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Opposite Effects of Stimulant and Antipsychotic Drugs on Striatal Fast-Spiking Interneurons

Alexander B Wiltschko[1], Jeffrey R Pettibone[1] and Joshua D Berke*[,1]

[1]*Department of Psychology, and Neuroscience Program, University of Michigan, Ann Arbor, MI, USA*

Psychomotor stimulants and typical antipsychotic drugs have powerful but opposite effects on mood and behavior, largely through alterations in striatal dopamine signaling. Exactly how these drug actions lead to behavioral change is not well understood, as previous electrophysiological studies have found highly heterogeneous changes in striatal neuron firing. In this study, we examined whether part of this heterogeneity reflects the mixture of distinct cell types present in the striatum, by distinguishing between medium spiny projection neurons (MSNs) and presumed fast-spiking interneurons (FSIs), in freely moving rats. The response of MSNs to both the stimulant amphetamine (0.5 or 2.5 mg/kg) and the antipsychotic eticlopride (0.2 or 1.0 mg/kg) remained highly heterogeneous, with each drug causing both increases and decreases in the firing rate of many MSNs. By contrast, FSIs showed a far more uniform, dose-dependent response to both drugs. All FSIs had decreased firing rate after high eticlopride. After high amphetamine most FSIs increased firing rate, and none decreased. In addition, the activity of the FSI population was positively correlated with locomotor activity, whereas the MSN population showed no consistent response. Our results show a direct relationship between the psychomotor effects of dopaminergic drugs and the firing rate of a specific striatal cell population. Striatal FSIs may have an important role in the behavioral effects of these drugs, and thus may be a valuable target in the development of novel therapies.
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INTRODUCTION

The striatum is the main input structure of the basal ganglia, a set of nuclei involved in the organization of thoughts and behavior (Cummings, 1993), and reinforcement-based learning (Graybiel, 1998). It has especially high concentrations of the neurotransmitter dopamine, and dopamine receptors (Boyson *et al*, 1986). Alterations in striatal dopamine neurotransmission are a major mechanism of action of many widely used drugs, including both psychiatric/neurological therapies and drugs of abuse (Hyman *et al*, 1995; Berke and Hyman, 2000; Olanow *et al*, 2009). Psychomotor stimulants such as amphetamine can cause large increases in striatal dopamine release, with accompanying behavioral activation (Lyon and Robbins, 1975). Typical antipsychotic drugs ('major tranquilizers') are dopamine D2 receptor antagonists (or inverse agonists; Strange, 2008), and their major acute effect is to suppress thoughts and actions.

*Correspondence: Dr JD Berke, Department of Psychology, and Neuroscience Program, University of Michigan, Ann Arbor, 530 Church Street, Ann Arbor, MI 48109-1043, USA, Tel: +734 615 2712, Fax: +734 763 7480, E-mail: jdberke@umich.edu
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Although these drug mechanisms are fairly well understood at the synaptic level, the steps leading from altered striatal dopaminergic signaling to altered behavior are not. In simplified models, dopamine is often considered to excite striatonigral projection cells through D1 receptors and inhibit striatopallidal cells through D2 receptors; however, the effects of dopamine signaling on neuronal ion channels are actually highly complex (for example, Moyer *et al*, 2007). Studies that directly examine the effects of stimulants on striatal neuron firing rates have found heterogeneous results (for example, Gardiner *et al*, 1988), which depend on many factors including the behavioral state of the animal (reviewed in Rebec 2006). Changes in overall striatal neuron firing rate have been observed following high doses of D2 antagonists such as haloperidol, but such changes appear to be modest in size, highly variable, and appear not to account for the profound drug-induced behavioral effects (Burkhardt *et al*, 2007). More broadly, it is now recognized that standard box-and-arrow models relating overall spike rate within components of cortical–basal ganglia circuits to behavior (Albin *et al*, 1989) are insufficient to account for neuronal firing changes observed after manipulations such as dopamine depletion (for example, Boraud *et al*, 2002). This has lead some basal ganglia investigators to shift focus from spike rate to patterns of neuronal spike timing and

synchronization instead (Bergman *et al*, 1998; Berke, 2009; Hammond *et al*, 2007).

However, another major potential reason for variable effects of dopaminergic drugs on striatal neurons is that existing studies have not distinguished between different striatal neuronal types. Although medium spiny projection neurons (MSNs) make up around 90–95% of striatal neurons, a large proportion of these are silent most of the time, so that active interneuron populations are disproportionately detected during *in vivo* recording studies (for example, Berke *et al*, 2004). Parvalbumin-positive (PV+) fast-spiking interneurons (FSIs) form a major striatal cell class that has come under increasing recent scrutiny. Both *in vitro* and anesthetized *in vivo* studies have shown that FSIs can provide powerful perisomatic inhibition of MSNs (Koos and Tepper, 1999; Mallet *et al*, 2005), whereas studies in behaving animals (Berke, 2008; Berke, 2009; Gage *et al*, 2008) and detailed network simulations (for example, Humphries *et al*, 2009; Moyer and Wolf, 2009) indicate that FSI:MSN interactions are more complex than simple inhibition (see Discussion). The exact role of FSIs in normal striatal information processing is unknown, although there is evidence that they are involved in the suppression of unwanted actions (Gage *et al*, 2008). Consistent with this idea, a reduction in the number of striatal PV+ cells has been observed in humans with Tourette syndrome (Kalanithi *et al*, 2005), for which a major therapy remains D2 receptor antagonists.

Although FSIs seem to be key components of local striatal microcircuits, and clinically important dopaminergic drugs act largely by modulating such circuits, no study has yet looked specifically at how dopaminergic drugs affect the firing rate of FSIs in behaving animals. In this study, we directly compare the responses of presumed MSNs and FSIs to multiple doses of the selective D2 receptor antagonist eticlopride or the psychostimulant amphetamine.

MATERIALS AND METHODS

Surgery and Electrophysiology

All procedures were approved by the University of Michigan Committee on Animal Use and Care. Three adult male Long-Evans rats (400–500 g) were each implanted with a drive containing 21 independently adjustable tetrode probes (constructed of four 12.5 μm wires twisted together), all targeting the right striatum. Tetrode tips were gold-plated to lower impedances to 200–250 MΩ. Skull screws were used to record electrocorticograms (ECoG) and serve as a recording reference site. After implantation, tetrodes were lowered into the striatum over the course of 1 week; entry into the striatum was clearly audible as a transition from the corpus callosum, which is comparatively very quiet and devoid of spiking activity. Signals were then checked daily for putative FSIs, identified by their characteristic brief waveform, and high firing rates (Berke *et al*, 2004; Berke, 2008). This study was intentionally biased to record from FSIs as they were the main focus of investigation; when an acceptable yield of stable FSIs was observed, the drug treatment protocol was begun. Wide-band signals (1–9000 Hz hardware bandpass) were digitized continuously at 31 250 samples(s) using a 96-channel system built around custom amplifiers (Boston University Electronics Design Facility) and LabView (National Instruments) software.

Drugs

D-amphetamine sulfate and *S*-(−)-eticlopride hydrochloride were both purchased from Sigma-Aldrich. Each experiment was 3.5 h long, and was conducted during the light period of a 12 : 12 light/dark housing cycle. Rats were placed on a familiar holding stool, and baseline activity was recorded. An IP saline injection was given after ~30 min; at 1 h after saline, a drug injection was given, and 2 h after drug, the experiment ended. After the experiment period, the animal remained briefly on the holding stool, while tetrodes without identifiable FSIs were moved deeper. Each rat received all four drug treatments (2 drugs × 2 doses), in a different order. Drugs were dissolved in saline and administered IP in a volume of 1 ml/kg of rat weight. For eticlopride, 'low' dose = 0.2 mg/kg and 'high' = 1.0 mg/kg. For amphetamine, 'low' = 0.5 mg/kg and 'high' = 2.5 mg/kg. After receiving each of the four drug treatments on successive days, two animals received additional drug injections with all tetrodes moved down by ~100 μm each day, to increase the number of distinct cells available for analysis (one rat received an additional 4 days of high amphetamine injections, and another received an additional 6 days of alternating low eticlopride and low amphetamine injections).

Histology

When experiments were complete, each rat was deeply anesthetized and the location of each tetrode was marked by a small electrolytic lesion (20 μA of current for 10 s). Rats were perfused with 4% paraformaldehyde (PFA) and the brains were then stored in PFA for 24 h, then moved to 30% sucrose in 1 × PBS. Brains were sliced in a microtome at 20–40 μm and Nissl stained. Final stereotaxic coordinates were calculated by mapping histology slice images onto a standard brain atlas (Paxinos and Watson, 2005) using Sqirlz Morph software.

Data Analysis

All analyses were performed using MATLAB (Mathworks) except where noted below. The continuously digitized signal was first wavelet-filtered (decomposition level 6; Wiltschko *et al*, 2008) to remove the local field potential, and then spike detection was performed using a threshold on the smoothed nonlinear energy (calculated with a 400 μs moving window) of the filtered signal (Mukhopadhyay and Ray, 1998). Detected waveforms were peak-aligned and manually clustered using OfflineSorter (Plexon). Units that did not show stable waveform size and shape throughout both the saline and drug injections were not analyzed. For measurements of waveform duration, spikes were re-extracted from the wide-band signal and wavelet-filtered at level 8 decomposition to minimize waveform distortion (Wiltschko *et al*, 2008).

For each neuron, we constructed a firing rate time series by first binning its spikes into nonoverlapping 1-min windows and then smoothing the resulting function with

a 3-point Gaussian kernel. Reported firing rates for baseline, saline, and drug are the mean of this time series within the following time blocks: baseline, from the start of recording to 5 min before the saline injection; saline, from 20 min after the saline injection to 5 min before the drug injection; drug, from 20 min after drug injection for a duration of 90 min. We identified episodes of high-voltage spindles (HVS; see main text) by manual inspection of local field potential and ECoG spectrograms (0–100 Hz, 1 Hz resolution), and repeated the firing rate analysis either excluding these episodes (the default option) or including them. As HVS episodes strongly affect neuronal firing patterns in the striatum (Berke *et al*, 2004), they were also excluded from the interspike-interval analysis.

A cell was labeled as 'significantly responding' to a drug treatment if two criteria were satisfied: (1) the saline and drug firing rates were significantly different at $p<0.01$ with a two-sample *t*-test, and (2) the saline and drug firing rates differed by at least 20%. A significant population response to a drug required $p<0.05$ using a paired *t*-test on saline and drug log firing rates. The distribution of saline and drug firing rates was verified to be log-normal using quantile–quantile plots (data not shown), justifying the use of a paired *t*-test for significance testing of whole population responses (Kass *et al*, 2005).

To assess whether intersession differences might have skewed our results, we used a Monte Carlo shuffling approach. This analysis treated each combination of drug, dose, and neuronal population separately, and included only cells that showed a significant drug-induced change in firing rate (either increased or decreased). We first computed the proportion of pairs of simultaneously recorded cells that changed firing rate in the same direction ('concordant' pairs). We then kept the number of cells in each session constant but randomly reassigned the cells to sessions, and we recalculated the proportion of concordant pairs. We repeated this shuffling 1000 times to obtain the expected proportion of concordant pairs if there were no differences between sessions, and to generate a 95% confidence interval around this mean.

Video Analysis

For a subset of sessions ($n=12$), we recorded the rats on video, and analyzed their locomotor activity. As a simple behavioral activity measure, we calculated the total absolute difference in pixel luminosity between each adjacent video frame, and used these differences to generate a time series. This time series was smoothed in exactly the same way as the neuronal firing rates (that is, averaged within 1 min bins, followed by convolution with a 3-point Gaussian kernel). Lastly, we normalized this time series to the mean value within the saline block (20 min after saline injection to 1 min before drug injection). Using this measure, all eticlopride treatments suppressed locomotion, and all amphetamine treatments increased locomotion (Supplementary Figure S4). For each neuron recorded together with video, we calculated the correlation coefficient *r* between the firing rate time series and the corresponding locomotor activity time series, using either the saline block (*x* axis of Figure 4a), drug block (*y* axis of Figure 4a), or both (Figure 4b).

RESULTS

Striatal Cell Classes and Recording Sites

We recorded from 306 striatal neurons during a baseline period, injection of saline, and injection of either amphetamine (0.5 or 2.5 mg/kg) or eticlopride (0.2 or 1.0 mg/kg). To classify striatal neurons, we examined the duration of their mean spike waveforms, using wavelet-filtered waveforms to minimize distortion (Wiltschko *et al*, 2008). As in our previous studies using other data sets (Berke, 2008; Berke *et al*, 2004; Gage *et al*, 2008), we found clearly separate groups of striatal neurons (Figure 1a and b). The group with extremely brief waveforms was presumed to be PV + FSIs, on the basis of the brief waveforms of such cells in previous *in vitro* and anesthetized *in vivo* studies (Kawaguchi, 1993; Mallet *et al*, 2005), and the known intrastriatal distribution and firing patterns of FSIs (discussed in Berke *et al*, 2004). The large group of cells with longer-duration waveforms was presumed to be MSNs; these cells typically had much lower mean firing rates (in no case >10 Hz) and were not tonically active (not shown). A few cells either resembled the 'O cells' of our previous studies ($n=4$; Berke *et al*, 2008; Gage *et al*, 2008), or did not readily fit any established classification ($n=17$), and were not analyzed further. Although neurons were recorded in a range of striatal subregions, most were in either the central or lateral (that is, sensorimotor) striatum (Figure 1c).

Drug Effects on Neuronal Firing Rates

We next compared the firing rates of each neuron after saline injection with drug injection (Figures 2 and 3). Consistent with previous studies, MSN responses were highly heterogeneous in each of the four drug conditions (Figure 3a and c). For both doses of amphetamine, individual MSNs were observed to increase, decrease, and not change the firing rate in roughly equal proportions (Figure 3c). After eticlopride, more MSNs decreased than increased firing rate, but increases were not uncommon. When the overall firing rates of the MSN population were examined, we found that of all drug treatments only the low eticlopride dose elicited a significant change in population firing rate (Figure 3e).

Compared with MSNs, FSIs had a far more homogeneous response to both drugs. Eticlopride caused FSIs to decrease their activity in a dose-dependent manner, with the high dose producing decreases in 100% (15 of 15) of FSIs (Figure 3b and d). Both doses of amphetamine caused increases, but no decreases, in individual FSI firing rates (Figure 3b and d). All drug treatments caused significant changes in FSI population firing rates compared with saline treatment—increased firing with amphetamine, and decreased firing with eticlopride (Figure 3f). The high uniformity of FSI cells was also apparent when we plotted cell waveform characteristics against drug responses (Supplementary Figure S1). This finding provides additional support for the use of waveform criteria to distinguish striatal cell classes with distinct functional responses.

We performed additional tests to ensure that this core result—relative homogeneity of FSIs compared with MSNs—was robust to some specific experimental and

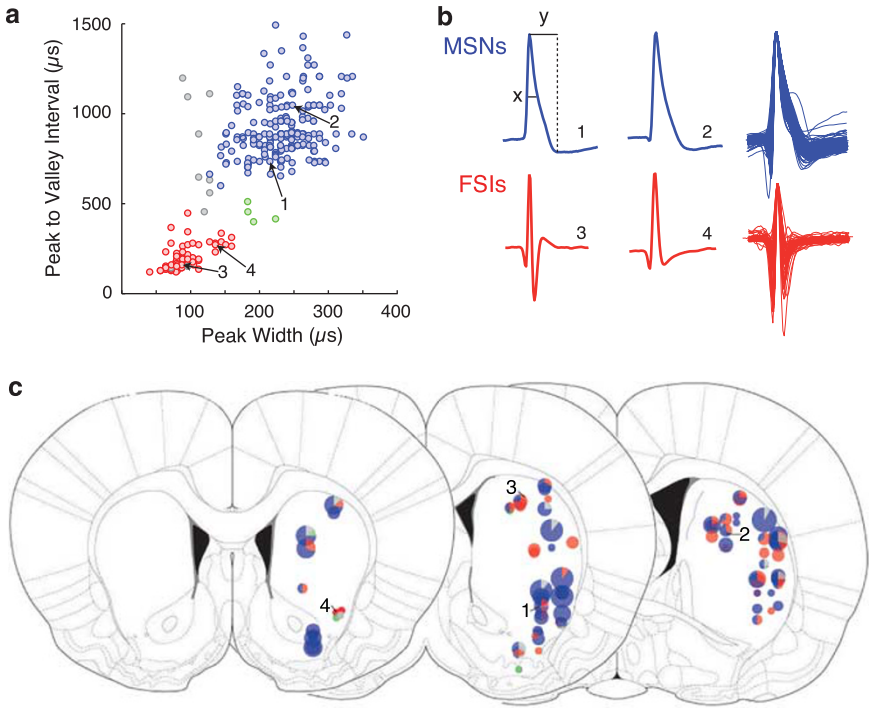

Figure 1 Identification of striatal cell subpopulations. (a) Waveform duration was measured using spike peak width at half-maximum ('x') and the peak-to-valley interval ('y'). A distinct cluster of striatal neurons with characteristic brief waveforms ($x < 200\,\mu s$, $y < 455\,\mu s$) and tonic activity (<2% of inter-spike intervals >1 s; baseline firing rates 2–100 Hz) are presumed FSIs (red; $n = 69$). The main cluster of cells with longer-duration waveforms are presumed MSNs (blue; $n = 203$). Cells not falling into these classes are indicated in gray ($n = 17$), except for a small cluster of cells (green; $n = 4$) with the distinctive waveform shape of the 'O-cells' in our previous studies (Berke *et al*, 2008; Gage *et al*, 2008). (b) Two examples each of mean MSN and FSI waveforms (left, central columns), with numbers indicating corresponding points in (a). Right column shows superimposed mean waveforms for all presumed MSNs and FSIs. (c) Recording locations for all striatal neurons. Each cell is indicated as a circle on a nearby atlas section (AP ranges for the three atlas sections: +2.75 to +1.55, +1.44 to +0.35, +0.12 to −0.24, all mm relative to bregma), using the same color code and numbering as (a, b). Circle areas are proportional to the number of neurons recorded from each site.

analysis procedures used. First, two of the subjects received some drug treatments repeatedly (see Materials and methods), and repeated drug treatments can alter brain circuits and behavior. When we performed our analysis including only cells recorded during the first time a rat received a particular treatment, our findings were unchanged (Supplementary Figure S2A). We did not record enough cells in completely drug-naive sessions to entirely rule out the possibility that MSN firing is more homogeneous on the first day of drug treatment, regardless of which drug is given.

We next considered the possibility that MSNs actually tend to show homogeneous drug responses within a given session, but variable responses between sessions. To do this, we compared the directions of drug-induced firing rate change in simultaneously recorded pairs of cells to pairs recorded in different sessions with the same drug treatment (see Materials and Methods). We found that simultaneously recorded MSN pairs were no more likely to change in the same direction than MSN pairs picked randomly from different sessions (for example, following high eticlopride, we had 46 simultaneous MSN pairs with 54.3% concordant, random pairs were 59.2% concordant; following high amphetamine, we had 62 simultaneous pairs with 51.6% concordant, and random pairs were 51.3% concordant; 95% confidence limits for random pairs were 47.5–70.9% and 39.4–63.1%, respectively). These results clearly show that MSN drug response variability is not the result of any form of intersession variability.

Different striatal subregions exhibit neurochemical differences and process different types of information, and FSIs are preferentially found in the lateral (sensorimotor) striatum. We therefore considered whether the differences between MSNs and FSI might reflect differences in intrastriatal recording locations. However, we found no significant relationships between location and drug response, for either cell population (Supplementary Figure S3).

The firing rate of striatal neurons can be affected by the presence of high-voltage spindles (HVS), an 'idling' network state that broadly engages the cortex, basal ganglia, and thalamus (for example, Berke *et al*, 2004; Dejean *et al*, 2007), and whose incidence is affected by changes in striatal dopamine transmission (Buzsáki *et al*, 1990). Although the results present above excluded HVS episodes, we found that including them did not substantially change our findings (Supplementary Figure S2B). Finally, our analyses above compared saline and drug time blocks of different durations, in order to increase data collection during the prolonged period of drug behavioral effect. However, using identically sized periods of analysis did not substantially change our results (Supplementary Figure S2C).

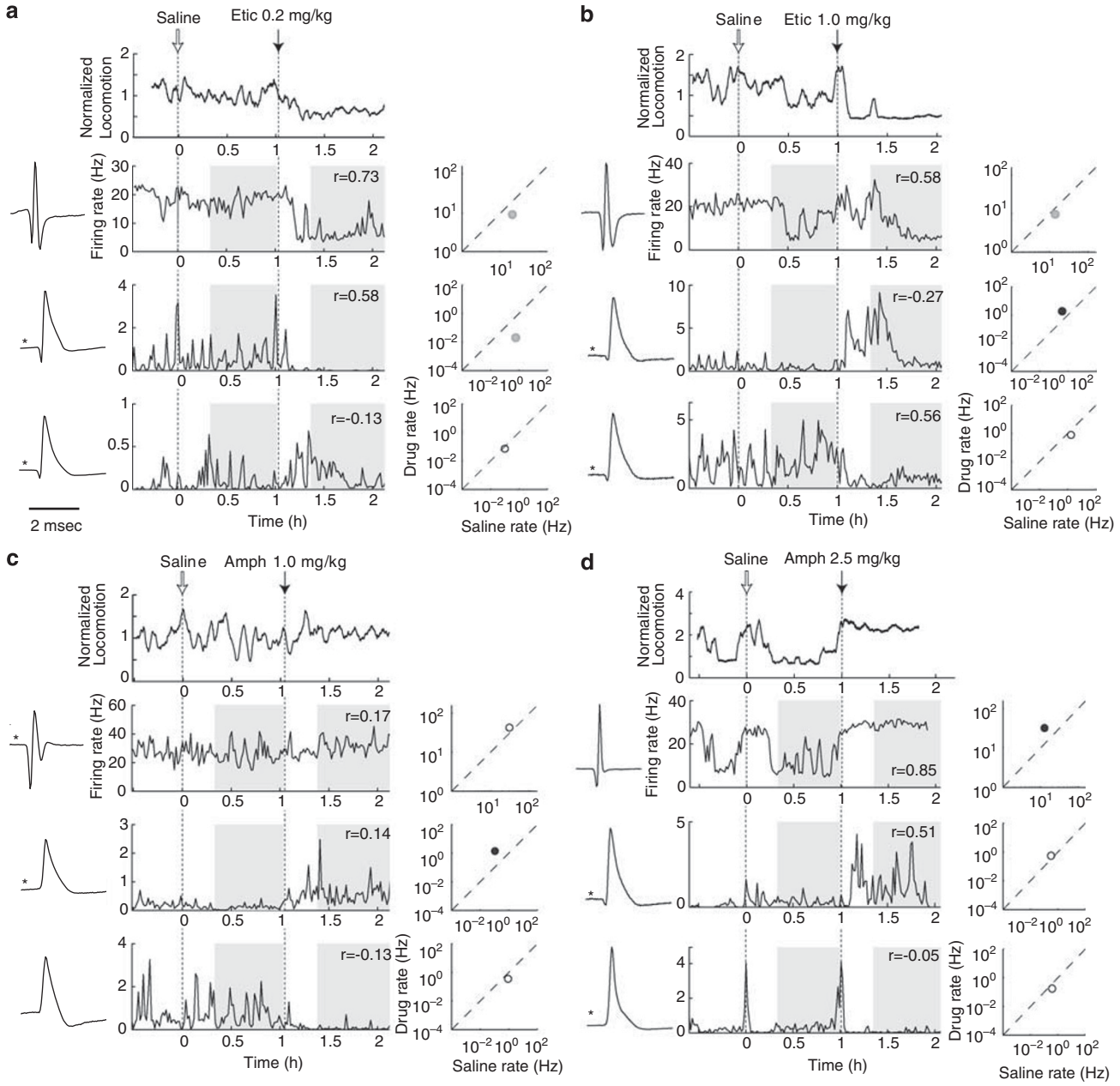

Figure 2 Examples of drug-induced firing rate change and relationships to locomotor activity. (a–d) Show data from four separate sessions (one for each drug treatment), in each case plotting locomotor activity above the corresponding firing rate of three simultaneously recorded cells (one FSI at top, and two MSNs; average normalized waveforms are shown at left). Asterisks indicate pairs of neurons that were recorded on the same tetrode. For each neuron, the indicated *r* value is the overall correlation coefficient between firing rate and locomotor activity (across both saline and drug epochs). Gray blocks over firing rate graphs indicate analyzed saline and drug epochs (drug epoch is truncated), and mean firing rate during these epochs is shown on the right (on a log scale). Black circles indicate cells with significantly higher firing rates under drug than control ($p < 0.01$, two-sample *t*-test), gray circles indicate cells with lower firing rates, and white circles indicate cells with similar firing rates under the two conditions. Low (a) and high eticlopride (b) cause a dose-dependent suppression of locomotor behavior, whereas low (c) and high (d) amphetamine cause a dose-dependent increase. Note that the firing rate of these FSI examples broadly tracks locomotor activity, and that MSN pairs recorded simultaneously from the same tetrode can show opposite directions of firing rate change.

Relationships between Neural Activity and Behavioral Activity

The results above show that dopaminergic drugs known to have opposite effects on psychomotor activation have corresponding opposite effects on FSI firing rates. We next examined the relationship between firing rate and behavior more directly, using video analysis of rat movement (see Materials and Methods). Although we recorded the video for only a subset of our experimental sessions, in every case we found that amphetamine increased locomotor activity and eticlopride decreased it, as expected (Supplementary Figure S4). Neurons recorded from these sessions showed a similar pattern of drug responses to our whole data set (Supplementary Figure S3D).

We found a striking minute-by-minute correlation between locomotor activity and the firing rates of FSIs, measured across both saline and drug epochs (Figure 4, for

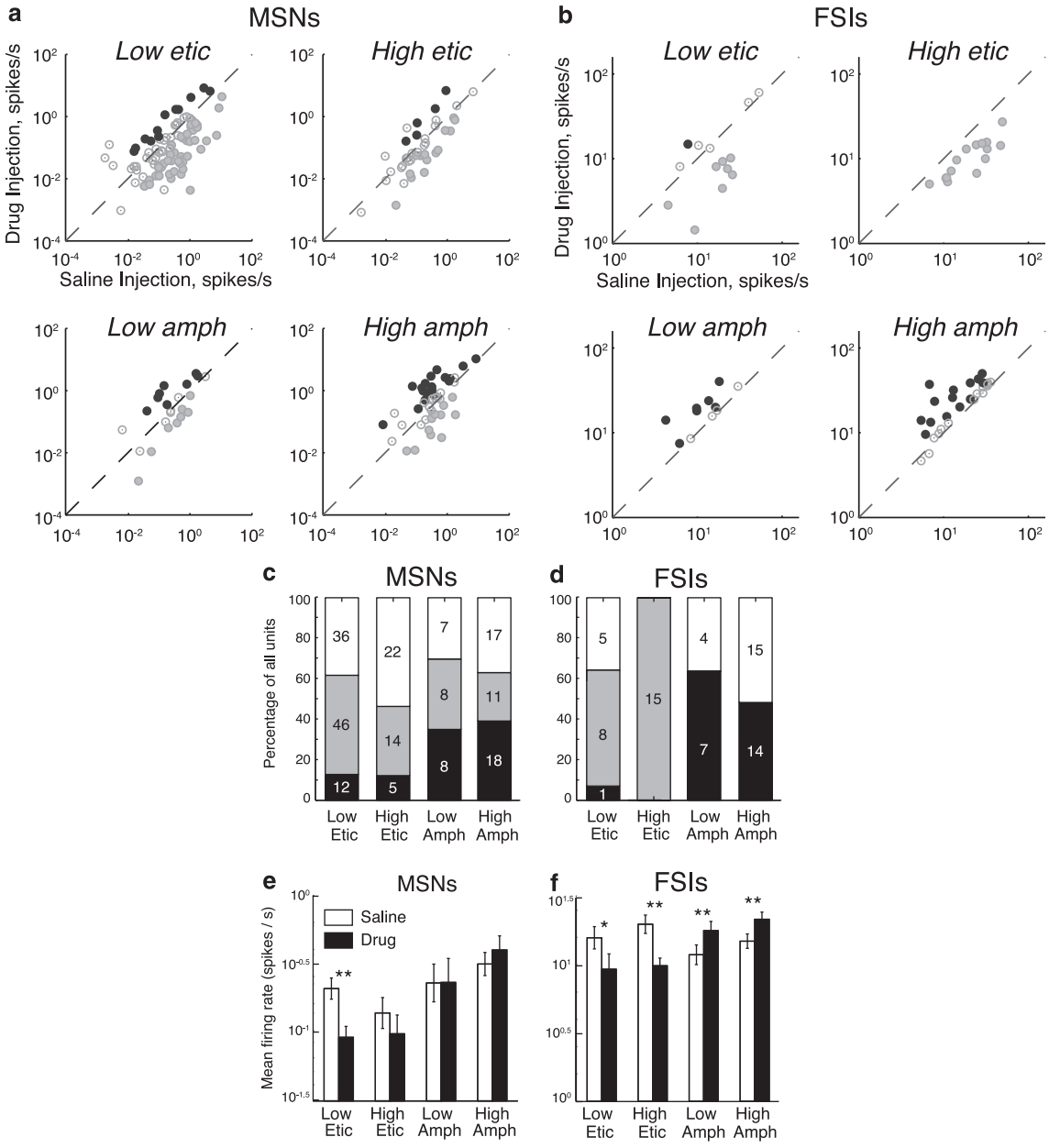

Figure 3 FSIs show consistent drug-induced firing rate changes, whereas MSNs do not. (a and b) Scatter plots comparing spike firing rates (log scale) for MSNs (a) and FSIs (b), after injection of drug *vs* saline control. 'Low etic' = 0.2 mg/kg eticlopride, 'high etic' = 1 mg/kg eticlopride, 'low amph' = 0.5 mg/kg amphetamine, 'high amph' = 2.5 mg/kg amphetamine. Circle color key is the same as Figure 2. (c and d) Proportions of MSNs, FSIs with each type of drug response. As in (a and b), black indicates increases, gray decreases, and white no change. Numbers on bars indicate absolute numbers of cells in each category. (e and f) Mean firing rates for the MSN, FSI populations during saline and drug time blocks. Asterisks indicate significant differences between saline and drug log firing rates (*$p<0.05$, **$p<0.01$ paired *t*-test).

examples, see Figure 2). The distribution of FSI correlation coefficients was strongly skewed toward positive values (Figure 4b), with a mean significantly different to zero (*t*-test, $p = 2.2 \times 10^{-7}$). Nineteen of 26 (73%) individual FSIs showed a significant positive correlation and none showed a negative correlation. By contrast, no consistent relationship was apparent between MSN firing rates and locomotor activity. Although we did observe more individual MSNs with positive correlation coefficients (51/142, 36%) than negative (28/142, 20%), the overall distribution appeared approximately Gaussian, with a mean that was not significantly different to zero (*t*-test, $p = 0.35$).

Drug-Induced Changes in Firing Pattern

Finally, we examined whether our drug treatments were producing changes in neuronal firing patterns, as well as firing rate. We found relatively little change in the distribution of inter-spike intervals (ISIs) following amphe-

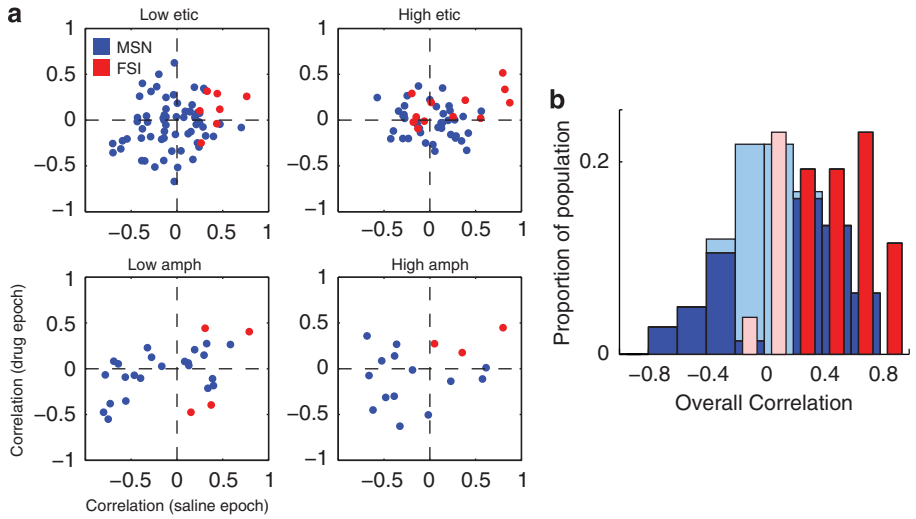

Figure 4 FSI firing rates are positively correlated with locomotor activity, whereas MSNs show no consistent relationship. (a) Scatter plots showing correlation coefficients (*r*) between individual neuron firing rates and locomotor activity, during each saline or drug epochs for which video was available. (b) Firing rate to locomotor activity correlation coefficients for all FSIs and MSNs. Darker colors indicate cells with significant ($p<0.05$) correlation. The distribution of FSI was markedly skewed toward positive values, whereas the MSN distribution was not.

tamine, except for a slight leftward shift that is consistent with increased firing rate (Supplementary Figure S5). By contrast, the high dose of eticlopride resulted in a more clearly bimodal ISI distribution, with an early peak around 2 ms indicating high-frequency burst firing. This pattern resembles the one that is found in striatal FSIs during (undrugged) slow-wave sleep (Berke *et al*, 2004; Berke, 2008), even though eticlopride-treated rats kept their eyes open (data not shown). A similar change to bimodality was not seen for the MSN population, although there was a tendency to get fewer spikes at 0.1–1 s intervals (Supplementary Figure S5).

DISCUSSION

In this study, we investigated the changes in striatal neuron firing that are produced by the systemic administration of widely used classes of dopaminergic drugs, and may be relevant to their behavioral and clinical actions in humans. Our primary finding is that presumed striatal FSIs show consistent drug-induced activity changes: decreased firing after the administration of a D2 antagonist that depresses psychomotor activity, and increased firing after a psychomotor stimulant. This clear relationship between firing rate and drug-induced behavior contrasts with the far more heterogeneous response of the larger population of MSNs, and illustrates the value of considering distinct neuronal cell types while exploring the systems-level physiology of drug actions.

The use of awake behaving animals was essential, not least because the physiological properties and responses of striatal neurons are highly dependent on behavioral state (for example, West, 1998; Berke *et al*, 2004; Rebec, 2006). Nonetheless, the approach we took has some substantial limitations. Although multiple, strong lines of evidence indicate that the brief-waveform striatal neurons are FSIs (discussed in Berke *et al*, 2004; Mallet *et al*, 2005), this identification cannot be made with certainty using extracellular recording alone. We cannot readily establish whether the observed activity changes were the cause of altered behavior, or merely a correlate. Finally, we cannot be certain of the sites of drug action responsible for changes in striatal neuron firing. This constraint is not readily overcome, as even with local striatal drug infusions we would not be able to disentangle, for example, eticlopride actions at multiple presynaptic and postsynaptic D2 receptor sites.

Despite these limitations, our results are quite consistent with the known mechanisms of dopamine action within the basal ganglia. Although FSIs do not typically express either of the main types of dopamine receptor (D1, D2; Bertran-Gonzalez *et al*, 2008), they do express the D5 receptor (Rivera *et al*, 2002; Centonze *et al*, 2003), which seems to be responsible for a depolarizing action of dopamine on FSIs *in vitro* (Bracci *et al*, 2002). In addition, dopamine decreases GABAergic synaptic input to FSIs through presynaptic D2 receptors (Bracci *et al*, 2002; Centonze *et al*, 2003; Sciamanna *et al*, 2009). This combination of depolarization and disinhibition can readily account for our observations of enhanced FSI firing following amphetamine-enhanced dopamine release, though the effects of amphetamine on other transmitters such as serotonin may also contribute (Blomeley and Bracci, 2009).

The GABAergic input to FSIs is provided largely by a broadly targeted feedback pathway from the globus pallidus (GP; Rajakumar *et al*, 1994; Spooren *et al*, 1996; Bevan *et al*, 1998). Many pallidostriatal cells express D2 receptors (Hoover and Marshall, 2004) and studies using the immediate-early gene marker *c-fos* suggest that D2 antagonists increase the activity of this cell population (Billings and Marshall, 2003). Eticlopride may thus also have affected FSI activity through a dual mechanism: acting in GP to enhance pallidostriatal firing rates while locally enhancing GABA release from the terminals of those same cells within

the striatum. Our observation of a general suppression of FSIs is also consistent with an eticlopride-induced reduction in the number of PV+ cells that express *c-fos* following cortical stimulation (Trevitt *et al*, 2005). Though supported by such prior evidence, our direct demonstration of firing rate changes is important because dopaminergic mechanisms can affect gene expression through multiple signal transduction pathways, which do not necessarily involve changes in spiking (Berke *et al*, 1998; LaHoste *et al*, 2000). Although we observed a bidirectional control of FSI firing rates with dopaminergic drugs, we note that a significant change in FSI firing rate was not observed after the broad removal of dopamine by 6-hydroxydopamine lesion (Mallet *et al*, 2006). However, those studies were performed under urethane anesthesia, leading to greatly depressed spontaneous FSI firing rates among other changes.

The MSN responses were highly heterogeneous under all drug treatment conditions. Part of this heterogeneity may reflect our inability to distinguish here between striatonigral and striatopallidal MSNs, or between different striatal compartments (patch/striosome *vs* matrix). In addition, some previous studies have found that 'motor-related' striatal neurons are disproportionately likely to increase firing rate following amphetamine (for example, Haracz *et al*, 1989), and there is evidence that this effect may be stronger than the predictable increase in motor-related firing with drug-enhanced motor activity (West *et al*, 1997). Nonetheless, it is striking that we obtained a quite uniform set of FSI drug responses without needing to subdivide this population using specific behavioral correlates.

As interneurons, FSIs affect behavior only through their influence over MSNs. If the high-firing-rate FSIs provide the constant powerful inhibition originally expected from anatomical and slice physiology results, then one might predict that the FSI and MSN populations would consistently change firing rate in opposite directions. This was certainly not the case, even for FSI:MSN pairs on the same tetrode (not shown). However, our results are in line with recent detailed modeling studies that have indicated unexpected, counter-intuitive properties of striatal microcircuits. In particular, the GABAergic input from FSIs seems not to greatly reduce overall MSN firing (Moyer and Wolf, 2009) and may even increase it (Humphries *et al*, 2009). FSIs are well placed to determine the fine timing of MSN spiking (Koos *et al*, 2004; Berke, 2009; Wilson, 2009) and may serve to control the correlations between members of MSN ensembles (Humphries *et al*, 2009) more than their gross firing rate. Another unanticipated property of FSIs is that, though coupled together by gap junctions, they do not normally show high synchrony (Berke, 2008; Hjorth *et al*, 2009; Humphries *et al*, 2009). We have found evidence that the striatal FSI network may switch from an unstable to a stable state as actions are selected, with gap junctions contributing to the stabilization of FSI firing (Lau *et al*, 2009). Speculatively, the increase in irregular, burst firing seen with eticlopride may thus mirror the reduced capacity of striatal networks to select actions, leading to psychomotor suppression.

The consistently positive correlation between the FSI population and our relatively crude measure of locomotor activity (on a minute-to-minute timescale) stands in contrast to the usually highly diverse FSI:behavior relationships reported during maze task performance (on a second-by-second timescale; Berke, 2008). Although we lack a detailed anatomical understanding of the excitatory inputs to FSIs, such idiosyncratic firing seems to reflect unique sets of cortical inputs from the cortex (Ramanathan *et al*, 2002). Recently, a more coordinated increase in FSI activity has been observed specifically around the moment of choice execution in an operant action-selection task, and this was accompanied by an overall decrease in GP activity (Gage *et al*, 2008). Those findings and the present results on drug effects together support a model in which cortical glutamate inputs supply detailed information patterns to FSIs, and GP GABAergic feedback serves as a more broadly distributed control over FSI firing rates.

Our findings help to reveal drug actions on the local circuit level that would be hard to detect using other methods such as neuroimaging, and are highly relevant to the understanding of widely prescribed drug treatments and their side effects. For example, the uniform suppression of FSI firing by eticlopride indicates that the therapeutic benefits of D2 antagonists in Tourette syndrome are unlikely to derive from increasing FSI firing to compensate for their reduced number (Kalanithi *et al*, 2005). Rather, given that a deficit in striatal FSIs is found in a rodent model of paroxysmal dystonia (Gernert *et al*, 2000), and that dystonias and dyskinesias are produced by local GABA blockade in striatum (for example, Yoshida *et al*, 1991; Worbe *et al*, 2009), FSI suppression is a strong candidate mechanism for the adverse behavioral effects of antipsychotic treatment (which include dystonias, dyskinesias, and akathesia; Hyman *et al*, 1995).

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DISCLOSURE

The authors declare no conflict of interest.

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