State-Dependence of Dopamine D1 Receptor Modulation in Prefrontal Cortex Neurons

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Dopamine makes an important yet poorly understood contribution to normal and pathological processes mediated by the prefrontal cortex. The present study proposes a hypothesis for the cellular actions of dopamine D1 receptors on prefrontal cortex neurons based on *in vitro* recordings and computational models. In deep layer V prefrontal cortex neurons, we show that D1 receptor stimulation: 1) increased evoked firing from rest 2) shifted the activation of a persistent Na^+ current and slowed its inactivation, 3) enhanced NMDA-mediated EPSCs and 4) enhanced $GABA_A$ IPSPs over many minutes. These changes had state-dependent effects on networks of realistically modeled prefrontal cortex neurons: spontaneous firing driven by low frequency inputs was decreased, while firing evoked by progressively stronger excitatory drive was enhanced and sustained following offset of an input. These findings provide insights into the paradoxical nature of dopamine's actions in the prefrontal cortex, and suggest how dopamine may modulate working memory mechanisms in networks of prefrontal neurons.

Despite over two decades of research into the pathophysiology of schizophrenia centered on the dopamine system and prefrontal cortex (PFC), the exact function of dopamine in normal and pathological processes has remained elusive. This is partly because manipulations of dopamine activity in the PFC have had such disparate effects. For instance, lesions of dopamine terminals in the PFC produced deficits on working memory tasks (Brozowski et al. 1979), but yielded improvements on attentional set shifting tasks (Roberts et al. 1994). Furthermore, both high levels of D1 receptor activation and D1 receptor blockade induced deficits on working memory tasks (Zahrt et al. 1997; Seamans et al. 1998; Sawaguchi & Goldman-Rakic 1994) while dopamine has been reported to both enhance (Yang & Mogenson 1990; Yang & Seamans 1996; Shi et al. 1996; Penit-Soria et al. 1987) and suppress (Gulledge & Jaffe 1998; Geijo-Barrientos & Pastore 1995) firing of PFC neurons *in vitro*.

Much of our understanding regarding the function of the PFC has come from single unit recording studies in primates performing delayed-response tasks. Dopamine via the D1 receptor, has been shown to consistently and significantly enhance delay and response-related activity of PFC neurons (Sawaguchi et al. 1990a,b). These findings are inconsistent with the classical view that dopamine is inhibitory in the PFC based on the finding that it reduces spontaneous firing in anesthetized rats (Bunney & Aghajanian 1976; Sesack & Bunney 1989). However, evoked activity is enhanced by dopamine in anesthetized rats (Yang & Mogenson, 1990) and the inhibitory actions of dopamine on spontaneous activity can often be occluded by blockade of $GABA_A$ receptors (Pirot et al. 1992). We examined some of the cellular, synaptic and network mechanisms potentially underlying these conflicting results. The present study used *in vitro* patch-clamp recordings and realistic models of PFC neurons to show that D1 receptor activation has both inhibitory and excitatory effects and collectively acts to intensify and stabilize activity patterns in PFC networks.

I*n vivo* the firing rate of PFC neurons is determined both by intrinsic ionic currents and synaptic inputs. D1 receptor agonists added to the bathing solution produced a slight depression of the non-isolated PSP in PFC neurons ($-17.4 \pm 13.6\%$, n=6, not shown) (Law-Tho et al. 1994). Since this response consisted of numerous components, the $GABA_A$, NMDA and non-NMDA responses were pharmacologically isolated. A significant enhancement of IPSP amplitude (Fig 1A,B) and IPSP initial slope was observed in 14/15 cells following application of high or low doses of a D1 agonist. The D1 agonist had no effect on IPSP amplitude ($-2.82 \pm 8\%$, n=5) in the presence of the D1 antagonist SCH23390 (5μM). Bath application of a D1 agonist also significantly increased the amplitude of the isolated NMDA EPSC in 12/15 cells (Fig 1A,B). This effect of a D1 agonist on NMDA amplitude was blocked by SCH23390 ($-3.5 \pm 10\%$, n=4). In contrast, a D1 agonist produced a reduction of non-NMDA EPSC amplitude in 10/14 cells (Fig 1A,B). However, the reduction was non-significant if the effects of normal response run-down were accounted for by subtracting off the average drop in EPSC amplitude for control cells recorded for 50 min in the absence of D1 agonists. Thus, while D1 receptor activation clearly enhanced $GABA_A$ and

NMDA-mediated responses, it had much smaller inhibitory effects on non-NMDA responses.

In addition to the effects on synaptic responses, D1 agonists caused a spike to be evoked by a previously subthreshold current pulse (Fig 1C). This effect could be due partly to D1-mediated changes in a persistent Na^+ current (I_{NAP}) (Yang & Seamans 1996) because this current strongly influences subthreshold excitability. In the present study, the pharmacologically isolated TTX-sensitive I_{NAP} was evoked by applying a voltage ramp from –70 mV to 10 mV. In the presence of a D1 agonist the inward current mediated by I_{NAP} peaked at more hyperpolarized membrane potentials (Fig 1D). Ten minutes following application of a D1 agonist more spikes were evoked by a suprathreshold current pulse (Fig 1C). Since I_{NAP} inactivation strongly influences spike accommodation in cortical neurons, the effects of D1 agonists on I_{NAP} inactivation were investigated. We found that the time-constant of inactivation was slowed in the presence of a D1 agonist (Fig 1D). Finally, the leak current was unaffected by D1 agonists in 17/18 PFC cells tested.

A biophysically realistic compartmental model (Fig 2A) was used to study the functional impact of the changes in synaptic currents and I_{NAP} in PFC neurons and networks. In the network simulations many of these neurons were connected to eachother and interneurons (Fig 2B), and all neurons received random excitatory and inhibitory 'background' inputs. The actions of D1 agonists were implemented in the model by altering intrinsic and synaptic currents in accordance with the data presented above. In the simulated D1 condition, a slight reduction in spontaneous activity occurred in all pyramidal neurons (Fig 2C vs D). However, when a high-activity state of the network was evoked in one neuron group, a significant and long-lasting enhancement in activity was observed for the simulated D1 condition (Fig 2C vs D). Conversely, the firing rate of neurons in the non-stimulated group decreased (Fig 2C vs D).

In order to evaluate these effects systematically we focused on one neuron and replaced the other 5 neurons in the group with 5 inputs to the neuron's basal dendrites. The frequency of these inputs were varied progressively from 0-90 Hz. Relative to the control condition, firing of the single neuron in the simulated D1 condition was depressed if input rates were below 10 Hz, but enhanced if input rates were above 30 Hz. The results were robust within ±10% shifts in synaptic conductances. However, if only intrinsic or only synaptic currents were modulated in the D1-condition, the attractor state broke down much sooner (<4s) and the average firing rate was ~50% lower. This indicated that it was the unique combination of changes in the D1 condition that contributed to the stable sustained activity. By modulating intrinsic and synaptic currents based on *in vitro* data, the model also replicated the paradoxical findings that D1-receptor activation can slightly reduce firing in relatively inactive networks such as anesthetized preparations (Yang & Mogenson 1990) but enhance firing in more active PFC networks (Sawaguchi et al. 1990a,b).

The present study focused on D1 receptors because they are more abundant than D2 receptors in the rat and primate PFC and make a far greater contribution to working memory processes (Bergson et al. 1995; Sawaguchi &

Goldman-Rakic 1994; Seamans et al. 1998; Vincent et al. 1995). Indeed only D1 and not D2 anatgonists consistently disrupt working memory performance in both rodents and primates (Seamans et al. 1998; Sawaguchi & Goldman-Rakic 1994; Sawaguchi et al. 1990b). On physiological measures, D2 receptor activation appears to have a faster onset, shorter duration and opposite effects to D1 receptor activation. While the functional contribution of the short-term inhibitory action D2 receptors on pyramidal neurons remains unclear, D1 receptors appear to enhance signal processing within the PFC.

In the present simulations, recurrent excitation in the D1 condition sustained firing only in those PFC neurons that were strongly activated, while the firing of weakly activated neurons was decreased. This type of differential enhancement of firing in active networks by D1 receptors may underlie the ability of neurons to enter a stable attractor state and sustain activity throughout a delay in the absence of sensory stimuli and perhaps even in the presence of distracting stimuli (Durstewitz et al. 1999; Goldman-Rakic 1995; Fuster 1997; Lisman et al. 1998; Sawaguchi et al. 1990a,b). Dopamine acting via the D1 receptor may therefore enhance the effects of strong excitatory inputs against a backdrop of noisy activity within small assemblies of PFC neurons, shifting a population of neurons from a state where several patterns of activity co-exist to a state where only a single pattern was maintained for an extended period. In extreme cases if the network were in the former state, random or disorganized modes of thought or behavior could result, while in latter state perseverative or stereotyped modes of behavior may prevail.

These predictions are consistent with experimental data showing that D1 agonists cause perseverative errors on a working memory task, while D1 antagonists or dopamine depletion in the PFC reverse amphetamine induced stereotypy, promote attentional set shifting on a modified version of the Wisconsin card sorting task and result in random and disorganized responding on a working memory task (Seamans et al. 1998; Zhart et al. 1998; Ellenbroek et al. 1989; Roberts et al. 1994). In spite of the differences in the PFC across species, the proposed D1-dependent enhancement in attractor dynamics may provide a generalized mechanism for the holding and manipulation of contextually-relevant information required to plan forthcoming action in complex sensory environments.

Methods

The brains of male Sprague-Dawley rats (14-28 days) were rapidly dissected and immersed for 1 minute in cold (4 °C) oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl (126), KCl (3), $NaHCO_3$ (26), $MgCl_2$ (1.3), $CaCl_2$ (2.3), glucose (10) at 30°C and 300μm slices containing the prelimbic/infalimbic region of the PFC were cut. Individual slices were perfused by gravity-fed ACSF (maintained at 30-34°C) at a rate of 1-3 ml per min. and viewed using differential interference contrast (DIC) optics. Thick-walled borosilicate pipettes were filled with (in mM): $KMeSO_4$ (140), HEPES (10), NaCL(4), EGTA(1), NaATP(4), TrisGTP(0.3) and Phosphocreatine (14) or K-gluconate (130), KCl (10), ethylene glycol-bis(β-aminoethyl ether)- N,N,N',N'-tetraacetic acid (EGTA) (1), $MgCl_2$ (2), NaATP (2), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES) (10). QX-314 (2mM) was added to pipettes in ~60% of experiments. Pipettes were connected to the headstage of an Axoclamp-2B or Axopatch-200B amplifier (Axon Instruments) with Ag/AgCl wire. Combinations of D(-) 2 Amino-5-phosphonopentanoic acid (AP5) (50μM) , (-) Bicuculline methiodide, l(S),9(R) (2-10μM) and DNQX or CNQX (2-10μM) were used to isolate synaptic responses. D1 receptor agonists (±)-SKF-38393, (±)-SKF-81297, R(+)-SKF-81297, or R(+)-SKF-82957 (0.5-50μM) (RBI, Natick Mass.) were applied in th dark.

A full model description can be obtained by contacting D. Durstewitz at dd@salk.edu. The network model consisted of identical reciprocally connected pyramidal neurons and interneurons which all received AMPA, NMDA and $GABA_A$ like inputs. AMPA and $GABA_A$ synaptic currents were modeled by alpha-functions and NMDA currents were modeled by the product of a double exponential function with a voltage-dependent activation gate (Spruston et al. 1995). The 20 compartment pyramidal cells reproduced the active behavior of PFC neurons through 12 active currents non-uniformly distributed throughout the neuron. The kinetics and distributions of two Na^+ (fast, and I_{NAP}) three Ca^{2+} (L, N, T-type), six K^+ (delayed rectifier, M-type, A-type, slowly-inactivating, C-type and afterhyperpolarizing) and an H-type mixed cationic current were based on data from both cortical and hippocampal neurons. The interneurons had 9 compartments and possessed a fast Na^+ current and K_{DR}. Each neuron received 6 connections from other neurons and connection strengths were adjusted to enable the small network to exhibit sustained activity via recurrent excitation. All neurons received 'background' synaptic inputs as described in Fig 2. The simulated D1 condition consisted of: 1) shifted I_{NAP} activation gate by 2-5 mV in the hyperpolarized direction; 2) increased inactivation time constant of I_{NAP} 1.4-fold; 3) reduced I_{KS} and I_{CaN} maximum conductance by 20-50% (Yang & Seamans 1996); 4) increased maximum NMDA conductance by 40%; 5) increased maximum $GABA_A$ conductance by 40%; and 6) increased firing frequency of $GABA_A$ synapses by 10% (Penit-Soria et al. 1987).

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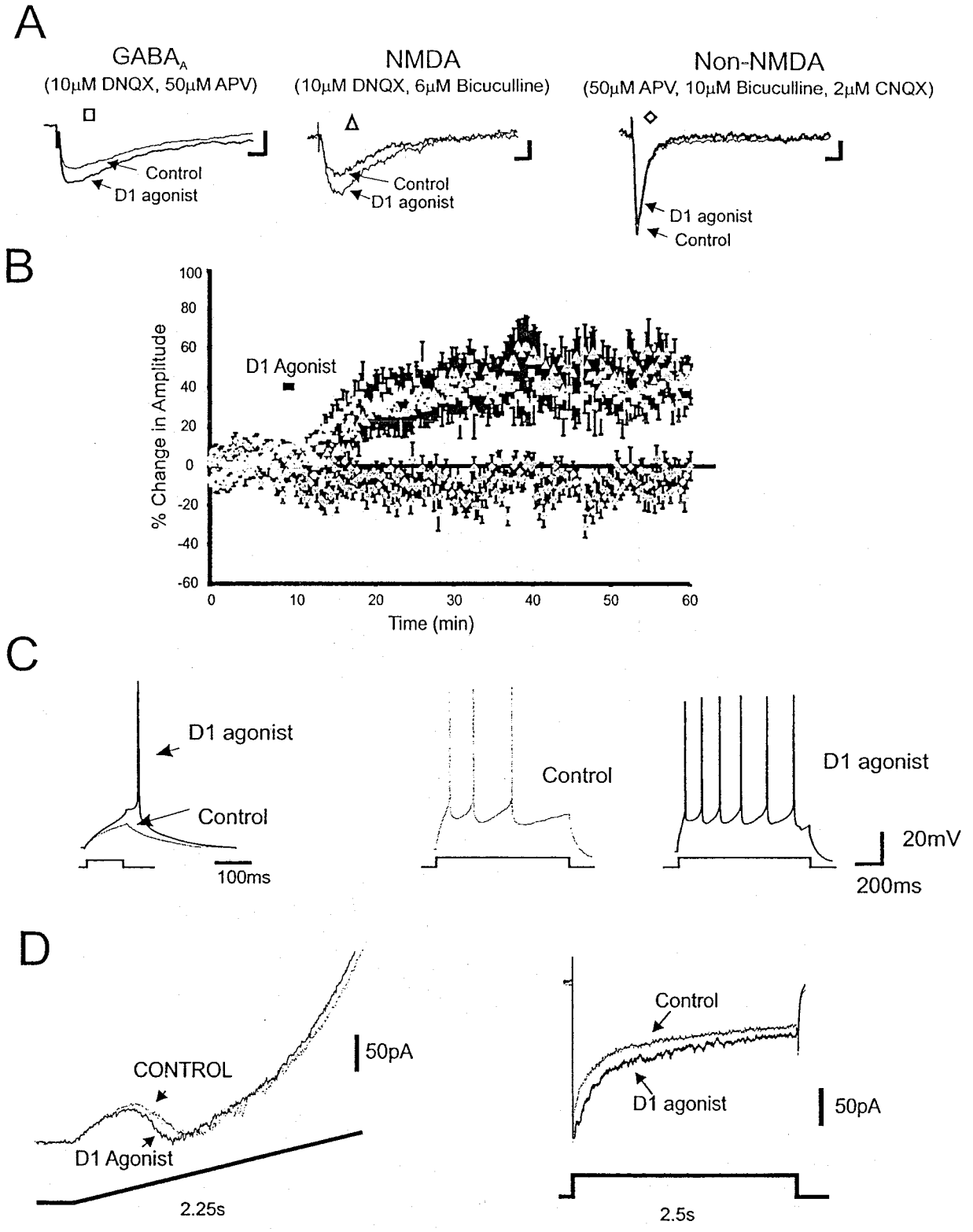

Figure 1. Action of a D1 agonist on synaptic and intrinsic responses of PFC neurons. **A)** Representative synaptic responses. **B)** Average percentage change (and SEM) in response amplitude over time. NMDA EPSC=triangles, $GABA_A$ IPSP=squares, Non-NMDA EPSC=diamonds. *statistical significance at $p<0.01$.**C)** (Left) Under current-clamp, a previously subthreshold (light trace) short current pulse evoked a spike 15min after application of a D1 agonist (black). Relative to control (light trace, middle), the D1 agonist (black, right) also caused more spikes to be evoked by a long suprathreshold current pulse (30 ± 13% increase in spiking 10-30 min after application). **D)** The same D1 agonist (black) caused a leftward shift relative to the control I_{NAP} (light trace, bottom). D1 agonists induced an average shift in I_{NAP} of 2.4 ± 0.3 mV (32 ± 11 %, n=19) at -50 mV. **E)** A D1 agonist also slowed the inactivation of I_{NAP} evoked by a long voltage-step (control = 1537 ± 237 ms, D1 agonist = 2211 ± 412 ms, 43.8 ± 18%, n=18).

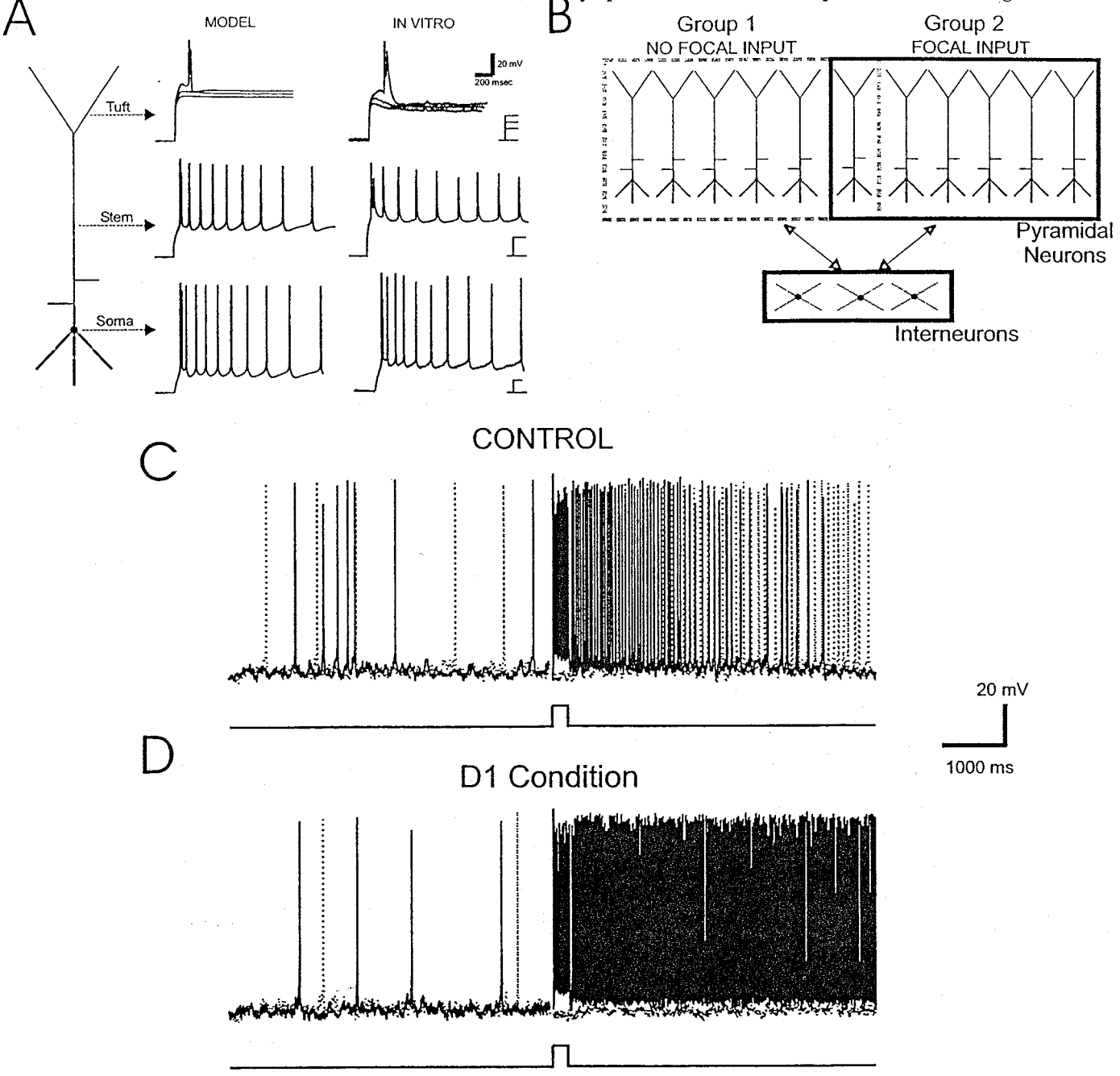

Figure 2. Dopaminergic modulation of activity states in the model network. **A)** A 20-compartment model could reproduce *in vitro* patch-clamp recordings (right) from the apical tuft (>500μm from soma, beyond the main bifurcation), apical stem (60-500μm from soma, middle) and soma (bottom) of a layer V intrinsically-bursting PFC pyramidal neuron. The model included 12 active ionic conductances distributed non-uniformly throughout all compartments. The kinetics and distributions of two Na^+ (fast, and I_{NAP}) three Ca^{2+} (L, N, T-type), six K^+ (delayed rectifier, M-type, A-type, slowly-inactivating, C-type and afterhyperpolarizing) and an H-type mixed cationic current were based on data from both cortical and hippocampal neurons. *In vitro* recordings were derived from 3 different neurons. **B)** Schematic of the network that included 2 groups of 6 identical interconnected pyramidal neurons, with one neuron connected to both groups. Three interneurons were reciprocally connected to both groups of pyramidal neurons. All cells received random Poisson background synaptic input (pyramidal: 4000 excitatory, 1000 inhibitory and interneuron: 1600 excitatory, 500 inhibitory). One set of pyramidal neurons received no additional input (gray box, Group 1) and the other set received a transient current input (black box, Group 2). **C)** In the control condition neurons from Group 1 (dashed trace) and Group 2 (solid trace) fired at a low rate (about 1.4Hz) until the focal input and reciprocal connections transiently increased the firing of Group 2 neurons to about 22Hz. This high activity fades out after about 1-2 sec and is not well separated from the activity of Group 1 neurons. **D)** In the simulated D1-condition the spontaneous firing rates for both Group 1 (dashed trace) and Group 2 (solid trace) neurons were reduced to about 0.6Hz. However, once activated, the firing rate of every Group 2 neuron increased about twofold compared to the control condition to around 44Hz, and this activity was maintained for long periods and was well separated from the totally suppressed activity of Group 1 neurons.