Selective modulation of excitatory and inhibitory microcircuits by dopamine

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Dopamine plays an important role in the working memory functions of the prefrontal cortex, functions that are impacted in age-related memory decline, drug abuse, and a wide variety of disorders, including schizophrenia and Parkinson's disease. We have previously reported that dopamine depresses excitatory transmission between pyramidal neurons in the prefrontal cortex. Here, using paired recordings, we have investigated dopaminergic modulation of excitatory transmission from pyramidal neurons to fast-spiking (FS) interneurons. In contrast to its effect on recurrent excitation, dopamine was without effect on excitatory transmission to FS interneurons. However, dopamine has directly enhanced the excitability of the FS interneurons to the extent that even a single excitatory postsynaptic potential could initiate spiking with great temporal precision in some of them. These results indicate that dopamine's effects on excitatory transmission are target-specific and that the axon terminals of pyramidal neurons can be selectively regulated at the level of individual synapses. Thus, dopamine's net inhibitory effect on cortical function is remarkably constrained by the nature of the microcircuit elements on which it acts.

pyramidal neuron | interneuron | excitatory transmission | *in vitro*

The functional architecture of the cerebral cortex is comprised of a large number of circuit elements. Among these, two basic types of local microcircuits, pyramidal cell connections with other pyramidal cells (P-P) and with nonpyramidal (P-NP) cells, respectively, are of particular interest because they constitute the elemental basis of all cortical operations (1). The monosynaptic connections between two or more pyramidal neurons are thought to provide the circuit basis for recurrent excitation in the cortex and the key mechanism underlying the persistent activity of prefrontal cortical neurons in working memory tasks (2). Connections between pyramidal and nonpyramidal neurons, on the other hand, play important roles in establishing neuronal specificity (3) and temporal integration (4) among other functionally important actions in the circuitry of working memory.

Recently, we have shown that P-P circuits are depressed by dopamine through a presynaptic mechanism (5). Dopaminergic depression of P-P excitation is also supported by *in vivo* recordings, as D1 agonists depress and D1 antagonists potentiate delay activity in prefrontal cortical neurons during performance of working memory tasks (6). On the other hand, dopamine's effects on P-NP connections have not been examined although its direct effects are known to enhance rather than depress interneuron excitability (7, 8, †). Here, we have examined dopaminergic modulation of excitatory synaptic transmission between pyramidal neurons and fast-spiking (FS) interneurons ($P\text{-}NP_{FS}$) and have compared its effects with that of recurrent excitation by means of paired recordings in layer V prefrontal circuits. This study provides evidence that dopamine's actions are circuit dependent rather than common to all excitatory synapses and provides further insight into its role in establishing a balance between excitation and inhibition in the output layer of the cerebral cortex (9–12). Preliminary findings have been reported in an abstract at a Society for Neuroscience annual meeting.‡

Materials and Methods

Physiological Recording and Drug Application. Young adult ferrets (3–4 mo) purchased from Marshall Farms (North Rose, NY) were carefully treated under National Institutes of Health animal use guidance and protocol approved by the Institutional Animal Care and Use Committee at Yale. The animals were anesthetized by overdose sodium pentobarbital and decapitated. Brains were quickly removed, and horizontal slices of 300 μm from medial prefrontal cortex were cut with Vibratome (Vibratome, St. Louis) in ice-cold Na^+-free sucrose solution (mM): KCl 2.5/NaH_2PO_4 1.25/$NaHCO_3$ 26/$CaCl_2$ 0.5/$MgSO_4$ 7.0/sucrose 213. The slices were incubated in oxygenated regular artificial cerebrospinal fluid (ACSF) containing (in mM) NaCl 124, KCl 2.5, NaH_2PO_4 1.25, $CaCl_2$ 2, $MgSO_4$ 1, $NaHCO_3$ 26, and dextrose 10, pH 7.4, at 35°C for 40 min, then kept at room temperature until being transferred to the recording chamber. Slices in the recording chamber were perfused with prewarmed ACSF (2 ml/min), and recordings were conducted at 32–34°C. Dual or quadruple whole-cell recordings in current clamp mode were used for analysis of pyramid-to-interneuron and pyramid-to-pyramid synaptic connections. The resistances of patch pipettes were ≈5–10 MΩ and were filled with intracellular solution containing (in mM) potassium gluconate 114, KCl 6, $CaCl_2$ 0.5, EGTA 0.2, ATP-Mg 4, Hepes 10, pH 7.25, and 0.3% biocytin (Molecular Probes). The signals were amplified and filtered at 2 kHz in bridge-balance mode with either IE-210 (Warner Instruments, Hamden, CT) or MultiClamp 700A amplifier (Axon Instruments, Foster City, CA), and acquired on a computer at sampling intervals of 20–100 μs through a DigiData 1200B interface using software PCLAMP 8.1 (Axon Instruments). The membrane potentials were not corrected for liquid junction potential. The access resistance was continuously monitored during recording. Baseline synaptic strength and all subsequent experiments were recorded at trial interval of 4–5 s (0.2–0.25 Hz).

Similar to our previous report (5), dopamine was applied either in puff through glass pipette (tip diameter, ≈1–2 μm; concentration, 0.1–1 mM; pressure, 6.9–13.8 Kp) or bath (10–30 μM) with addition of the 10 μM antioxidant ascorbic acid.

Data Analysis. Excitatory postsynaptic potential (EPSP) amplitudes were measured between the onset and peak of EPSP from the average of 20–40 traces by using CLAMPFIT software (Axon Instruments). To identify synaptic mechanism, we have calculated the failure rate and coefficient of variation (CV). The percentage of synaptic failure to the evoked presynaptic spike was determined individually for each recording. Failure was defined as an event in which the EPSP amplitude was below the limit of 1.5× noises. The mean amplitude and standard deviation ($Mean_{EPSP}$ and SD_{EPSP}) were obtained from 40 successive

Abbreviations: P-P, pyramidal-pyramidal; P-NP, pyramidal-nonpyramidal; FS, fast-spiking; EPSP, excitatory postsynaptic potential; CV, coefficient of variation; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid.

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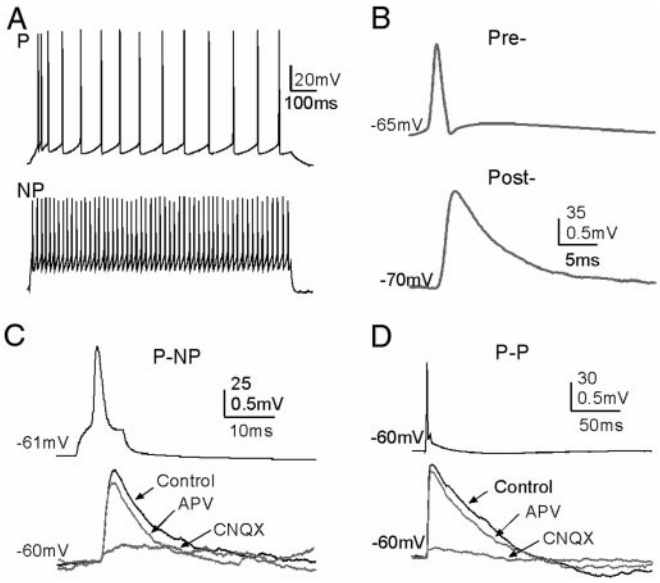

Fig. 1. Pharmacological and physiological properties of excitatory synapses. (*A*) Patterns of action potentials in response to current injection in pyramidal neuron and FS interneuron, respectively. (*B*) Action potentials were induced with current injection into the presynaptic pyramidal neuron, and the resultant characteristic EPSP was recorded in a postsynaptic FS interneuron and averaged from 20 traces (hold ≈−70 mV). (*C* and *D*) At membrane potentials of ≈−60 mV, bath application of 2-amino-5-phosphonovaleric acid (50 μM), an *N*-methyl-D-aspartate (NMDA) receptor antagonist, slightly reduced EPSP amplitude (≈10%), whereas AMPA receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (20 μM) almost completely blocked the voltage, indicating an AMPA-dominated EPSP in both P-NP_{FS} and P-P connections. Traces were averages of 20 individual trials without failures. Please note the different time constant in P-NP_{FS} and P-P EPSPs.

sweeps. The CV of EPSP amplitude for control and dopamine application were therefore computed as $SD_{EPSP}/Mean_{EPSP}$ (13). The data are analyzed by either ANOVA or Student's *t* test and presented as mean ± SE.

Histology. To confirm the morphology of recorded postsynaptic interneurons, slices were immediately submerged in cold 4% paraformaldehyde for fixing 3–5 days after recording and then were directly reacted in 3% hydrogen peroxide for 25 min. After thorough rinsing, avidin-biotinylated enzyme complex reactions were conducted overnight, and then completed in nickel-diaminobenzidine (Ni-DAB) reaction. The slices were resectioned into 150 μm, mounted in phosphate buffer, and covered with water-soluble mounting media. Selected neuronal pairs were reconstructed with NEUROLUCIDA software (Microbrightfield, Williston, VT), and the morphologies of remaining cells were directly examined under microscope.

Results

Comparison of EPSPs Between P-NP_{FS} and P-P Connections in Prefrontal Cortical Neurons. Somatic dual or quadruple whole cell recordings were obtained from 21 P-NP_{FS} pairs and 13 P-P pairs. The FS interneurons were initially identified by their round or oval somata and multipolar oriented principal dendrites under differential interference contrast videomicroscopy and were further confirmed by their characteristic narrow action potentials, deep after-hyperpolarization, and high frequency firing pattern without adaptation (Fig. 1*A*; refs. 14 and 15). The half width of NP_{FS} interneuron action potentials was ≈0.5 ms, consistently less than that of spikes generated by pyramidal cells, which was ≈1.0 ms. These characteristic morphological and electrophysiological properties are easily distinguished from pyramidal cells that possess pia-oriented apical dendrites and usually express regular or intrinsic burst spiking firing patterns. Several other types of interneuronal connections were also found but not in sufficient numbers, and they were therefore not included in this study.

The amplitudes of EPSPs initiated by injecting small current into presynaptic pyramidal cells averaged 1.71 ± 0.16 mV in P-NP_{FS} pairs ($n = 21$; range, 0.8–4.2 mV including failures), compared with 0.86 ± 0.12 mV in P-P connections ($n = 13$; range, 0.40–1.83 mV with failures). The P-P values are in line with our earlier study of P-P connections (5). EPSP kinetics of P-NP, measured at resting membrane potentials of postsynaptic interneurons averaging −69.0 ± 1.31 (Fig. 1*B*), were faster than those in P-P synaptic connections (16, 17). EPSP rise time (20–80%) in FS cells was 1.17 ± 0.08 ms, significantly faster than that of 1.84 ± 0.21 in pyramidal neurons ($P < 0.005$). The decay time constant, which was fitted by a standard exponential function with the CLAMPFIT program, was also significantly faster in interneurons (10.7 ± 0.83 ms) than in pyramidal cells (51.1 ± 7.59 ms, $P < 0.001$, Fig. 1 *B–D*, Table 1). Moreover, synaptic transmission in P-NP_{FS} connections was highly reliable, with a release probability of 93.1% (failure rate 6.9 ± 1.80%), similar to that of P-P pairs recorded in the present study under control conditions (5.4 ± 1.74% failure). Fig. 1 illustrates the physiological properties of both P-P and P-NP_{FS} EPSPs. The EPSPs were identified as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor-dominated glutamatergic synapses in both types of connections as they were only slightly reduced by the *N*-methyl-D-aspartate (NMDA) receptor antagonist 2-amino-5-phosphonovaleric acid (50 μM) but almost completely abolished by an AMPA receptor antagonist, 6-cyano-7-nitroquinoxaline-2,3-dione (20 μM; Fig. 1 *C* and *D*).

Table 1. Physiological properties and dopaminergic modulation of EPSP in P-NP_{FS} and P-P connections

	P-NP_{FS}			P-P		
	Control ($n = 21$)	DA ($n = 21$)	*P* value	Control ($n = 13$)	DA ($n = 13$)	*P* value
EPSP amplitude, mV	1.71 ± 0.16	1.67 ± 0.19 (−2.34%)	0.577	0.86 ± 0.12**	0.57 ± 0.13 (−35.7%)	0.001*
EPSP delay, ms	0.68 ± 0.08	—	—	1.39 ± 0.20**	—	—
EPSP 20–80% rise time, ms	1.17 ± 0.08	—	—	1.84 ± 0.21**	—	—
EPSP decay, ms	10.7 ± 0.83	15.3 ± 0.79	0.121	51.1 ± 7.59**	47.6 ± 6.48	0.619
Failure rate, %	6.9 ± 1.80	9.7 ± 2.20	0.190	5.4 ± 1.74	22.7 ± 5.71	0.002*
CV	0.35 ± 0.02	0.36 ± 0.02	0.684	0.46 ± 0.05	0.86 ± 0.18	0.013*
Membrane potential of postsynaptic neurons, mV	−69.0 ± 1.31	−67.5 ± 1.25	0.043*	−65.0 ± 1.40	−62.7 ± 1.69	0.007*
Threshold of postsynaptic cells, mV	−47.4 ± 2.32	−48.7 ± 2.27	0.295	−40.8 ± 1.26**	−42.3 ± 1.60	0.272
After hyperpolarization (AHP)	12.0 ± 0.97	12.4 ± 1.09	0.127	—	—	—

*, $P < 0.05$ between control and dopamine (DA); **, $P < 0.05$ between controls of P-NP_{FS} and P-P.

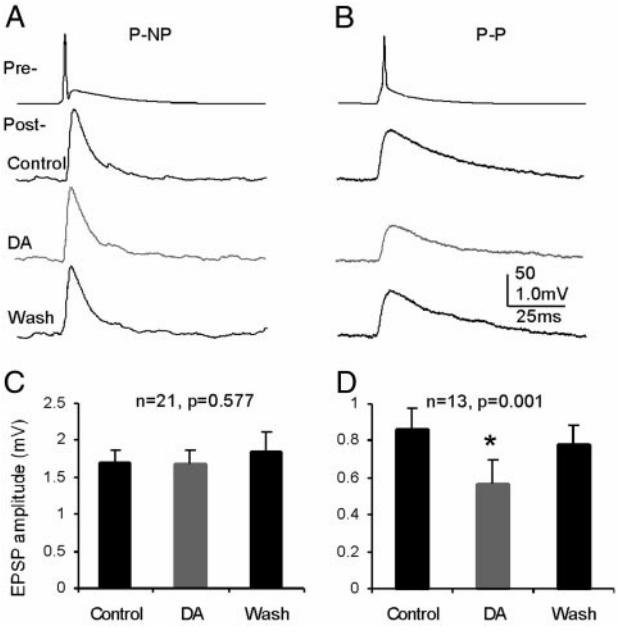

Fig. 2. Dopamine (DA) differentially modulates excitatory synaptic transmission between P-NP_{FS} and P-P connection. (*A* and *B*) Dopamine does not significantly affect P-NP_{FS} synapses in most of the pairs recorded (18/21, 85.7%), but significantly reduces the EPSP amplitude in most of P-P pairs (9/13; 69.2%), possibly through presynaptic D1 receptors as reported. (*C* and *D*) Summary histograms showing dopaminergic effects on P-NP_{FS} ($n = 21$, $P = 0.577$) and P-P connections ($n = 13$, $P < 0.001$), respectively.

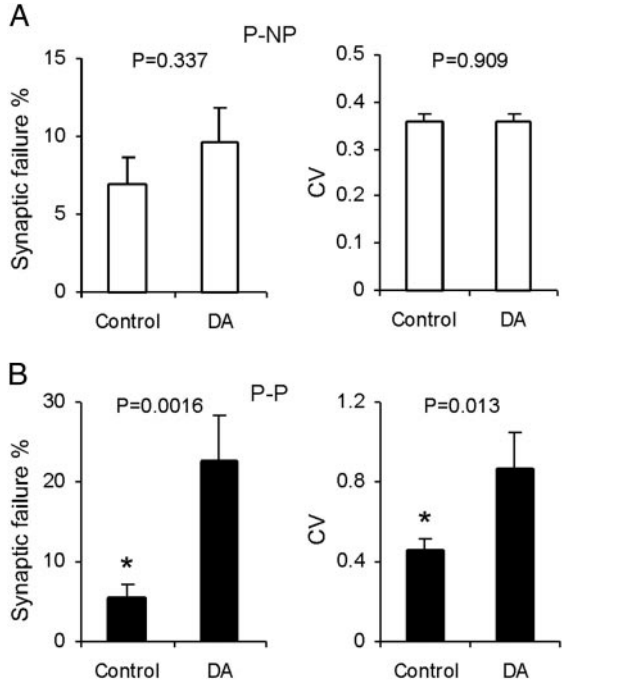

Fig. 3. Mechanism identification of dopamine (DA) modulation of excitatory transmission. (*A*) Dopamine has no significant effects in both synaptic failure and CV in P-NP_{FS} synapses ($P = 0.337$ and 0.909, respectively). (*B*) In contrast to P-NP_{FS} connections, dopamine significantly increases the failure rate ($n = 13$, $P < 0.005$) and CV ($P < 0.05$) in P-P synapses, suggesting a selective presynaptic modulation of excitatory transmission in a manner that was target cell-specific.

Differential Actions of Dopamine on Excitatory Terminals Impinging on Pyramidal Cells and FS Interneurons. As predicted by, and replicating, previous results (5), dopamine reduced EPSP amplitudes in the newly identified P-P pairs by 35.7 ± 6.31% ($n = 13$, $P < 0.001$, Table 1, Fig. 2), whether administered in the bath or by puff. This result was also statistically significant in individual recordings at the $P < 0.05$ level. Only 1 pair of the 13 pairs examined failed to evidence amplitude change (2.7%) whereas the percent amplitude reduction in all other pairs ranged between 17.5% and 71.7%. In sharp contrast to dopamine's consistent effects on P-P pairs, on the other hand, we failed to observe changes in P-NP_{FS} pairs. The mean EPSP amplitude of 21 P-NP_{FS} pairs remained unchanged after dopamine application (control, 1.71 ± 0.16 mV; dopamine, 1.67 ± 0.19; $P = 0.577$; Fig. 2 *A* and *C*). Nor was there evidence of a significant EPSP amplitude change in 85% ($n = 18/21$) of the P-NP_{FS} pairs examined on an individual basis. However, a few exceptions were noted: significant EPSP amplitude reduction of considerable size was observed in 2 of the 21 pairs (29.8% and 42.3%, respectively) whereas one pair exhibited a substantial 41.6% increase, presumably reflecting some heterogeneity in the FS interneuronal target population examined. Because dopamine is known to directly depolarize FS interneurons (7, 8, †), we also used voltage clamp recordings in 2 P-NP_{FS} pairs. Consistent with the current clamp findings, dopamine also failed to induce any changes in EPSCs with membrane potential clamped at −70 mV (data not shown).

In accord with the lack of change in EPSP amplitude, we were also unable to find significant changes in measures of synaptic failure and CV in P-NP_{FS} connections (Fig. 3*A*, Table 1). These negative findings contrasted with clear increases in P-P connections, not only in failure rate (5.4 ± 1.74% in control conditions and 22.7 ± 5.70% after dopamine, $P < 0.005$), but also in CV (0.46 ± 0.05 in control and 0.86 ± 0.18 after dopamine, $P < 0.05$, Fig. 4*B*). Dopamine's modulatory effect was also explored with stimulus trains at 10 Hz. In agreement with the single pulse experiments, dopamine was without significant effect in P-NP_{FS} connections ($n = 12$, Fig. 4 *A* and *C*) but significantly reduced all five EPSP responses in P-P synapses ($n = 5$, Fig. 4 *B* and *D*), further supporting the circuit-specific modulation of dopamine.

Additional evidence of dopamine's differentiated actions was obtained in a quadruple recording experiment in which a single pulse in a presynaptic pyramidal neuron (P1) simultaneously elicited EPSPs in two postsynaptic cells, one, a pyramidal cell (P2), and the other, an FS interneuron (NP_{FS}) (Fig. 5 *A* and *B*). A reconstruction of these biocytin-labeled neurons is shown alongside their respective firing patterns in Fig. 5*B* (the fourth cell in the group was recorded but no synaptic connection was found). After dopamine application, the FS interneuron was slightly depolarized by 1.9 mV, and action potentials were induced in 9 of 20 sweeps (9/20, 45%), with many spikes precisely located on the rising phase of the EPSPs (data not shown, but see below). EPSP amplitudes were almost identical in control and dopamine conditions after eliminating traces with firing spikes (mean control, 2.31 ± 0.13 mV; mean dopamine, 2.32 ± 0.14 mV; $P = 0.972$, ANOVA, Fig. 5*C*). Further, neither synaptic failure (0% in both control and during dopamine) nor CV change (0.41 ± 0.01 before dopamine vs. 0.39 ± 0.01 in dopamine) was observed during dopamine application, in agreement with the dual recording data. In contrast to the responses of P1-NP_{FS} synapses, the EPSP amplitude of the P1-P2 connection was again predictably reduced by 26.7% in the presence of dopamine (0.90 ± 0.10 mV in control conditions and 0.66 ± 0.07 mV after dopamine, $P < 0.05$). Additionally, we also found a 20% increase in failure rate (5% in control and 25% in dopamine) and 32.4% increase in CV (0.37 ± 0.01 in control and 0.49 ± 0.01, $P < 0.001$) in this P-P connection. These results provide strong direct evidence that dopamine decreases glutamate release only at presynaptic terminals contacting pyramidal neurons and has little effect on feed-forward excitation to FS nonpyramidal neurons.

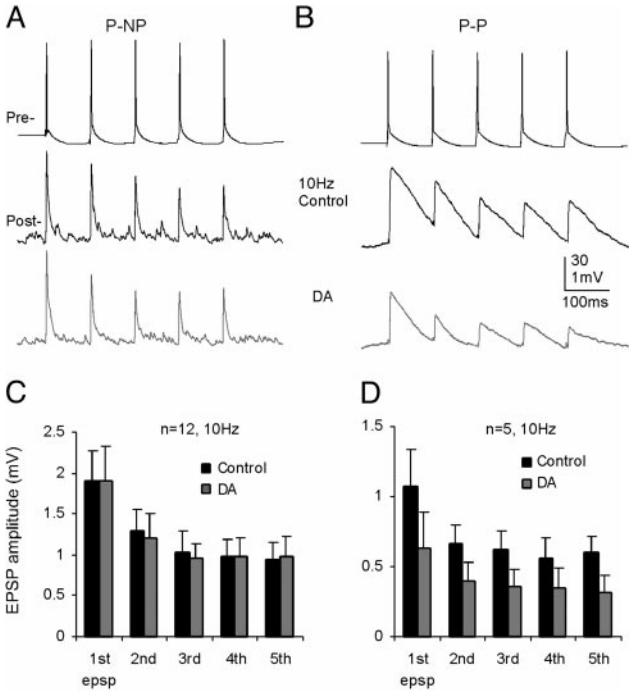

Fig. 4. Excitatory synapses response to train of five pulses in dopamine (DA). (*A* and *B*) Samples of dopaminergic modulations in an individual pair of P-NP_{FS} connections and a pair of P-P at a five-pulse train in 100-ms intervals (10 Hz). (*C* and *D*) Summary histograms indicate that dopamine does not have an effect in the P-NP_{FS} pair (n = 12) but clearly reduces all EPSP amplitudes in P-P synapses (n = 5), similar to the responses to single pulse.

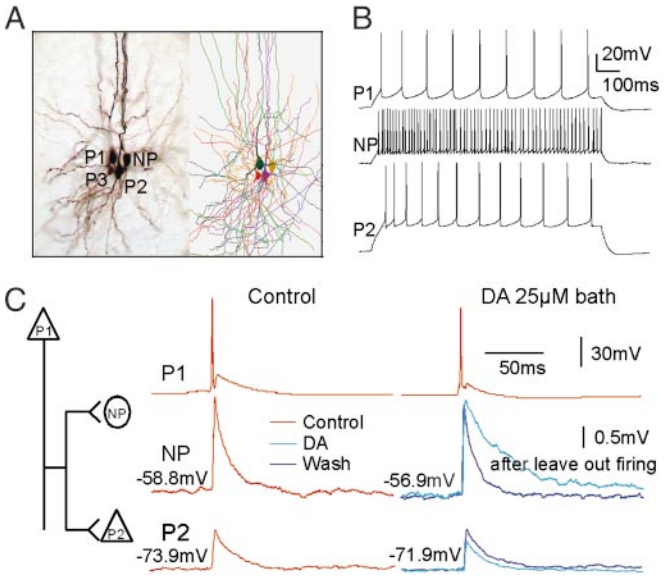

Fig. 5. Sample of quadruple recording showing differential dopamine (DA) modulation of transmitter release from terminals arising from an individual axon that contacts different cell types. (*A*) A light microscopic photograph of four biocytin-filled neurons and Neurolucida reconstructions of connected neurons. The pyramidal cell (P1) innervated another pyramidal neuron (P2) and FS interneuron (NP). (*B*) Action potential traces showing the physiological properties of three connected neurons. Both pre- (P1) and postsynaptic (P2) pyramidal neurons exhibited a regular spiking firing pattern, whereas the interneuron (NP) showed a typical pattern of fast spiking with high frequency firing without adaptation. (*C*) Averaged EPSPs showing differential dopaminergic modulation of excitatory transmission from a single axon of P1. Dopamine depolarized interneuron (NP) by 1.9 mV and induced firing in the peak of EPSP in 9 of 20 traces (45%), but the average EPSP amplitude after leaving out firing was almost identical except for a prolonged time constant in P-NP_{FS} synapses. In contrast, dopamine reduced the EPSP amplitude of P-P connection by 26.7% and increased synaptic failure by 20% and CV by 32.4%, indicating a presynaptic regulation. It should be noted that, although EPSP amplitude in P-NP_{FS} was not affected, the decay time constant was obviously slower after dopamine (τ = 11.9 ms in control vs. 32.1 ms in dopamine), probably due to the activation of Na^+ currents (17, 32–34).

Direct Effects of Dopamine on FS Interneurons and Precise Timing of Spike Transmission. In view of the evidence that dopamine increases the excitability of FS interneurons (7, 8, †), the lack of a significant effect on excitatory transmission between pyramidal cells and FS interneurons reported here indicates that dopamine's indirect effects on synaptic actions are different from its direct effects. This difference was further amplified by comparing dopamine's influence on FS interneuron and pyramidal cell membrane potentials and spike initiation under current-clamp conditions. Most FS cells (16/21, 76.2%) were reversibly depolarized by dopamine on average at 3.0 ± 0.44 mV (range, 0.3–6.4 mV), whereas the remaining 5 cells were hyperpolarized on average at 3.01 ± 0.81 mV (range, 0.1–4.3 mV). Membrane potential change was not correlated with percent EPSP amplitude change (R^2 = 0.064) and depolarization was also not accompanied by a proportional change in threshold (R^2 = 0.006). However, as shown by the smaller currents needed to elicit action potentials, and in agreement with previous studies, we observed increased excitability in the majority of interneurons tested (16/21, 76.2%, Fig. 6 *A* and *B*). Indeed, dopamine induced firing in many of these cells (9/24, 37.5%), and, in some of them (3/9), spiking was so robust that we were unable to measure EPSPs. These cells were therefore excluded from analysis. At the same time, however, there was little evidence of EPSP amplitude change (1.3–9.2%) in the remaining group of cells in which firing was induced, after excluding the traces with firing spikes. Nor was the EPSP amplitude change of these interneurons correlated with changes in threshold (R^2 = 0.009), indicating that dopamine-induced firing in interneurons is a threshold phenomenon.

A novel and potentially functionally important finding with respect to dopamine's direct actions on interneurons was that it increased coupling between EPSPs and action potential initiation such that even a single EPSP was capable of inducing an action potential with a probability of 0.3–0.6 (Fig. 6*C*). Most of the FS cells responded to dopamine, but EPSP-initiated action potentials occurred only in about one-third of them. In these interneurons, the delay between EPSP onset and action potential peak was very brief (<6 ms) in 84% of 50 sweeps, corresponding to the rising phase of the EPSP (Fig. 7 *A* and *C*; refs. 16 and 17). In contrast, the latency of action potential discharge in pyramidal cells was considerably more variable and uncorrelated to EPSPs when positive currents were injected to elicit firing spikes (Fig. 7 *B* and *D*). The mean EPSP-spike delay of 12.7 ± 4.07 ms for interneurons was significantly shorter than the value of 102.2 ± 8.61 ms for pyramidal cells (50 sweeps; $P < 0.0001$). It should be noted that, in the presence of dopamine, the membrane potential at which EPSPs triggered cell firing in these interneuron averaged −65.7 ± 1.96 mV (n = 6, −66.1 ± 2.17 mV in control, P = 0.831), almost 20 mV lower than their threshold (−46.9 ± 2.66 mV, $P < 0.005$). In contrast, EPSPs failed to induce firing in any of 50 pyramidal neurons examined in dopamine (including pre- and postsynaptic cells). Injection of positive current was needed to elicit firing, and the membrane potential for a firing probability of 0.5 was on average of −41.4 ± 1.96 mV (n = 5), very close to their threshold (−40.9 ± 1.33 mV in control, P = 0.285, Fig. 7*B*). Altogether, these findings provide strong evidence that a majority of FS interneurons in layer V are sensitive to dopamine and a subpopulation (≈40%) of these cells are hypersensitive to this neurotransmitter.

Discussion

Target Cell-Specific Modulation of Transmitter Release at Terminals from a Single Axon. The present study of excitatory transmission in prefrontal elemental circuits has led to two major conclusions

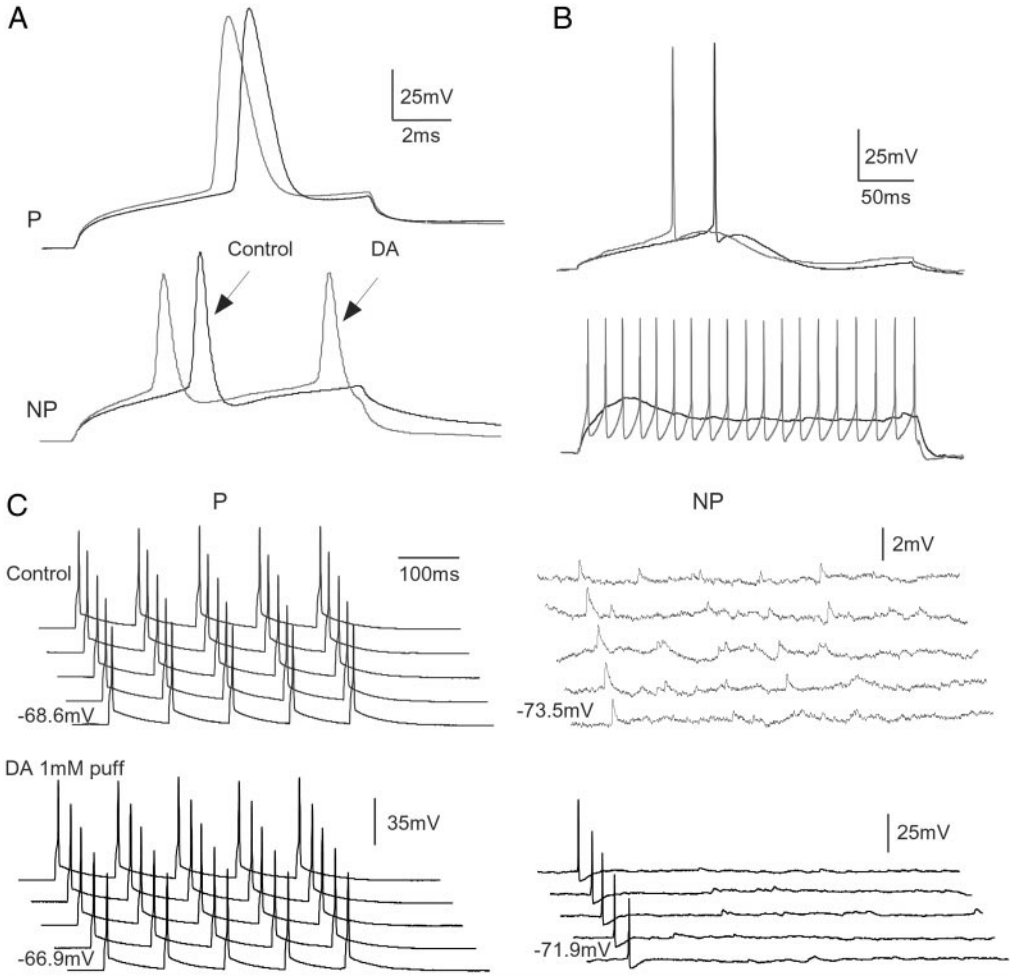

Fig. 6. Dopamine (DA) increases the excitability of postsynaptic FS interneurons and elicits firing in the rising phase of EPSPs in P-NP_{FS} synapses. (*A*) Small current injection in short duration (10 ms) induces action potentials in both pyramidal cell and interneuron. Dopamine application only slightly shortens the latency of pyramidal cell but increases the spikes of interneuron significantly. (*B*) Another example of P-NP_{FS} pair with current injection of 300 ms duration. The threshold of postsynaptic interneuron was significantly reduced, with the same amount of current injection-induced firing, clearly, but not before dopamine application. (*C*) A train of five pulses at intervals of 100 ms (10 Hz), applied in presynaptic pyramidal neurons, reliably induces EPSPs in postsynaptic interneuron in control condition. Spikes were initiated from the rising phase of first EPSPs in FS interneuron during dopamine application.

relevant to cortical function. First, dopamine's effects on excitatory transmission are target-specific: it selectively depresses excitatory synapses on pyramidal cell targets yet is without comparable effect on FS interneurons. These effects were observed not only by comparison of dopamine's actions in independent P-P and P-NP_{FS} pairs but also in the dual targets of a single axon arising from the same neuron. Second, we support previous findings that direct application of dopamine enhances interneuron excitability in a majority of FS interneurons, and add to these reports that dopamine can increase the precision of coupling between EPSPs and action potential generation in some FS interneurons. Thus, dopamine powerfully facilitates inhibition in prefrontal layer V, the output layer of the cortex. However, as these local circuit components are yet part of a larger network of local connections, it remains to be determined how their net inhibitory effects alter the projection cells of this layer.

Target Specificity. Target cell-specific regulation of synaptic signaling has previously been reported in short-term modification of postsynaptic potentials (PSPs; refs. 18–20) and long-term plasticity (21), as well as in synaptic release (22) in both hippocampal and neocortical excitatory connections (see ref. 23 for a review). However, the present finding provides previously undocumented evidence that target specificity applies to dopamine modulation of cortical circuit function. Thus, dopamine's actions can no longer be considered as nonspecific and global, as traditionally assumed, but seem to be remarkably focused and constrained to a particular role in specific local circuits. The selective depression of P-P connections and absence of action at P-NP_{FS} connections in the present study suggest that pyramidal axon terminals on other pyramidal cells may possess dopamine receptors whereas those that innervate interneurons may not. Indeed, our previous study of pyramid-pyramid pairs provided evidence for a presynaptic action of dopamine at D1 receptors on excitatory axon terminals (5). In support, recent immunoelectron microscopic observations in this laboratory have revealed that dopamine D1 receptors are more frequently observed at putative excitatory terminals impinging onto spines of pyramidal neurons and are rare on axon terminals on parvalbumin-containing interneurons (L. Negessy and P.S.G.-R., unpublished observations; §). In agreement, metabotropic glutamate receptors, among other receptor systems, have also

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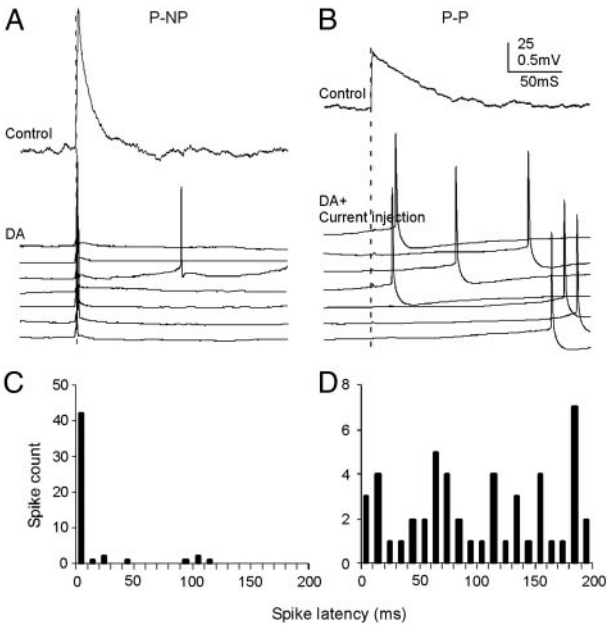

Fig. 7. EPSP-spike coupling in FS interneurons and pyramidal cells in dopamine (DA). (*A* and *B*) Upper traces: averaged EPSPs of 20 trials from P-NP_{FS} and P-P connections in control conditions. Lower traces: sample traces showing that spikes were always initiated from the location of EPSPs in a typical FS interneuron during dopamine application, whereas, in pyramidal neurons, spikes were often evoked after the peak of EPSPs. The threshold holding potential was very different between interneurons and pyramidal cells. In interneuron, dopamine was able to induce firing directly by small depolarization, whereas, in pyramidal cell, membrane potential must be held to its threshold level to elicit firing. (*C* and *D*) Spike latency histograms constructed from 50 trials of three interneurons in P-NP_{FS} connections and three pyramidal cells in P-P pairs, showing the different variability of initiated spikes in two kinds of postsynaptic neurons (latency average, 12.7 $\pm$ 4.07 ms in interneuron and 102.2 $\pm$ 8.61 ms in pyramidal cells, $P < 0.0001$).

been shown to be differentially located in axon terminals of the same neuron, depending on their postsynaptic targets (22, 24).

Excitability Increase of FS Interneurons. An apparent paradox arises from the finding that, whereas dopamine's direct effect on FS cells is to increase their excitability (7, 8, †), it depresses evoked inhibitory postsynaptic potentials or inhibitory postsynaptic currents (25–27). Currently, this effect is thought to be due to a postsynaptic action on potassium currents (8). An additional possibility is suggested by our recent finding that dopamine depresses γ-aminobutyric acid (GABA) release by a presynaptic mechanism analogous to its presynaptic effects on excitatory transmission (27). Thus, if dopamine also depresses GABA release at axon terminals targeting at interneurons, this result could provide a simple explanation for the increased excitability of interneurons with direct dopamine application. This disinhibitory action would therefore indicate that a general property of dopamine modulation in the cerebral cortex may be to regulate neurotransmitter release at both inhibitory and excitatory terminals, in addition to whatever other actions it may have. Finally, it should be recalled in the present study that 25% of FS interneurons examined did not show an increase in excitability in the presence of dopamine, probably reflecting the well-known diversity of FS interneuron subtypes (28, 29).

Physiological Implications for Cognition. Extracellular recordings have revealed the involvement of inhibitory neurons in the basic properties of spatial tuning (3), temporal integration (4), and neuronal synchronization (30). Dopamine's actions on cortical circuitry would seem to provide the basis for a net inhibitory action on one or more of these cortical functions. However, the intralaminar and nearby afferents of the layer V neurons investigated in this study represent only a minority of these neuron's inputs. Moreover, layer V pyramidal neurons transmit to non-FS as well as FS interneurons, both of which project to pyramidal neurons as well as other interneurons. Thus, dopaminergic depression of pyramidal cell output could lead via multisynaptic transmission to disinhibition at distant sites. Dopamine's direct modulatory actions on FS interneurons may also have disinhibitory effects in NP-NP connections (31). Thus, it would be an oversimplification to draw any conclusion regarding dopamine's net effect on layer V output neurons. The differentiated actions of dopamine on specific circuit elements described here indicate that the pharmacological investigation of additional cortical circuits may provide the basis for determining which cortical functions are facilitated by dopamine, and which are not, as well as providing important insights into the therapeutic actions of dopamine agonist and antagonist drugs used to treat a variety of cognitive disorders.

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