice were trained to perform an operant 20-s fixed-interval timing task (Fig. 1) (20, 23) motivated by 85–90% food restriction. First, animals learned to make operant nose pokes to receive rewards (rats, 45-mg sucrose pellets; mice, 20-mg grain pellets). After fixed-ratio training, animals were trained in a 20-s fixed-interval timing task in which rewards were delivered for responses after a 20-s interval (starting at the previous reinforced response). Early responses were unreinforced. A stimulus light was used during training. After animals learned the 20-s interval, as indicated by a peak in their time-response histogram inflection point in the curvature of response functions, stimuli were turned off for experimental sessions. Of note, there is no intertrial interval in this task, and “early responses” during the early phase of the interval (0–5 s) likely reflect responses from the previous trial. Locomotion was measured by calculating the elapsed time between nose pokes on one side of the chamber and reward collection on the opposite chamber wall. Time-response histograms were normalized to maximum response rate to investigate timing independent of response rate, and curvature statistics were calculated from smoothed time-response histograms (21). Response rates were compared between experimental sessions via *t* tests; for optogenetics experiments, paired *t* tests were used within subjects between sessions with and without photostimulation. *P* values less than 0.05 were interpreted as statistically significant.

Cannula. Trained rats were anesthetized and cannulated bilaterally in the prefrontal cortex using coordinates and procedures described previously (43) [anterior–posterior (AV) +3.2, medial–lateral (MV) ±1.4, dorsal–ventral (DV) –4.0 at 10° laterally]. After 1 wk of recovery, animals were briefly anesthetized (isoflurane) and infused with vehicle or drug (0.5 μ L of 1 μ g/ μ L of sulpiride, SCH23390, muscimol, or vehicle dissolved in PBS with glacial acetic acid added dropwise to a pH of 6.09 for drug and vehicle infusions; five total infusions per animal: SCH23390, sulpiride, muscimol, and two vehicle infusions) (42, 43). Infusions were made with a 33-gauge cannula (Plastics One) that protruded 0.2 mm from the tip of the guide cannula. Injectors were inserted into the guide cannula and 0.5 μ L of infusion fluid was delivered per

site at a rate of 15 μ L/h via a syringe infusion pump (KDS Scientific). Fluid was infused via 0.38-mm-diameter polyethylene tubing (Intramedic) that connected the injector to a 10- μ L Hamilton syringe (Hamilton). Injections were confirmed by monitoring movement of fluid in the tubing via a small air bubble. After injection was complete, the injector was left in place for 2 min to allow for diffusion. Thirty minutes following infusion, animals performed fixed-interval timing tasks. After muscimol sessions, animals were tested in a “recovery” session that demonstrated behavior identical to that in vehicle sessions.

Viral Methods. Viral production for decreasing tyrosine hydroxylase and optogenetics was accomplished using a triple-transfection, helper-free method and purified as described in detail previously (19). Hairpin RNA was designed to target specific regions of tyrosine hydroxylase mRNA according to methods described in detail previously (19). The hairpins were designed such that the antisense strand came before the sense strand during transcription. The hairpin was shTH (top, 5'-TTTGAA GCTGAT TGCAT AGATTG CCTCC CAAGGC AATCTC TGCAAT CAGCTT CTTTT -3'; bottom, 5'-CTAGAA AAAGAA GCTGAT TGCAGA GATTGC CTTGGG AAGGCA ATCTAT GCAATC AGCTT-3'). The pAAV plasmid was designed to coexpress EGFP under the control of an independent RNA polymerase II promoter and terminator. Control virus was made by removing EGFP from the pEGFP-N1 plasmid (Clontech) and ligating it first into pCMV-MCS (Stratagene) then into the pAAV plasmid using the *Acl*I and *Sma*I restriction sites. Trained rats were injected with virus into the VTA (–5.6 AP, –2.3 ML, –8.0 DV at 12° laterally from bregma), and fixed-interval timing performance was assessed 4 wk later to allow for robust viral expression.

Optogenetics. We used an AAV viral construct with floxed inverted channelrhodopsin (ChR2) or halorhodopsin (NpHR 3.0) along with EYFP [UNC Viral Core; AAV-EF1a-DIO-hChR2(H134R)-EYFP and AAV-EF1a-DIO-eNpHR 3.0-EYFP] (44). When delivered to transgenic D1-Cre+ mice, Cre recombination leads to high expression driven by an EF-1a promoter selectively in neurons expressing D1 receptors. Mice were injected with AAV-ChR2 or AAV-Halo into the prefrontal cortex (mouse: AP: +1.8, ML –0.2, DV –2.8), with immediate placement of an optical fiber cannula (200- μ m core, 0.22 NA; Doric Lenses). The injection consisted of 0.5 μ L of $\sim 10^{11}$ infectious particles per milliliter. Neural activity was assessed using expression of the immediate early gene *c-Fos*; sites ipsilateral to photostimulation or photoinhibition were compared with homologous contralateral brain areas.

On test day, D1-Cre+ mice were briefly anesthetized and the incoming fiberoptic was connected to the optical cannula. Light was generated from either a 473-nm or 590-nm laser source (OEM Optics), and an optical rotary joint (Doric Lenses) was used to facilitate animal rotation during performance of the timing task. During testing, each mouse performed the fixed-interval timing task for 1 h with light delivered randomly in half of the trials. In channelrhodopsin sessions, light was delivered from 10 to 20s during the fixed-interval at 20 Hz with a power density of under 75 mW/mm² at the fiber tip. Because halorhodopsin has distinct dynamics (45), in halorhodopsin sessions constant light was delivered from 1 to 20 s at a power density of under 75 mW/mm² at the fiber tip. Task performance was compared between illuminated and unilluminated trials within each animal on the test day.

Histology. Animals were anesthetized with 100 mg/kg sodium pentobarbital and then transcardially perfused with either 10% (vol/vol) formalin or 4% (wt/vol) paraformaldehyde. Brains were coronally sectioned on a freezing microtome and either mounted on slides or free-floating in 1 \times PBS with 0.01% sodium azide. Cannula locations were visualized and translated to their placement in a coronal brain atlas. Immunohistochemistry was performed according to methods described previously (19) on mounted and free-floating sections. Immunofluorescent staining for TH (mouse anti-TH, 1:1,000–1:2,000; Abcam), GFP (chicken anti-GFP, 1:200; Abcam), or rabbit anti-*c-Fos* (Santa Cruz) with secondary antibodies (Alexa 488 or 555, 1:500; Invitrogen) was performed in normal donkey serum. Tissue was visualized using a fluorescent microscope (Zeiss) using standard FITC and TRITC filters. TH was quantified by counting TH+ cell bodies or axons and comparing to control animals. Images were selected for *c-Fos* quantification based on position of fiber cannula. *c-Fos* labeling was determined by standard threshold settings in ImageJ (National Institutes of Health) and quantified without knowledge of ipsilateral vs. contralateral side.

ACKNOWLEDGMENTS. The authors thank Madeline Whittle and Catherine Brayton for their technical contributions. This work was supported by National Institutes of Health Grants UL-DE19586 and RL1AA017537 (to R.J.D.) as well as by the State of Connecticut, Department of Mental Health and Addiction Services (R.J.D.).

1. Buhusi CV, Meck WH (2005) What makes us tick? Functional and neural mechanisms of interval timing. *Nat Rev Neurosci* 6(10):755–765.
2. Matell MS, Meck WH, Nicolelis MAL (2003) Interval timing and the encoding of signal duration by ensembles of cortical and striatal neurons. *Behav Neurosci* 117(4):760–773.
3. Jahanshahi M, et al. (2010) Dopaminergic modulation of striato-frontal connectivity during motor timing in Parkinson's disease. *Brain* 133(Pt 3):727–745.
4. Drew MR, Fairhurst S, Malapani C, Horvitz JC, Balsam PD (2003) Effects of dopamine antagonists on the timing of two intervals. *Pharmacol Biochem Behav* 75(1):9–15.
5. Malapani C, et al. (1998) Coupled temporal memories in Parkinson's disease: A dopamine-related dysfunction. *J Cogn Neurosci* 10(3):316–331.
6. Church RM (1984) Properties of the internal clock. *Ann N Y Acad Sci* 423:566–582.
7. Meck WH (2006) Neuroanatomical localization of an internal clock: A functional link between mesolimbic, nigrostriatal, and mesocortical dopaminergic systems. *Brain Res* 1109(1):93–107.
8. Kurti AN, Matell MS (2011) Nucleus accumbens dopamine modulates response rate but not response timing in an interval timing task. *Behav Neurosci* 125(2):215–225.
9. Drew MR, et al. (2007) Transient overexpression of striatal D2 receptors impairs operant motivation and interval timing. *J Neurosci* 27(29):7731–7739.
10. Miller EK, Cohen JD (2001) An integrative theory of prefrontal cortex function. *Annu Rev Neurosci* 24:167–202.
11. Narayanan NS, Laubach M (2006) Top-down control of motor cortex ensembles by dorsomedial prefrontal cortex. *Neuron* 52(5):921–931.
12. Huang C, et al. (2007) Metabolic brain networks associated with cognitive function in Parkinson's disease. *Neuroimage* 34(2):714–723.
13. Hinton SC, Harrington DL, Binder JR, Durgerian S, Rao SM (2004) Neural systems supporting timing and chronometric counting: An fMRI study. *Brain Res Cogn Brain Res* 21(2):183–192.
14. Narayanan NS, Laubach M (2009) Delay activity in rodent frontal cortex during a simple reaction time task. *J Neurophysiol* 101(6):2859–2871.
15. Javoy-Agid F, Ploska A, Agid Y (1981) Microtopography of tyrosine hydroxylase, glutamic acid decarboxylase, and choline acetyltransferase in the substantia nigra and ventral tegmental area of control and Parkinsonian brains. *J Neurochem* 37(5):1218–1227.
16. Javoy-Agid F, Agid Y (1980) Is the mesocortical dopaminergic system involved in Parkinson disease? *Neurology* 30(12):1326–1330.
17. Dymecki J, Lechowicz W, Bertrand E, Szpak GM (1996) Changes in dopaminergic neurons of the mesocorticolimbic system in Parkinson's disease. *Folia Neuropathol* 34(2):102–106.
18. Ko JH, et al. (2012) Prefrontal dopaminergic receptor abnormalities and executive functions in Parkinson's disease. *Hum Brain Mapp*, 10.1002/hbm.22006.
19. Hommel JD, Sears RM, Georgescu D, Simmons DL, DiLeone RJ (2003) Local gene knockdown in the brain using viral-mediated RNA interference. *Nat Med* 9(12):1539–1544.
20. Caetano MS, Church RM (2009) A comparison of responses and stimuli as time markers. *Behav Processes* 81(2):298–302.
21. Fry W, Kelleher RT, Cook L (1960) A mathematical index of performance on fixed-interval schedules of reinforcement. *J Exp Anal Behav* 3:193–199.
22. Drago J, et al. (1998) Targeted expression of a toxin gene to D1 dopamine receptor neurons by cre-mediated site-specific recombination. *J Neurosci* 18(23):9845–9857.
23. Balci F, et al. (2008) Interval timing in genetically modified mice: A simple paradigm. *Genes Brain Behav* 7(3):373–384.
24. Bullitt E (1990) Expression of c-fos-like protein as a marker for neuronal activity following noxious stimulation in the rat. *J Comp Neurol* 296(4):517–530.
25. Boyden ES, Zhang F, Bamberg E, Nagel G, Deisseroth K (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nat Neurosci* 8(9):1263–1268.
26. Goldman-Rakic PS, Castner SA, Svensson TH, Siever LJ, Williams GV (2004) Targeting the dopamine D1 receptor in schizophrenia: Insights for cognitive dysfunction. *Psychopharmacology (Berl)* 174(1):3–16.
27. Durstewitz D (2003) Self-organizing neural integrator predicts interval times through climbing activity. *J Neurosci* 23(12):5342–5353.
28. Staddon JER (2005) Interval timing: Memory, not a clock. *Trends Cogn Sci* 9(7):312–314.
29. Ungerstedt U, Arbuthnott GW (1970) Quantitative recording of rotational behavior in rats after 6-hydroxy-dopamine lesions of the nigrostriatal dopamine system. *Brain Res* 24(3):485–493.
30. Hosp JA, Pekanovic A, Rioult-Pedotti MS, Luft AR (2011) Dopaminergic projections from midbrain to primary motor cortex mediate motor skill learning. *J Neurosci* 31(7):2481–2487.
31. Fiorillo CD, Newsome WT, Schultz W (2008) The temporal precision of reward prediction in dopamine neurons. *Nat Neurosci* 11:966–973.
32. Schultz W (2001) Reward signaling by dopamine neurons. *Neuroscientist* 7(4):293–302.
33. Radcliffe RA, Erwin VG (1996) Alterations in locomotor activity after microinjections of GBR-12909, selective dopamine antagonists or neurotensin into the medial prefrontal cortex. *J Pharmacol Exp Ther* 277(3):1467–1476.
34. Cheung THC, et al. (2007) Evidence for the sensitivity of operant timing behaviour to stimulation of D1 dopamine receptors. *Psychopharmacology (Berl)* 195(2):213–222.
35. Williams GV, Goldman-Rakic PS (1995) Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* 376(6541):572–575.
36. Narayanan NS, Laubach M (2009) Methods for studying functional interactions among neuronal populations. *Methods Mol Biol* 489:135–165.
37. Aarsland D, et al. (2010) Mild cognitive impairment in Parkinson disease: A multi-center pooled analysis. *Neurology* 75(12):1062–1069.
38. Caballol N, Martí MJ, Tolosa E (2007) Cognitive dysfunction and dementia in Parkinson disease. *Mov Disord* 22(Suppl 17):S358–S366.
39. Merchant H, Luciana M, Hooper C, Majestic S, Tuite P (2008) Interval timing and Parkinson's disease: Heterogeneity in temporal performance. *Exp Brain Res* 184(2):233–248.
40. Harrington DL, et al. (2011) Neurobehavioral mechanisms of temporal processing deficits in Parkinson's disease. *PLoS ONE* 6(2):e17461.
41. Marks WJ, Jr., et al. (2010) Gene delivery of AAV2-neurturin for Parkinson's disease: A double-blind, randomised, controlled trial. *Lancet Neurol* 9(12):1164–1172.
42. Narayanan NS, Horst NK, Laubach M (2006) Reversible inactivations of rat medial prefrontal cortex impair the ability to wait for a stimulus. *Neuroscience* 139(3):865–876.
43. Seamans JK, Floresco SB, Phillips AG (1998) D1 receptor modulation of hippocampal-prefrontal cortical circuits integrating spatial memory with executive functions in the rat. *J Neurosci* 18(4):1613–1621.
44. Cardin JA, et al. (2009) Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* 459(7247):663–667.
45. Zhang F, Aravanis AM, Adamantidis A, de Lecea L, Deisseroth K (2007) Circuit-breakers: Optical technologies for probing neural signals and systems. *Nat Rev Neurosci* 8(8):577–581.