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Neural Substrates of Dopamine D2 Receptor Modulated Executive Functions in the Monkey Prefrontal Cortex

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Dopamine D2 receptors (D2R) play a major role in cognition, mood and motor movements. Their blockade by antipsychotic drugs reduces hallucinatory and delusional behaviors in schizophrenia, but often fails to alleviate affective and cognitive dysfunctions. The prefrontal cortex (PFC) expresses D2R and is altered in schizophrenia. We investigated how D2R modulate behavior and PFC function in monkeys. Two monkeys learned new and performed highly familiar visuomotor associations, where each cue was associated with a saccade to a right or left target. We recorded neural spikes and local field potentials from multiple electrodes while injecting the D2R antagonist eticlopride in the lateral PFC. Blocking prefrontal D2R impaired associative learning and cognitive flexibility, reduced motivation, but left the performance of familiar associations intact. Eticlopride reduced saccade-direction selectivity of prefrontal neurons, leading to a decrease in neural information about the associations, and an increase in alpha oscillations. These results, together with our recent study using a D1R antagonist, suggest that D1R and D2R in the primate lateral PFC cooperate to modulate several executive functions. Our findings help to gain insight into why antipsychotic drugs, with strong antagonistic actions on D2R, fail to ameliorate cognitive and emotional deficits in schizophrenia.

Keywords: dopamine D2 receptors, learning and memory, neural oscillations, prefrontal cortex, schizophrenia

Introduction

The lateral prefrontal cortex (PFC) is central to executive functions and motivation (Fuster 2001; Miller and Cohen 2001). In primates, dopamine neurons from the ventral tegmental area and the substantia nigra pars compacta send widespread afferents to PFC (Fallon 1988; Lewis 1992; Williams and Goldman-Rakic 1998) and modulate PFC function via multiple receptor subtypes, including dopamine D2 receptors (D2R) (Lidow et al. 1998; de Almeida et al. 2008). D2R are preferentially expressed in subpopulations of layer V pyramidal neurons (Lidow et al. 1998; Santana et al. 2009), where they can increase or decrease their spiking activity (Gulledge and Jaffe 1998; Wang et al. 2004; Lavin et al. 2005; Gee et al. 2012). In rodents, prefrontal D2R contribute to memory-related tasks, cognitive flexibility, and decision making (Druzin et al. 2000; Floresco et al. 2006; St Onge et al. 2011; Watson et al. 2012; Floresco 2013). But whether D2R are involved in working memory and other executive functions in primates is less clear (Sawaguchi and Goldman-Rakic 1991, 1994; Wang et al. 2004; von Huben et al. 2006; Vijayraghavan et al. 2007; Floresco 2013).

Classical antipsychotics are potent antagonists of D2R, and are very efficient in reducing the hallucinatory and delusional behaviors (positive symptoms) in schizophrenia (Artigas 2010; Meltzer 2013). Schizophrenia patients, however, show other impairing symptoms such as poor long-term planning and disorder of thought, loss of motivation and anhedonia (cognitive and negative symptoms), which often do not respond adequately to antipsychotic treatment (Harvey and Keefe 2001; Vingerhoets et al. 2013). The classical dopamine hypothesis is that the positive symptoms in schizophrenia are caused by hyperstimulation of D2R in the mesolimbic system, whereas the cognitive and negative symptoms follow hypostimulation of cortical D1R (Abi-Dargham and Moore 2003). Interestingly, it has been recently proposed that imbalanced D1:D2 receptor activation in the PFC with overstimulation of prefrontal D2R could lead to the emergence of positive, cognitive, and negative symptoms in schizophrenia (Durstewitz and Seamans 2008). Thus, understanding the cellular mechanisms of D1R and D2R in PFC function may help unravel why most antipsychotic drugs have poor capacity to ameliorate the cognitive and negative symptoms in schizophrenia (Harvey and Keefe 2001; Vingerhoets et al. 2013).

We trained 2 monkeys on an associative learning and memory task and blocked PFC D2R while recording spikes and local field potentials (LFPs) from multiple electrodes. We recently showed that blocking D1 receptors (D1R) impairs associative learning and cognitive flexibility in monkey lateral PFC (Puig and Miller 2012). Here, we show that blocking D2R also impairs associative learning and cognitive flexibility, and provide the neural correlates of this modulation.

Materials and Methods

Animals

Animal protocols were approved by the National Institutes of Health and the Massachusetts Institute of Technology Animal Care and Use Committee. Stimulus presentation and behavioral monitoring were controlled with the CORTEX real-time system (http://dally.nimh.nih.gov/). Eye position was tracked optically with an infrared camera (EyeLink 1000 system).

Behavioral Task

Two rhesus monkeys (*Macaca mulatta*; LA and LK) were trained to learn, by trial and error, associations between visual cues and saccades to the right or left (Fig. 1*A*). Also included were highly familiar cues (>1 year of training) and their associations. Trials were blocked in pairs of initially novel cues on 80% of the trials (novel trials), and pairs of highly familiar cues on 20% of the trials (familiar trials). Once a pair of new associations was learned (at least 80% correct and 30 correct trials per cue), 2 new cues replaced the previously novel cues, and a new block of trials started. In each session, monkeys first completed several preinjection (baseline) blocks. Then, 3 μL of the D2R antagonist eticlopride were unilaterally pressure-injected into the left lateral

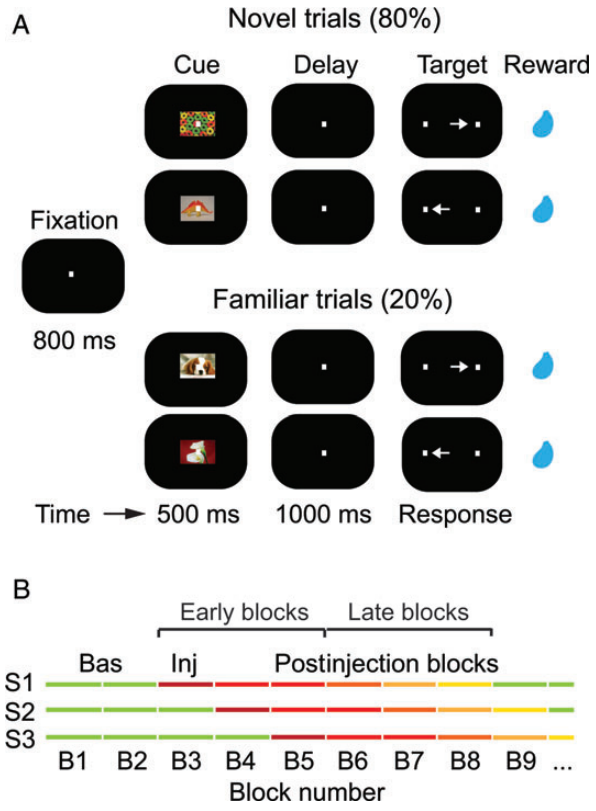

Figure 1. Delayed associative learning and memory task. (*A*) Monkeys performed a delayed associative learning and memory task, similar to that described previously (Puig and Miller 2012). Animals fixated to start trial. A cue object was followed by a brief memory delay and presentation of 2 target dots. Saccade to the target associated with the cue was rewarded with juice drops. Trials were blocked in pairs of novel cues (novel trials; 80% of trials), and pairs of familiar cues (familiar trials; 20% of trials). When performance of novel trials reached the learning criteria (80% correct and 30 correct trials per novel cue), novel cues were replaced and a new block started. The performance of familiar associations was not considered in the learning criteria. (*B*) Monkeys first completed several Baseline blocks (Bas; first green lines). Then, 3 μL of eticlopride (30 or 1 μg diluted in saline) were pressure-injected in the left lateral PFC (Inj; injection block). The performance during postinjection blocks was compared with baseline blocks and saline controls to assess any actions of eticlopride on behavior and neural activity. Eticlopride was injected after different numbers of baseline blocks in different sessions (S1–S3) to account for any confounds generated by a systematic behavior of the monkeys. We classified blocks as baseline, "early" (injection block and first 2 postinjection blocks), or "late" (postinjection blocks 3–5).

PFC at 0.3 µL/min. High (30 µg) or low (1 µg) concentrations of eticlopride (ETI30 and ETI1, respectively) were injected in separate sessions (see below). Saline controls, eticlopride, and SCH23390 (D1R antagonist) experiments were interleaved. Thus, the controls used in this study are the same as in our previous study of D1R (Puig and Miller 2012).

Behavioral Analysis

In order to examine the time-course of the behavioral effects, blocks completed after the start of the injection were classified as "early" (the injection block and the first 2 postinjection blocks) or "late" (postinjection blocks 3–5) with respect to the time of injection (Fig. 1*B*). Eticlopride effects on learning were examined by comparing the mean learning rates during baseline blocks with learning rates of "early" and "late" blocks. Learning rates were estimated for each block by fitting a sigmoid curve to the trial-by-trial binary responses (1 for correct and 0 for incorrect trials) using a logistic regression model (Williams and Eskandar 2006; Puig and Miller 2012), and the learning rates were the slopes of the fitted distributions. As previously reported (see Supplementary Fig. 1 in Puig and Miller 2012), monkey LA developed a behavioral bias with training by trying first a saccade to the left target, whereas monkey LK was extremely proficient at this task and needed very few trials to learn the associations. As a consequence, monkeys' performance was over 50% correct at the beginning of some blocks, thus learning curves in these blocks were naturally shallower. In order to eliminate this potential confound, sigmoid curves were fitted ignoring the performance of the first 3 trials and forcing the curves to start at 50%. Cognitive inflexibility was estimated by calculating the percentage of perseverative (consecutive error) trials, error trials that were preceded by another error trial of the same cue (Clarke et al. 2008; Caetano et al. 2013). Overall, the monkeys were overtrained in the task and made very few errors after the initial learning phase in drug-free conditions.

Drugs and in Vivo Pharmacology

Saline and SCH23390 (D1R antagonist) groups were recently reported in Puig and Miller (2012). We used the dopamine D2R antagonist "eticlopride" that has preferential affinity for D2R over D3R (which are much less expressed than D2R in monkey lateral PFC; Lidow et al. 1998) and D4R (Martelle and Nader 2008). Eticlopride has also some affinity for D1R and alpha1-adrenoceptors. Eticlopride was purchased from Sigma/RBI and dissolved in commercially available sterile saline (0.9% NaCl) at 10 µg/µL (26.4 mM) or 0.33 µg/µL (0.9 mM) under strict sterile conditions and stored at −20°C. The pH was corrected to be around 6.0. A total of 30 or 1 µg of eticlopride was infused in one injection site in 3 µL of saline. The procedure is described in detail elsewhere (Puig and Miller 2012).

Electrophysiological Recordings

Electrode penetration sites were determined using MRI scans. The recording chamber was positioned stereotaxically over the left lateral PFC of each animal overlying the principal sulcus. Electrophysiological signals were recorded simultaneously from 7 to 15 dura-puncturing tungsten microelectrodes (FHC Instruments), located 1 or 2 mm away from the cannula. Electrodes were lowered each day using screw-driven microdrives mounted on a plastic grid (Crist Instruments), with spacing of 1 mm between adjacent locations. Neuronal activity was amplified, filtered, and stored using an integrated multichannel recording system (Plexon Neurotechnology Research Systems). To minimize any sampling bias of neuronal activity, we did not prescreen activity for any visual responsiveness. From each electrode, we simultaneously recorded spiking activity and the LFP. The spike signal (passband 154 Hz to 8.8 kHz) was threshold-triggered to separate neuronal spikes from background noise, and individual spike waveforms were stored at 40 kHz. LFPs (passband 0.7–300 Hz) were recorded continuously with a sampling rate of 1 kHz.

Spike-Rate Analysis

Recorded waveforms were sorted with Offline Sorter (Plexon). All spike-rate analyses were performed on correct trials with custom software written in MATLAB (Mathworks). Saccade direction selectivity was assessed by comparing the spiking activity of right versus left trials during 2 epochs of the trial: "cue" (100–600 ms after cue onset) and "delay" (100–1000 ms after cue offset), using the ω^2 percentage of explained variance (ωPEV) statistic (Olejnik and Algina 2003; Buschman et al. 2011),

$$\omega^2 = \frac{\mathrm{SS}_{\text{Between groups}} - \mathrm{df} \times \mathrm{MSE}}{\mathrm{SS}_{\text{Total}} + \mathrm{MSE}}$$

where $\mathrm{SS}_{\text{Total}} = \Sigma_i^N (xi - \bar{x})^2$, $\mathrm{SS}_{\text{Between groups}} = \sum_{\text{Group}}^{G} n_{\text{Group}} (\bar{x}_{\text{Group}} - \bar{x})^2$, df is the degrees of freedom (number of groups G −1), and MSE is the mean squared error $= \sum_i^N (x_i - \bar{x}_{\text{Group}})^2$. ω^2 is an unbiased measure, resulting in a near zero mean when there is no saccade direction information. To identify neurons selective to novel and/or familiar associations, we combined all right correct trials and all left correct trials of baseline blocks. Because familiar trials were only presented on 20% of the trials, only neurons with 10 or more correct (familiar) trials per cue were used. To determine whether ωPEV was significantly different from chance, we used a randomization test. Right and left saccade trials were randomly shuffled and ωPEV was recalculated. By repeating this process

1000 times, a null distribution was constructed. ωPEV was then compared with this null distribution to determine the likelihood of the observed ωPEV, and significance level was set to $P<0.05$. For neurons selective to novel and/or familiar associations, we calculated the ωPEV z-score with the mean and standard deviation of the null distribution in baseline, early, and late blocks. Because the z-score is sensitive to the number of observations used, to compare ωPEV z-score in novel and familiar trials, we combined all familiar trials in a block and matched the same number of randomly selected novel trials. Firing rates were normalized by the spiking rate during fixation (300 ms before the cue onset) and smoothed with a Gaussian kernel.

Spectral Analysis

Spectrograms were built for correct trials using a continuous wavelet transform between frequencies 1 and 128 Hz and a Morlet function as mother wavelet (Torrence and Compo 1998). Power spectra during the 1 s delay were computed with a multi-taper approach with the Chronux Package (www.chronux.org), and were estimated with a time-bandwidth product of $TW=3$ and $k=5$ tapers. To enhance readability of the LFP power at high frequencies, which are masked by the $1/f^n$ power-law decay, we normalized the power by the frequency. To investigate which LFP activity reflects signal components independent to trial events, we subtracted from each trial the LFP signal averaged across all of the trials. Sixty hertz noise was digitally filtered with a Butterworth filter.

Results

Prefrontal D2R Contribute to Learning of New Associations and Cognitive Flexibility, but not Performance of Familiar Associations

The injection of 30 µg of eticlopride (ETI30; $n=10$ sessions) in the lateral PFC impaired associative learning. The number of blocks of trials successfully completed (by reaching learning criterion) after ETI30 was reduced during the first hour postinjection compared with baseline (from 2.4 ± 0.4 to 1 ± 0.4 blocks) and saline (from 2.6 ± 0.2 to 3.5 ± 0.2 blocks, two-way ANOVA with treatment [saline, ETI30] and time [baseline, postinjection] as factors, $F_{1,50}>25$, $P<0.0008$ for treatment and interaction, Tukey's least significant difference post hoc test). This was not significantly different from D1R blockade with 30 µg SCH23390 (from 2.6 ± 0.2 to 1.6 ± 0.2 blocks; $P=0.08$; Puig and Miller 2012). However, in 8 of 10 sessions, monkeys stopped working <20 min after the ETI30 injections and, in 4 sessions, they could not complete any postinjection block. This was not observed during D1R blockade.

Figure 2*A* shows the average learning curves across all sessions during the first 60 trials of each block (the minimum block length) of the ETI30 sessions. We measured the learning rate of each block completed by the monkeys from the slope of a fitted sigmoid distribution using a logistic regression model (Williams and Eskandar 2006). Importantly, monkeys' behavioral biases at the beginning of some blocks were taken into consideration for the computation of learning rates, allowing for a more accurate comparison of performances between blocks (see Materials and Methods). The learning rates were significantly smaller (i.e., slower) in the injection and first 2 postinjection blocks ("early" blocks) relative to baseline blocks and relative to the same postsaline blocks (ANOVA as above; $F_{1,83}>5.8$, $P<0.02$ for both factors, $P=0.08$ for the interaction; $P=0.0025$ for baseline vs. early blocks; $P=0.0035$ for saline vs. ETI30 learning rates in early blocks; Fig. 2*B*). This reduction of learning rates after ETI30 in early blocks was observed

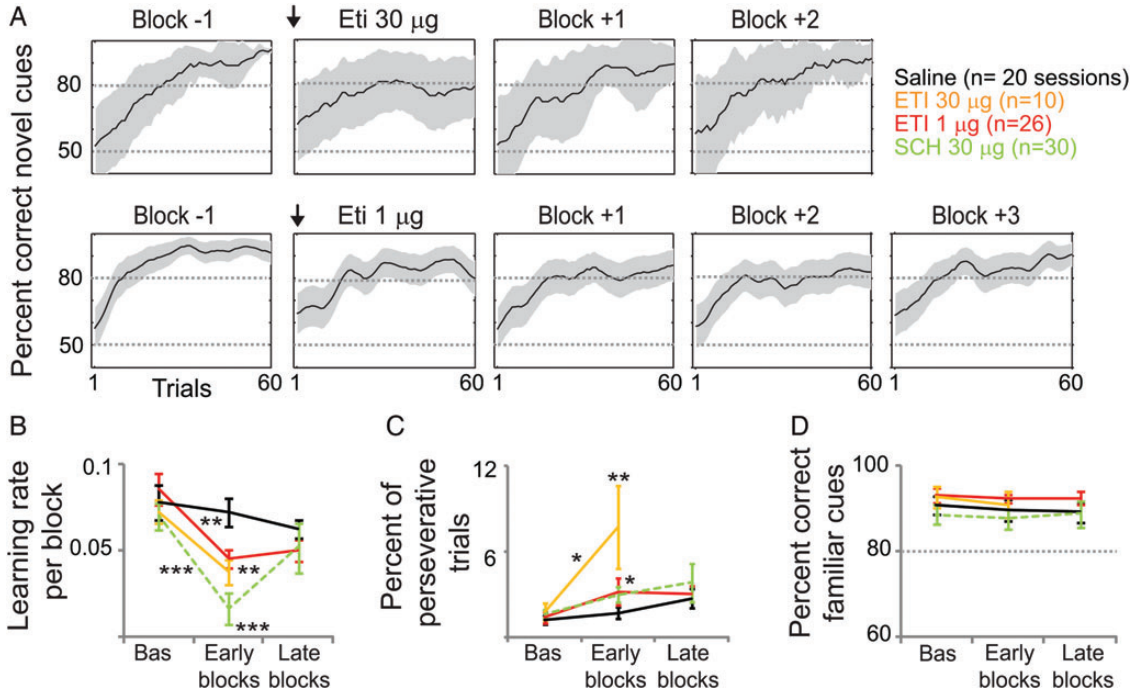

Figure 2. D2R in the lateral PFC are involved in the learning of new associations and cognitive flexibility, but not the performance of familiar associations. (*A*) Average percent correct performance across sessions during the first 60 correct and incorrect trials of blocks (the minimum block length) for the preinjection baseline block (Block −1), the injection block, and the first (Block +1), second (Block +2), and third (Block +3; only available in the ETI1 group) postinjection blocks. (*B*) Average learning rates across sessions during the baseline, early, and late blocks. Learning rates decreased significantly after the injection of both ETI30 and ETI1 compared with baseline and postsaline blocks, but less than after SCH23390 (D1R antagonist). (*C*) Average percent of perseverative errors (consecutive error trials) during baseline, early, and late blocks. Perseverative errors increased significantly after the injection of both ETI30 and ETI1 compared with baseline and postsaline blocks. (*D*) Average percent correct of familiar trials. Dashed line depicts the 80% threshold used as learning criterion. Eticlopride did not affect the performance of familiar associations. Two-way ANOVA for treatment and blocks as factors (see main text). $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$, Tukey's least significant difference post hoc test. Data for saline and SCH23390 were taken from Puig and Miller (2012). Shown are the mean and SEM in all figures.

in the 2 monkeys (Supplementary Fig. 1). Learning rates were higher after ETI30 (when the monkeys did not stop working) than after SCH23390 (ANOVA for treatment and block, $F_{1,128} > 12$, $P < 0.0009$ for both factors and interaction; $P = 0.04$ in early blocks).

To collect neurophysiological data, we needed the animals to perform a sufficient number of trials. Thus, because 30 μg of eticlopride typically caused the monkeys to stop working, we switched to a smaller concentration of eticlopride. We implemented several pilot experiments with 10, 5, and 1 μg of eticlopride and observed that 10 and 5 μg still caused the monkeys to stop working. Thus, we used the lower concentration (ETI1, 1 μg). Learning rates in the injection and the first 2 ETI1 postinjection (early) blocks were significantly lower than both baseline blocks ($P = 9 \times 10^{-5}$, as above) and saline controls ($F_{1,256} > 5$, $P < 0.007$ for block factor, $P = 0.0078$ in early blocks; Fig. 2*B*). This effect of ETI1 was observed in both monkeys individually (Supplementary Fig. 1). Learning rates, in general, decreased over each session (see saline, Fig. 2*B*). By postinjection blocks 3–5 (late blocks), learning rates following ETI1 were not different from saline (ANOVA as above, $P > 0.05$), although they were lower than the baseline blocks ($P = 0.04$).

Part of the learning impairment was due to increases in perseveration (consecutive repeats of the same error). Figure 2*C* shows that the increase in perseveration was highest after ETI30 (ANOVA for treatment [ETI30 vs. saline] and block, $F_{1,104} > 14$, $P < 0.0004$ for both factors and interaction, $P = 0.0003$ in early blocks). It also increased after ETI1 in the early blocks ($F_{1,252} > 10$, $P < 0.05$ for block, $P = 0.001$ in early blocks) to a similar extent as SCH23390 ($P = 0.79$). The perseveration increase in early blocks was more pronounced after ETI30 than after SCH23390 ($F_{1,128} > 12$, $P < 0.05$ for both factors and interaction, $P = 0.0005$). Errors after eticlopride were not caused by increased impulsivity (premature saccades) or abnormal eye movements, and reaction times did not change significantly after eticlopride (Supplementary Fig. 1).

In contrast, and like SCH23390, eticlopride did not impair the performance of familiar associations compared with baseline (ETI30 [baseline, early blocks]: $P = 0.6$; ETI1 [baseline, early, late blocks]: $F_{2,143} < 0.2$, $P > 0.8$) or saline (vs. ETI30: $F_{1,106} < 1$, $P > 0.37$; vs. ETI1: $F_{1,239} < 1$, $P > 0.5$; Fig. 2*D*), nor did it increase perseveration in familiar trials (saline vs. ETI30: $F_{1,104} < 0.2$, $P > 0.6$; saline vs. ETI1: $F_{1,252} < 0.3$, $P > 0.7$).

Neural Information During Associative Learning is D2R-Dependent

Here, we report the effects of ETI1 on PFC spiking activity during correctly performed trials. As previously seen (Pasupathy and Miller 2005; Puig and Miller 2012), during normal learning many prefrontal neurons showed an increase of activity during the cue and/or memory delay that predicted the direction of the forthcoming saccade associated with the cue. We assessed saccade-direction selectivity using the ω^2 percent explained variance or ωPEV. ωPEV provides an unbiased estimation of neural information about saccade direction, higher ωPEV indicating more information (see Materials and Methods). We focused our analyses on the cue and delay intervals, when the monkeys could, with learning, predict the saccade direction to be made at the end of the trial. During one or both of these intervals, 27% (69 of 259) of neurons showed neural selectivity for saccade direction during learning trials in baseline blocks.

For this neuron population, ETI1 significantly reduced the average difference in activity to preferred versus nonpreferred saccade directions during the early learning blocks (see Fig. 3*A* for a single-neuron example, and Fig. 3*B* for the population average; ANOVA, saccade direction and block, $F_{1,380} > 2.4$, $P = 0.001$ for direction). This reduction seemed to be due to an increase in activity to the nonpreferred direction ($P = 0.005$; Fig. 3*B*). Correspondingly, ETI1 decreased neural information (ωPEV *z*-score) about saccade direction during learning in early blocks compared with baseline blocks and saline (ANOVA for treatment and block as above, $F_{1,441} > 7$, $P < 0.007$ for treatment; $P = 0.0004$ for baseline vs. early blocks; $P = 0.04$ for ωPEV postsaline vs. post-ETI1 early blocks; Fig. 3*C*). In late blocks, the amount of neural information about the forthcoming saccade direction did not differ from baseline ($P = 0.06$) or saline ($P = 0.2$), but it also did not differ from post-ETI1 early blocks ($P = 0.7$).

An overlapping but distinctive population of neurons showed saccade-direction selectivity to familiar cue associations during the cue and/or memory delay (63 of 259 neurons [25%]; ANOVA of all correct trials per saccade direction in baseline blocks; 27 neurons were also selective to novel associations [43% overlap]). Note that this is a conservative estimate because familiar cues were only shown to the monkeys on 20% of the trials, thus limiting statistical power. ETI1 also reduced the difference in neural activity to preferred versus nonpreferred saccade directions in early blocks, again, by increasing activity to the nonpreferred direction (ANOVA for direction and block as above; $F_{1,358} > 5$, $P < 0.006$ for both factors and the interaction; $P = 0.001$ for baseline vs. early blocks in the nonpreferred direction; Fig. 3*D*). During the late blocks, there was a recovery of neural effects, a significant difference in activity to the preferred and nonpreferred directions ($P = 0.0004$). ETI1 reduced saccade-direction ωPEV to familiar cues during early and late blocks compared with baseline (ANOVA for block; baseline vs. early blocks, $P = 0.008$; baseline vs. late blocks, $P = 0.03$), where it was not different from novel trials (ANOVA for trial type [novel, familiar] and block; $F_{1,322} > 7$, $P < 0.008$ for both factors; $P = 0.3$ and $P = 0.4$ for novel vs. familiar in early and late blocks, respectively; Fig. 3*E*).

Blocking Prefrontal D2R Increases Alpha Oscillations During Associative Learning

As previously reported (Puig and Miller 2012), we observed a robust alpha-beta band in prefrontal LFPs during the fixation and delay epochs of correct trials (Fig. 4*A*). During the memory delay, there were 2 peaks in the power spectra at ~12 Hz (alpha) and ~22 Hz (beta), and a smaller peak at 2 Hz (delta). ETI1 increased the power of alpha oscillations, but not beta and delta oscillations (Fig. 4*B*). This increase occurred in both novel and familiar trials, and during both early and late blocks (Wilcoxon ranked test, $P < 0.05$; early blocks: novel trials: 9–14 Hz; familiar trials: 8–14 Hz; late blocks: novel trials: 8–19 Hz; familiar trials: 8–17 Hz).

Discussion

By using the potent D2R antagonist eticlopride (Martelle and Nader 2008), we show that dopamine D2R in the monkey

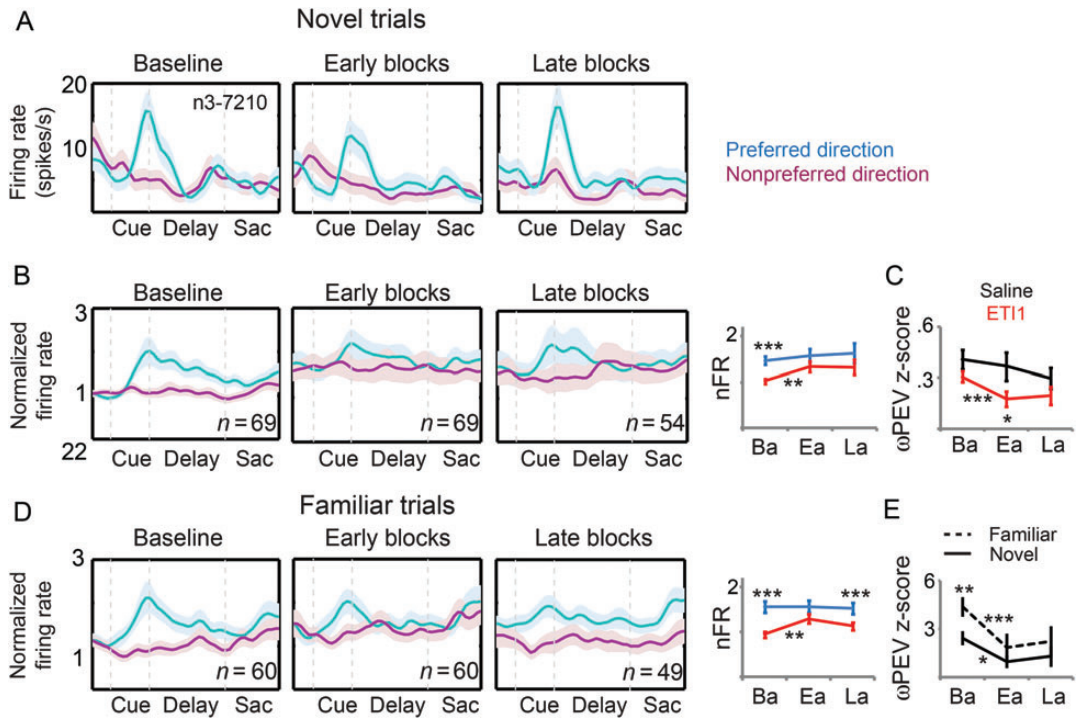

Figure 3. Cue and delay selectivity of association-selective neurons is D2R-dependent. (*A*) Single-neuron example showing a reduction of selectivity after injection of ETI1. Preferred (blue traces) and nonpreferred (magenta traces) saccade direction trials during baseline, early, and late blocks are shown. (*B*) Normalized firing rate during learning (all correct trials per block) of neurons selective to novel associations, and corresponding quantification during baseline, early, and late blocks (nFR, cue epoch for cue-selective neurons and delay epoch for delay-selective neurons; two-way ANOVA for preferred direction and block). Spiking activity was normalized by the mean firing rate during the fixation period (300 ms before cue presentation) in baseline blocks. *n* depicts the number of neurons used for the analyses. (*C*) Quantification of the strength in direction selectivity as monkeys learned the associations (proportion of explainable variance by direction factor ωPEV normalized with a *z*-score, all correct trials per cue; see Materials and Methods) during the cue or delay epochs comparing the ETI1 group (red) with saline controls (black). Two-way ANOVA for treatment and block. (*D*) Normalized firing rate of neurons selective to familiar associations, and quantification during the cue or delay epochs. (*E*) Effects of ETI1 on novel and familiar trials. Novel trials were randomly selected to match the number of familiar trials in a block. Two-way ANOVA for novel versus familiar trial and block. Data for saline was taken from Puig and Miller (2012).

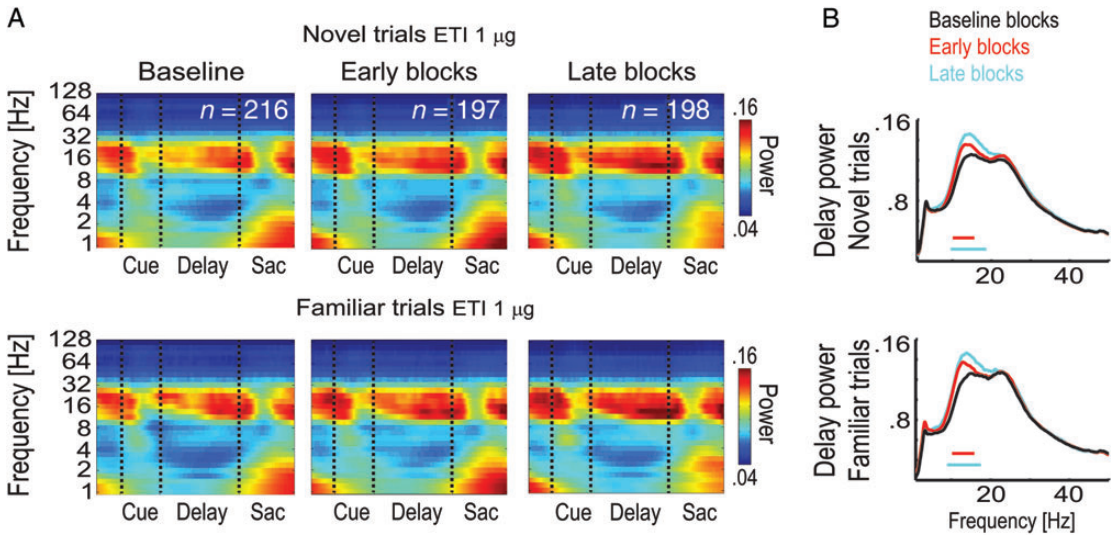

Figure 4. Blocking prefrontal D2R increases the power of alpha oscillations. (*A*) Time–frequency representation of the average LFP power using wavelets for correct trials during baseline, early, and late blocks, novel and familiar trials. *n* depicts the number of recording sites used for the analyses (the same electrodes were used for novel and familiar trials). (*B*) Corresponding power spectra of the memory delay (1000 ms after cue offset). Wilcoxon ranked test, $P < 0.05$.

lateral PFC are involved in the modulation of several executive functions essential for advanced cognition. D2R likely contribute to associative learning (the monkeys learned new cue-response associations slower after eticlopride), although their blockade induced a smaller impairment compared with blockade of D1R, even with a high concentration of the D2R

antagonist. The effects of blocking D2R on the monkeys' learning performance are striking because D2R are several times less abundant than D1R in PFC and are confined to layer V neurons (Lidow et al. 1998; de Almeida et al. 2008). D2R may also be involved in cognitive flexibility (eticlopride increased perseverative errors), a core feature of PFC function that depends on dopamine neurotransmission (Chudasama and Robbins 2006; Floresco et al. 2006; Gruber et al. 2010; Floresco 2013). Perseveration was more pronounced after a high concentration of eticlopride than after a high concentration of the D1R antagonist. This dissociation between D1R/D2R roles in PFC function (predominant role of D1R in associative learning and D2R in cognitive flexibility) support the notion that D1R activation allows the stabilization of new representations once an effective strategy has been identified (robust stimulus-response maintenance after initial learning of novel associations), whereas D2R activation destabilizes PFC network states favoring the exploration of new strategies (flexible processing; Durstewitz and Seamans 2008). Given that learning of novel associations requires initial behavioral flexibility, a fine balance between D1R and D2R activation may be necessary to perform this task. Our study also suggests that prefrontal D2R do not influence the performance of highly familiar associations.

The reduction in learning rates produced by the high and low concentrations of eticlopride was not different. This may reflect the smaller number of experiments carried out with the high concentration ($n = 10$ vs. $n = 26$ with the low concentration) due to the strong demotivation shown by the animals. It may be that the animals "gave up" on many of the high concentration sessions because they were so severely impaired, but on the minority of sessions in which they managed to keep working, their learning impairment was no worse than on the lower concentration sessions. The higher concentration could have also affected motivation per se. Dopamine is key for motivational processes (Wise 2004), and prefrontal neurons integrate information about cognitive and motivational context (Watanabe and Sakagami 2007). It is also plausible that D2R modulate associative learning less than D1R, and the deleterious effects of eticlopride reach a "ceiling" with a lower concentration (i.e., a higher concentration does not impair learning further).

In monkeys, systemic blockade of D2R, but not D1R, impairs cognitive flexibility (reversal learning) without affecting new learning (Lee et al. 2007). Our work using local administration of D1R/D2R antagonists in PFC suggests that both receptors contribute differently to new learning and cognitive flexibility. We measured cognitive flexibility as the percentage of consecutive error (perseverative) trials. Our task was not intended to examine the actions of D2R on different types of cognitive flexibility. Future work will have to be conducted to determine if blockade of PFC D2R affects other types of cognitive flexibility like reversal learning or attentional-set shifting (Chudasama and Robbins 2006; Robbins and Arnsten 2009).

Blocking D2R reduced neural information of association-selective neurons (both during cue and delay epochs of the trial) largely via an increase in spiking rates for nonpreferred directions. This is similar to blocking of D1R in associative learning (Puig and Miller 2012) and spatial working memory tasks (Sawaguchi and Goldman-Rakic 1991, 1994; Williams and Goldman-Rakic 1995; Vijayraghavan et al. 2007), but in contrast with a lack of D2R modulation of delay activity in spatial working memory (Wang et al. 2004). Differences in task demands and specific recording/injection sites may account for this discrepancy. We have previously shown that neurons and networks in the PFC can multitask (Cromer et al. 2010; Buschman et al. 2012). It is therefore likely that association-selective neurons modulated by D2R participate in (overlapping) cortical and subcortical neural networks encoding associative learning and cognitive flexibility. Because our recording technique does not allow for an unambiguous identification of neuron populations, it is challenging to speculate which neuron subtypes account for these effects. However, D2R are primarily expressed by layer V pyramidal neurons of the PFC (Lidow et al. 1998; Santana et al. 2009) where they can enhance (Wang and Goldman-Rakic 2004) or suppress (Gulledge and Jaffe 1998) excitability. Neural information for familiar associations was also reduced by eticlopride, even though their performance was not affected. Thus, performance of familiar associations likely depends on other brain structures such as the striatum, where they could have become habits (Graybiel 2008).

At a network level, eticlopride increased the power of alpha oscillations (~8–14 Hz) both in novel and familiar trials. But D2R exerted less of an influence on oscillatory activity than D1R. Blocking D1R boosted alpha and beta oscillations and spike hypersynchronization, reflected as sharp seizure-like deflections in the LFP signals (Puig and Miller 2012). This was never observed during the D2R blockade. This may be due to a combination of 2 factors. First, eticlopride does not induce enough increase of neural activity to favor hypersynchronization; and second, D2R are not as broadly expressed as D1R in primate cortex. Noteworthy, aberrant alpha oscillations have been associated with inattention (Jensen et al. 2002; Buschman et al. 2012), and are abnormal in neurodegenerative and psychiatric disorders, such as Alzheimer's, bipolar disorder, and schizophrenia (see for review Basar-Eroglu et al. 2008; Basar and Güntekin 2008; Uhlhaas and Singer 2010).

Eticlopride has a strong affinity for D2R and D3R (Martelle and Nader 2008), but D3R are much less expressed than D2R in the monkey PFC (Lidow et al. 1998; de Almeida et al. 2008). It binds to a lesser extent to D4R, D1R, and alpha1-adrenoceptors. Therefore, it is possible that the effects observed here (especially after a high concentration of eticlopride) had been elicited in part by receptors other than D2R. Future work examining the actions of selective antagonists for D3R, D4R, D1R, and alpha1-adrenoceptors will be necessary to establish the specificity of effects of eticlopride.

Collectively, our work shows that blocking prefrontal D1R and D2R modulate associative learning and cognitive flexibility in a cooperative manner. Importantly, the impairing actions of a D2R blockade are congruent with the poor capacity of antipsychotic treatments to ameliorate the cognitive and negative symptoms in schizophrenia (Harvey and Keefe 2001; Vingerhoets et al. 2013). In fact, they may deteriorate PFC-dependent associative learning, cognitive flexibility, and motivation even further.

Supplementary Material

Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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