Behavioral/Cognitive

Dopamine Regulates Two Classes of Primate Prefrontal Neurons That Represent Sensory Signals

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The lateral prefrontal cortex (PFC), a hub of higher-level cognitive processing, is strongly modulated by midbrain dopamine (DA) neurons. The cellular mechanisms have been comprehensively studied in the context of short-term memory, but little is known about how DA regulates sensory inputs to PFC that precede and give rise to such memory activity. By preparing recipient cortical circuits for incoming signals, DA could be a powerful determinant of downstream cognitive processing. Here, we tested the hypothesis that prefrontal DA regulates the representation of sensory signals that are required for perceptual decisions. In rhesus monkeys trained to report the presence or absence of visual stimuli at varying levels of contrast, we simultaneously recorded extracellular single-unit activity and applied DA to the immediate vicinity of the neurons by micro-iontophoresis. We found that DA modulation of prefrontal neurons is not uniform but tailored to specialized neuronal classes. In one population of neurons, DA suppressed activity with high temporal precision but preserved signal/noise ratio. Neurons in this group had short visual response latencies and comprised all recorded narrow-spiking, putative interneurons. In a distinct population, DA increased excitability and enhanced signal/noise ratio by reducing response variability. These neurons had longer visual response latencies and were composed exclusively of broad-spiking, putative pyramidal neurons. By gating sensory inputs to PFC and subsequently strengthening the representation of sensory signals, DA might play an important role in shaping how the PFC initiates appropriate behavior in response to changes in the sensory environment.

Introduction

All neuronal systems are subject to neuromodulation, which can profoundly alter the properties of target circuits (Marder, 2012). The primate lateral prefrontal cortex (PFC), a hub of higher-level cognitive functioning (Fuster, 2008; Bongard and Nieder, 2010; Eiselt and Nieder, 2013), receives particularly strong projections from dopamine (DA) neurons in the midbrain (Williams and Goldman-Rakic, 1998; Björklund and Dunnett, 2007). DA neurons fire phasic bursts of action potentials with short latencies of 100–150 ms in response to behaviorally relevant sensory events (Schultz, 1998; Matsumoto and Hikosaka, 2009). Therefore, it has been suggested that DA could prepare its higher-order target areas for the processing of incoming signals (Redgrave and Gurney, 2006; de Lafuente and Romo, 2011). How might DA influence recipient prefrontal neurons to control information relayed to this important cortical structure?

Prefrontal DA regulates many frontal lobe functions, such as set-shifting and behavioral flexibility (Floresco et al., 2006), association learning (Puig and Miller, 2012), and the maintenance of stimuli in working memory (Brozoski et al., 1979). Much of what is known about the mechanisms of DA action in PFC stems from electrophysiological studies on memory-related activity, i.e., in the absence of sensory stimulation (Williams and Goldman-Rakic, 1995). In rhesus monkeys engaged in a spatial working memory task, PFC neurons active in the delay period of the task showed improved tuning to preferred remembered locations when stimulated with DA receptor agonists (Vijayraghavan et al., 2007). Therefore, it is believed that the principal function of DA in PFC is to strengthen mental representations (Arnst, 2011).

In contrast, little is known about how DA modulates prefrontal sensory signals that precede and give rise to such sustained activity. Anecdotal evidence indicates that visual stimuli used to cue a target to be remembered are also influenced by DA (Sawaguchi et al., 1990; Williams and Goldman-Rakic, 1995), but quantitative analysis and an in-depth investigation of the cellular mechanisms are lacking. Because phasic DA activity that is time-locked to relevant sensory stimuli seems particularly suited to regulate the representation of these shorter-lived signals, it has been proposed that DA might serve as a gating signal that controls inputs to PFC (Servan-Schreiber et al., 1990; D’Ardenne et al., 2012). By assigning salience to prefrontal sensory inputs, phasic DA could strongly influence subsequent cognitive processing in PFC. Visual signals, for example, are passed through lower-level cortical areas in a feedforward manner and reach the PFC within 100–150 ms (Thorpe and Fabre-Thorpe, 2001). The PFC collects this sensory information to form subjective judgments, such as regarding the presence or absence of sensory stimulation (de Lafuente and Romo, 2006). Recent electrophysiological studies have demonstrated that the physical intensity of tactile and visual...
stimuli is represented in single neurons of the primate PFC along-
side their perceived intensity, i.e., the animal’s subjective experi-
ence of a stimulus (de Lafuente and Romo, 2005; Merten and

Here, we investigate in trained rhesus monkeys how DA con-
trols the prefrontal representation of such brief sensory stimuli
that must be detected by the animals (Merten and Nider, 2012,
2013). We found that DA strengthens visual signals by modulat-
ing activity in two distinct classes of neurons. Our results suggest
that prefrontal DA may play an important role in determining
how the PFC orchestrates behavioral responses triggered by sen-
sory events.

Materials and Methods

Surgical procedures

Two male rhesus monkeys (Macaca mulatta) were implanted with a tita-
nium head post and one recording chamber centered over the principal
sulcus of the lateral PFC, anterior to the frontal eye fields (right hemi-
sphere in monkey H, right and left hemispheres consecutively in monkey
M). Surgery was conducted using aseptic techniques under general anes-
thesia. Structural magnetic resonance imaging was performed before
implantation to locate anatomical landmarks. All experimental proce-
dures were in accordance with the guidelines for animal experimentation
approved by the local authority, the Regierungspräsidium Tübingen.

Behavioral protocol

Task. The monkeys were trained to report the presence or absence of
visual objects flashed at varying contrast levels centered on their percep-
tual threshold. The animals initiated each experimental trial by grasping
a lever and fixating a central fixation target (fixation period). After 500
ms, a stimulus was displayed for 100 ms in half of the trials (stimulus
period). In the other half, no stimulus was shown. Both trial types were
randomly intermixed. After the delay period (2700 ms), a colored rule
cue instructed the monkey how to respond. If a stimulus was presented,
a red square cue required the monkey to release the lever within 1000 ms
to receive a fluid reward, whereas a blue cue indicated to the monkey
to keep holding the lever for 1200 ms. The rule applied in the inverse way if
no stimulus was presented.

CORTEX software (National Institute of Mental Health, Bethesda,
MD) was used for experimental control and behavioral data acquisition.
The animals maintained fixation throughout the fixation, stimulus, and
delay periods within 1.75° of visual angle of the central fixation target
(ISCAN).

Visual stimuli

The stimulus consisted of a gray object (4° of visual angle in diameter) presented at seven levels of contrast close to perceptual
threshold, determined individually for each animal (monkey H: 7.3, 8.7,
10.6, 11.6, 19.9, 24.9, and 28.0%; monkey M: 9.1, 9.8, 11.8, 12.5, 14.7,
16.7, and 17.4%), measured with an LS-100 luminance meter (Konica
Minolta). The shape of the object was chosen randomly from a set of two objects: hexagon and circle for monkey H; cross and rhomboid for mon-
key M. The area of the object was kept constant to maintain the same
visual contrast across different shapes.

Visual contrasts were determined for each animal individually to yield
approximately the same data points on the psychometric curve. To pool
data for analysis, visual contrasts were normalized to an ordinal scale of
1–7 (1 corresponding to the lowest and 7 to the highest stimulus contrast
presented to each animal, regardless of the actual physical intensity). Salient stimuli analyzed in Figures 3 and 5 denote the three highest con-
trasts (5–7).

Electrophysiology

In each recording session, up to three electrodes (see below, Iontopho-
thesis) were inserted transdurally using a modified electrical microdrive
(NAN Instruments). Neurons were recorded at random; no attempt was
made to preselect neurons according to particular response properties.
Signal acquisition, amplification, filtering, and digitalization were ac-
complished with the MAP system (Plexon). Waveform separation was
performed offline (Offline Sorter; Plexon).

Iontophoresis

DA was applied iontophoretically (MVCs iontophoresis system; npi
electronic) using custom-made tungsten-in-glass electrodes flanked by
two pipettes each (Thiele et al., 2006). Electrode impedances were 1–3
MΩ (measured at 500 Hz; Omega Tip Z; World Precision Instruments).
Pipeette resistances depended on the pipette opening diameter, drug, and
solvent used. Typical resistances were 15–50 MΩ (full range, 15–150 MΩ).
Pilot in vitro experiments (DA iontophoresis into NaCl, concentrations
quantified by HPLC) determined the smallest holding current that en-
sured good retention without accumulation of dead space and thus al-
lowed for rapid delivery of DA after switching to ejection currents.
Retention currents were —7 to —10 nA. Ejection currents for DA (200 mx
in double-distilled water, pH 4.0 with HCl; Sigma-Aldrich) were +25–100
nA (median, +50 nA). Control experiments with 0.9% NaCl, pH 7, used
+50 nA. Ejection currents were chosen to match the values reported to
be maximally effective, i.e., in the peak range of the inverted-U function
(Sawaguchi, 2001; Vijayaraghavan et al., 2007). DA currents were varied
only during experiments to determine whether the ratio of inhibition/ excitation depended on the applied concentration. Otherwise, we did not attempt to investigate dosage effects.

One pipette per electrode was filled with DA solution, and the other
contained 0.9% NaCl. Electrode impedance and pipette resistance were
measured after each recording session. DA was applied continuously for
12–15 min, depending on the number of trials completed correctly by the
animal. The first block was always the control condition. Given the fast
DA application verified by HPLC (see above), we did not automatically
exclude data at the current switching points.

Data analysis

Data analysis was performed with MATLAB (Mathworks). None of the
reported analyses depended on the exact choice of trials to include or
time windows to analyze. Repeating analyses with a different choice of
parameters yielded comparable results.

Excitability modulation. Neurons stimulated with DA were excluded
from additional analysis if their baseline (fixation period) discharge rates
were <1 Hz in the control or DA phase. Baseline firing rates of each
neuron were pooled for the control condition and the DA condition
and compared with a rank-sum test (Mann–Whitney U test). If the median
firing rate in the DA condition was significantly (p < 0.05, two-sided
test) larger than in the control condition, the neuron was classified as
excited, and if the median was lower, the neuron was classified as inhib-
ited by DA.

Receiver operating characteristic analyses. Neuronal coding strength
was quantified using receiver operating characteristic (ROC) analysis
(Green and Swets, 1966). The area under the ROC curve (auROC) is a
nonparametric measure of the discriminability of two distributions. It
denotes the probability with which an ideal observer can tell apart a
meaningful signal from a noisy background. ROC is a separation, and values of 1 signal perfect discriminability. The auROC
takes into account both the difference between distribution means as well
as their widths and is therefore a more suitable indicator of signal quality
than other, simpler measures of signal/noise ratio (Servan-Schreiber
et al., 1990; Parker and Newsome, 1998; Herrero et al., 2008).

Stimulus-responsive neurons. A two-way ANOVA was calculated with
main factors stimulus contrast (salient/absent) and iontophoresis condi-
tion (control/DA) using firing rates after stimulus presentation (300 ms
time window aligned to the individual response latency of the neuron; see
below), including correct trials only. Neurons with a significant stimulus
main effect (p < 0.05) were classified as stimulus responsive. Salient
stimulation was defined as the three highest visual contrasts.

Except for the analysis in Figure 7B (see below), visual response laten-
cies were calculated using sliding ROC analysis with a window size of
50 ms, step of 1 ms. For each window, we calculated the auROC by
comparing the firing rates between correct salient stimulus trials (hits)
and correct absent stimuli trials (correct rejections). To test whether the
auROC was significantly different from 0.5, bootstrapping was used to
construct 999 resamples by randomly sampling the data with replace-
ment and maintaining the original number of trials per condition. The
latency of a neuron was defined as the time after stimulus onset but no
later than 500 ms, when the auROC exceeded the 95% confidence interval of the bootstrapped data for 50 consecutive windows. The response latency was determined separately for the control and DA conditions. If no value could be determined, a default latency corresponding to the median response latency of all neurons in the respective condition was used (228 and 217 ms for the control and DA conditions, respectively). The choice of these parameters ensured that the analysis window (see below, Neuronal signal metrics) covered the stimulus response in all neurons.

To directly compare visual response latencies between the population of inhibited and excited stimulus encoding neurons (see Fig. 7b), response latency was defined as two consecutive significant auROC values using a window size of 50 ms, step of 10 ms. This choice of parameters was more sensitive to the actual onset of the stimulus response so that latencies were reliably determined in all stimulus neurons (i.e., no default latencies were used).

For single-cell spike density histograms, the average firing rate in salient trials and trials without visual stimulation (correct trials only) was divided by the SD of the baseline firing rates in the control condition and dividing by the SD of the baseline firing rates in the control condition.

Stimulus responses calculated using sliding ROC analysis (window size of 300 ms, step of 50 ms) quantified the discriminability between the firing rate distributions of correct salient trials and correct rejection trials.

**Neuronal signal metrics.** All analyses were performed using data from a 300 ms window aligned to individual visual response latencies. This ensured that stimulus responses were adequately captured in all neurons. To distinguish between additive and multiplicative operations, the difference between the mean firing rate in hit trials and correct rejections was divided by the mean baseline firing rate for all (normalized) contrasts and both iontophoresis conditions (Vijayraghavan et al., 2007). Neurometric curves were determined by calculating the auROC between discharge rates in hit trials and correct rejections for all (normalized) visual contrasts. Neuronal variability was quantified by the Fano factor (FF), i.e., the ratio of trial-by-trial spike count variance and mean spike count (Churchland et al., 2010).

To determine whether DA modulated a signal metric, multiple linear regression analysis was applied to the population data (Merten and Nieder, 2012). Linear functions were fitted to the factors normalized visual contrast and iontophoresis condition (control and DA) using the model for the signal metric (S): \( S = a_0 + a_{stim} \times STIM + a_{ion} \times ION \)

where \( a_{stim} \) and \( a_{ion} \) are the coefficients that quantify the signal metric dependence on the normalized stimulus contrast (STIM) and the iontophoresis condition (ION). To assess DA effects on the analyzed signal metric, \( p \) values for the factor iontophoresis condition were used (t statistics for the coefficient \( a_{ion} \)).

DA modulation of neuronal variability was also quantified by multiple linear regression analysis. Linear functions were fitted to the relationship between mean spike count of each contrast and neuron (COUNT) and variance of the spike count of each contrast and neuron (VAR) separately for each iontophoresis condition (ION), i.e., control and DA. An interaction term was included to analyze changes in the slope of the linear functions induced by DA (VAR \( \times ION \)). The model term was \( COUNT = a_0 + a_{VAR} \times VAR + a_{ION} \times ION + a_{INT} \times VAR \times ION \), \( p \) values for the interaction term \( a_{INT} \) were used to assess DA effects on neuronal variability.

**Kinetics of excitability.** Exponential functions were fitted to the baseline firing rates of all trials recorded within 6 min of switching to the ejection current (temporal resolution of one trial, i.e., one data point per 5 s). Neurons with bad fits (e.g., fitted parameters out of bounds; \( n = 1 \) inhibited cell, \( n = 4 \) excited cells) were excluded from additional analysis. If several DA phases were recorded, baseline firing rates were aligned to all instances of switching to the ejection current and averaged using bins of 5 s. The amplitude of DA modulation was estimated by the mean baseline firing rate in the first or second half of the DA condition for inhibited and excited neurons, respectively. The time course of the baseline firing rate (FR) was expressed as \( FR = A \times (1 - \exp(-x/\tau)) \), where \( A \) is the estimated amplitude and \( \tau \) the parameter fitted using nonlinear least squares. The population time course was calculated by averaging the normalized baseline discharge rates from all trials recorded within 6 min before and after switching to DA application using bins of 5 s and smoothed with a Gaussian kernel (width of 10 s, step of 5 s).

**Extracellular action potential waveforms.** Recoded single units were categorized into narrow-spiking (NS) and broad-spiking (BS) neurons, i.e., putative interneurons and pyramidal cells, using a linear classifier (k-means, \( k = 2 \), squared Euclidean distance) (Dieter and Nieder, 2008). For each single unit, the template waveform was extracted with the Plexon Offline sorter. Only neurons with a downward voltage deflection followed by an upward peak were included. Units with a minimum outside 200–400 μs or a maximum before 300 μs after reaching the initial threshold were excluded (\( n = 3 \) of 60 units). Waveforms were normalized by their difference between maximum and minimum voltage deflection and aligned to their minimum. Units in the cluster with the smaller mean spike width constituted the population of NS neurons, and units in the cluster with the larger mean spike width constituted the BS neurons. Interdependence between modulation type (excited or inhibited by DA) and waveform type was tested with Fisher’s exact test.

**Results**

To determine how DA regulates sensory signals in PFC, we presented brief flashes of visual stimuli at varying contrasts to two rhesus macaque monkeys (Macaca mulatta). The animals were trained to detect the stimuli and report their subjective perceptual judgment about the presence or absence of visual stimulation (Merten and Nieder, 2012) (Fig. 1a). The rule-based task design ensured that neuronal activity in the delay period after the stimulus was free of preparatory motor signals. While the monkeys performed this task, we recorded single units from the lateral PFC. During recordings, trial blocks without pharmacological manipulation (control) alternated with blocks in which DA was applied to the vicinity of the recorded cells by micro-iontophoresis (Fig. 1b). As expected, we did not observe changes in the monkeys’ behavior as a consequence of micro-iontophoretic drug application (Fig. 1c,d), because transmitter application with this method is very focal (Herz et al., 1969).

**Two classes of DA-sensitive prefrontal neurons**

We recorded 110 neurons that entered the analysis (60 neurons from monkey M, 50 neurons from monkey H). Application of DA influenced the excitability of prefrontal neurons. We compared fixation period activity in the control condition with the DA condition (rank-sum test, \( p < 0.05 \); Fig. 2a). DA suppressed discharge rates in 32 neurons (DA-inhibited neurons; single-neuron example in Fig. 2b). Activity increased in 28 neurons (DA-excited neurons; single-neuron example in Fig. 2c). Discharge rates were unaffected in 50 neurons (DA-unmodulated neurons; data not shown). The changes in excitability were independent of the iontophoretically applied DA dosage. The proportion of DA-inhibited to DA-excited neurons was not altered when the cell counts were determined separately for lower (+25–50 nA) and higher (+75–100 nA) ejection currents (23/22 versus 9/6, respectively; Fisher’s exact test, \( p = 0.8 \)). None of the physiological parameters analyzed in the following changed in DA-unmodulated cells. This indicates that the effects reported for DA-excited and DA-inhibited neurons were not the result of nonspecific electrical currents.

Inhibitory and excitatory DA effects showed different time courses in the two groups of neurons. In a representative inhibited neuron, DA-mediated suppression of spiking activity was fast and reversed equally rapidly (Fig. 2b). In a typical excited neuron, DA caused much slower, undulating changes in firing
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very consistent across all DA-excited single cells (eight neurons increased, one neuron unchanged, one neuron decreased; mean \( \Delta \text{auROC} \) pooled across contrasts, \( +0.0748 \pm 0.0258 \); signed-rank test, \( p < 0.05 \)). Mean population auROC values, separated into individual contrasts, increased as a function of stimulus contrast and were significantly higher with DA compared with the control condition (multiple linear regression; factor contrast, \( p < 0.001 \); factor iontophoresis condition; \( p < 0.001 \); Fig. 5f). No changes were induced by DA in DA-unmodulated neurons (multiple linear regression; factor contrast, \( p < 0.001 \); factor iontophoresis condition; \( p = 0.14 \); data not shown). These results demonstrate that prefrontal DA does not uniformly modify visual coding strength but selectively enhances the capacity to discriminate stimuli from background in the class of DA-excited neurons.

**Prefrontal DA reduces neuronal variability in excited neurons**

To investigate which mechanisms could give rise to the strengthening of cortical processing by DA, we determined whether a reduction in neuronal noise (discharge rate variability; Shadlen and Newsome, 1998) might be a contributing factor as hypothesized frequently (Winterer and Weinberger, 2004; Durstewitz and Seamans, 2008; Rolls et al., 2008). To do so, we analyzed the correlation between mean spike counts after stimulus presentation (correct trials) and spike count variance across trials for all neurons in a given class. For quasi-Poisson spiking processes, the data should cluster along the first diagonal (McAdams and Maunsell, 1999). This was the case for DA-inhibited neurons under both control and DA conditions (multiple linear regression, interaction term, \( p = 0.64 \); Fig. 6a). On a single-cell level, no systematic DA effects on response variability were observed as measured by FF (spike count variance divided by mean; seven neurons increased, seven neurons decreased; mean \( \Delta \text{FF} \) pooled across contrasts, \( −0.1498 \pm 0.1709 \); signed-rank test, \( p = 0.63 \)). Mean population FFs for each contrast were unchanged in DA-inhibited neurons after application of DA (multiple linear regression; factor iontophoresis condition, \( p = 0.31 \); Fig. 6b).

In contrast to the findings for DA-inhibited neurons, trial-to-trial variability decreased significantly in DA-excited neurons under the influence of DA (multiple linear regression, interaction term, \( p < 0.01 \); Fig. 6c). The FF reduction was consistent across single cells (eight neurons decreased, two neurons increased; mean \( \Delta \text{FF} \) pooled across contrasts, \( −0.391 \pm 0.1931 \); signed-rank test, \( p < 0.05 \)). Mean population FFs, separated into individual contrasts, were significantly reduced by DA compared with the control condition (multiple linear regression, factor iontophoresis condition; \( p < 0.05 \); Fig. 6d). No changes were induced in DA-unmodulated neurons (multiple linear regression; factor iontophoresis condition; \( p = 1.0 \); data not shown). Thus, DA rendered prefrontal processing more reliable by reducing noise at the level of DA-excited neurons.

**Inhibition and excitation control distinct prefrontal processing stages**

To further characterize the two DA-responsive neuron classes, we analyzed the extracellular action potential waveforms of the cells. Electrophysiological recordings have suggested that longer waveforms might be primarily associated with pyramidal cells (BS neurons), whereas shorter waveforms could be more typical of interneurons (NS neurons) (Henze et al., 2000; Diester and Nieder, 2008; Hussar and Pasternak, 2009; Vigneswaran et al., 2011). We calculated the average normalized waveform for each single
Figure 3. DA modulation of prefrontal visual signals is neuron-class specific. a, b, Responses of an example DA-inhibited neuron to salient (highest 3 contrasts) and absent visual stimuli in the control (a) and DA (b) conditions. Activity is aligned to the start of a trial (fixation period). The gray shaded area marks the stimulus presentation. Top, Dot raster plot; bottom, spike density histogram. Visual coding is preserved at shifted response levels. c, Sequence of control and DA periods in the same example DA-inhibited neuron. DA-mediated effects are reversible. d–f, Conventions as in a–c for an example DA-excited neuron. Visual responses are enhanced by DA. g, h, Population mean responses of DA-inhibited neurons in control (g) and DA (h) trials. i, j, Conventions as in g and h, for DA-excited neurons. Shaded areas in g–j indicate SEM across neurons.

Figure 4. Subtraction and multiplication of activity in DA-inhibited and DA-excited neurons. a, Stimulus-evoked change in firing rate normalized to baseline activity in the fixation period, computed as shown by schematic on the left, for DA-inhibited neurons under control and DA conditions. Shifts to larger values indicate that DA offsets activity (additive operation), i.e., the firing rate difference is retained at lower baseline firing rates (subtraction). The animals’ perceptual threshold (on the rising slope of the psychometric function; Fig. 1c,d) corresponds to normalized stimulus contrasts 1–4. b, Conventions as in a for DA-excited neurons. Superimposed curves indicate that DA increases gain (multiplicative operation), i.e., the firing rate difference increases in proportion to baseline firing rates. Error bars indicate SEM across neurons.
neuron and used a linear classifier to objectively separate BS from NS cells. BS and NS waveforms were distributed differently in the classes of DA-excited and DA-inhibited neurons (Fisher’s exact test, \( p < 0.05 \); Fig. 7a). All stimulus-encoding DA-excited cells were BS neurons (putative pyramidal neurons, \( n = 10 \)). In contrast, there were equal numbers of BS and NS cells (putative interneurons) in the class of stimulus-responsive DA-inhibited neurons (\( n = 7 \) each). Thus, in the group of DA-inhibited neurons, there were more putative interneurons than to be expected by their frequency in neocortex (20–30%; Markram et al., 2004), and there were significantly more putative pyramidal cells in the class of DA-excited neurons. Interestingly, all stimulus encoding putative interneurons that were responsive to DA were inhibited (\( n = 7 \)). The same pattern was found when all DA-responsive neurons were analyzed (DA-excited neurons: 22 BS, 3 NS; DA-inhibited neurons: 17 BS, 15 NS; \( p < 0.01 \)). In accord with the strongly biased distribution of putative interneurons toward DA-inhibited cells, baseline firing rates under control conditions were higher in this group of neurons compared with DA-excited cells, although the difference did not reach significance (8.3 ± 1.4 vs 5.4 ± 0.8 spikes/s for DA-inhibited and DA-excited neurons, respectively; rank-sum test, \( p = 0.13 \)). In the instances in which multiple DA-modulated neurons were recorded at the same electrode (12 of 45 electrodes), we more often recorded cells from the same class than from different classes (eight vs four electrodes, respectively). These results support the notion that DA-mediated changes in excitability were characteristic of distinct neuronal populations.

We finally explored whether DA-inhibited and DA-excited neurons might be involved at different stages of prefrontal sensory processing. Under control conditions, prefrontal neurons that were inhibited by DA encoded visual signals significantly earlier than DA-excited neurons (mean stimulus response latency, 165 ± 18 and 261 ± 27 ms for DA-inhibited and DA-excited neurons, respectively; rank-sum test, \( p < 0.05 \); Fig. 7b; see also Figs. 3g,d, 5b,e). DA-inhibited neurons were driven more strongly by sensory input: under control conditions, visual coding strength was higher in this population compared with DA-excited cells across all contrasts (multiple linear regression; factor neuron class, \( p < 0.001 \); Fig. 7c).

Closuer inspection of the population spike density histograms of DA-excited neurons revealed that activity after omission of a stimulus was not a simple continuation of activity in the fixation period when DA had been applied (Fig. 3, compare i, f). To examine whether DA-excited neurons represented not just physical stimulus intensity but possibly a processing stage more remote from sensory input, we compared baseline activity in the fixation period with firing rates in trials without stimulation, calculated in the same 300 ms analysis window as previously (Fig. 7d). A deviation from zero could suggest that absent stimulation was not encoded as a “default” condition (i.e., a continuation of baseline activity; to be expected for sensory-driven neurons) but instead actively in a potentially more advanced processing step. In DA-inhibited neurons, there were no significant differences between baseline activity and activity after the omission of a stimulus in either control or DA conditions (signed-rank test, \( p = 0.39 \) and \( p = 0.54 \), respectively; signed-rank test for difference between control and DA conditions, \( p = 0.95 \); Fig. 7d, left). However, in DA-excited neurons, DA application disclosed a deflection from zero could suggest that absent stimulation was not encoded as a “default” condition (i.e., a continuation of baseline activity; to be expected for sensory-driven neurons) but instead actively in a potentially more advanced processing step. In DA-inhibited neurons, there were no significant differences between baseline activity and activity after the omission of a stimulus in either control or DA conditions (signed-rank test, \( p = 0.39 \) and \( p = 0.54 \), respectively; signed-rank test for difference between control and DA conditions, \( p = 0.95 \); Fig. 7d, left). However, in DA-excited neurons, DA application disclosed a deflection from baseline in trials without visual stimulation that was not evident under control conditions (signed-rank test, \( p = 0.19 \) and \( p < 0.01 \), for control and DA conditions, respectively; signed-rank test for difference between control and DA conditions, \( p < 0.01 \); Fig. 7d, right). This result suggests that the absence of visual stimulation was represented differently in the two DA-responsive neuron classes.

Discussion

We report here that DA regulates the representation of sensory information in the primate PFC. We found that prefrontal DA affects two distinct neuronal populations involved in visual coding. DA controlled neurons with short visual response latencies
by suppressing neuronal activity. In neurons with longer response latencies, DA acted as an excitatory modulator and strengthened the representation of visual inputs.

**Modes of operation**
Inhibition was implemented principally in the form of a subtractive shift in response levels [additive operation (Silver, 2010; Figs. 3g,h, 4a], whereas excitation in the second population resulted from an increase in gain [multiplicative operation (Silver, 2010); Figs. 3i,j, 4b]. In the rodent visual cortex, subtraction is induced by dendrite-targeting interneurons, whereas somatotargeting interneurons regulate gain (Wilson et al., 2012). *In vitro* experiments in the ferret PFC have demonstrated that these classes of interneurons are modulated by DA (Gao et al., 2003). Thus, DA would subtract activity by modulating dendrites and increase gain by controlling the soma (Yang and Seamans, 1996). Our results now suggest that subtraction and multiplication by DA target not the same prefrontal neuron but instead early and late, possibly functionally specialized, processing stages, respectively (Fig. 7).

**DA-inhibited neurons**
Control over sensory inputs by inhibition and subtraction of response levels offers a major computational advantage, namely response normalization (Carandini and Heeger, 2012). Inhibitory conductances can adaptively rescale the input of a neuron to match its dynamic range (Mitchell and Silver, 2003) and therefore maximize information transmission (Brenner et al., 2000; Fairhall et al., 2001). Our data indicate that DA afferents to the PFC might constitute an important pathway to fine-tune and facilitate downstream processing.

DA could also filter distracting, nonpreferred signals by modulating neurotransmission at the dendritic arbor of input layer neurons (“gating”) (Durstewitz et al., 2000; Gao et al., 2003). Neurons extracting behaviorally relevant information from a multitude of competing signals would necessarily show temporally precise modulation. Given their rapid responsiveness to DA (Fig. 2g), DA-inhibited neurons would be ideal recipients of the phasic signals, e.g., prediction errors, DA neurons relay to the PFC (Redgrave et al., 2008). With a mean stimulus response latency of 165 ms (Fig. 7b), these cells closely follow the discharge of midbrain DA neurons that typically occurs between 100 and 150 ms (Donnem et al., 2005; de Lafuente and Romo, 2012). Therefore, DA-inhibited neurons are maximally active at peak extracellular DA concentrations (Schultz, 2007). Thus, DA might reinforce or block signals reaching the PFC and segregate important from distracting information (Servan-Schreiber et al., 1990; D’Ardenne et al., 2012). Interestingly, we found that all putative interneurons were inhibited by DA (Fig. 7a). Interneurons are thought to play an important role in the control of information flow in cortex (Constantinidis et al., 2002) and would constitute an ideal target for rapid gating by DA.

At present, the cellular mechanisms by which DA could mediate fast inhibition are unclear (Seamans and Yang, 2004). Inhibitory DA effects are generally reported on longer timescales as a result of technical constraints, such as bath application of dopaminergic drugs. It is also conceivable that the applied DA binds to non-dopaminergic receptors, such as adrenergic receptors, especially at higher concentrations. Iontophoresis is nonquantitative and generally does not provide reliable assessments of the drug concentrations reaching individual neurons. Therefore, additional experiments are required to resolve the issue of pharmacological specificity as well as to determine whether the observed decrease in excitability is indeed the result of phasic, time-locked signaling or generated by longer-lasting mechanisms.

In behaving nonhuman primates, neuronal inhibition has been identified as an important mechanism by which DA affects prefrontal signal processing. DA suppresses neuronal activity in spatially tuned prefrontal neurons engaged in memory-guided saccade tasks and enhances tuning for the remembered saccade target location (“sculpting inhibition”; Williams and Goldman-Rakic, 1995; Vijayraghavan et al., 2007; Arnsten, 2011). Because subtraction sharpens stimulus selectivity, i.e., tuning (Wilson et al., 2012), we propose that the spatially tuned cells described previously belong to the class of DA-inhibited neurons identified here. Although the ROC measures we used are well suited for analyzing binary yes–no, e.g., stimulus present–absent decisions (Green and Swets, 1966), we did not detect an increase in signal/noise ratio as defined by the auROC in DA-inhibited neurons.

Figure 6. Prefrontal DA reduces response variability in excited neurons. *a*, Mean spike count after stimulus presentation versus spike count variance across trials for DA-inhibited neurons. Each data point represents one neuron and stimulus contrast. Straight lines indicate fits to data. *b*, FFs (spike count variance divided by mean) for all stimulus contrasts in DA-inhibited cells. No changes in response variability are observed after DA application. *c, d*, Conventions as in *a* and *b* for DA-excited neurons. The slope of the fitted line is significantly smaller in DA trials compared with control conditions. DA reduces response variability across all contrasts in DA-excited cells. Error bars indicate SEM across neurons.
DA modulates distinct prefrontal processing stages. a. Normalized average waveforms of stimulus-encoding DA-inhibited and DA-excited neurons. All DA-excited cells were BS neurons, and NS neurons were all inhibited by DA. b. Visual response latencies of DA-inhibited and DA-excited neurons under control conditions. DA-inhibited neurons encode visual signals significantly earlier. c. Visual coding strength of DA-inhibited and DA-excited neurons under control conditions (auROC values comparing firing rates between trials with and without visual stimulation). DA-inhibited neurons are driven more strongly by visual stimulation across all contrast levels. d. Normalized difference between baseline activity in the fixation period and activity after omission of a stimulus. Firing rates were identical in DA-inhibited neurons in both control and DA trials. In DA-excited neurons, absence of visual stimulation induced a deflection from baseline when DA had been applied. Error bars indicate SEM across neurons.

DA-excited neurons

In DA-excited neurons, stimulus responses increased in proportion to baseline activity, indicating a multiplicative increase in gain (Servan-Schreiber et al., 1990; Thurley et al., 2008; Figs. 3i,j, 4b). Although the strength of sensory inputs was unchanged in DA-inhibited neurons, DA selectively increased signal/noise ratio in excited cells (Fig. 5a–c). Other response characteristics that are not adequately captured by signal detection theory, e.g., sharpening of tuning curves, might nevertheless create advantages for cortical processing. We also considered the possibility that inhibited neurons were the result of higher intrinsic DA tone and excited neurons were subject to lower DA levels. However, this is unlikely because the ratio of inhibition to excitation was independent of iontophoretic DA dosage, and we did not observe more inhibited neurons at higher DA currents. More experiments tapping different behavioral demands are needed to determine whether the benefits conveyed by DA-induced inhibition lie primarily in rescaling and gating inputs to PFC or whether DA can also affect signal strength per se at this stage.

Implications for mental diseases

DA is strongly linked to neuropsychiatric diseases that involve the frontal lobes, such as attention-deficit hyperactivity disorder or...
schizophrenia (Arnsten, 2011). By strengthening sensory inputs, prefrontal DA could be a critical factor in resolving ambiguous sensory events or maintaining the focus of attention. It is tempting to speculate that the observed DA effects could help safeguard the healthy mind, e.g., from hallucinations and intrusions of thought that are characteristic of these mental diseases (Winterer and Weinberger, 2004; Rolls et al., 2008; Fletcher and Frith, 2009). For example, it is frequently hypothesized that the symptom relief conveyed by antipsychotic drugs targeting the DA system, in particular the D₂R results from the fact that they decrease noise in prefrontal circuits (Winterer and Weinberger, 2004; Rolls et al., 2008). Our experiments now provide evidence on a cellular level that DA indeed controls neuronal variability in the primate brain.

In conclusion, we have demonstrated that DA neuromodulation in PFC is not uniform but tailored to functionally specialized neurons in the prefrontal processing stream (Arnsten et al., 2012). By controlling sensory inputs to the PFC, DA could be a powerful determinant of how the primate brain uses these signals to generate intelligent behavior in interactions with its sensory environment.

References


