Dopamine Enhances Spatiotemporal Spread of Activity in Rat Prefrontal Cortex

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Bandyopadhyay, Susanta, Carlos Gonzalez-Islas, and John J. Hablitz. Dopamine enhances spatiotemporal spread of activity in rat prefrontal cortex. J Neurophysiol 93: 864–872, 2005. First published October 6, 2004; doi:10.1152/jn.00922.2004. Dopaminergic modulation of prefrontal cortex (PFC) is important for neuronal integration in this brain region known to be involved in cognition and working memory. Because of the complexity and heterogeneity of the effect of dopamine on synaptic transmission across layers of the neocortex, dopamine’s net effect on local circuits in PFC is difficult to predict. We have combined whole cell patch-clamp recording and voltage-sensitive dye imaging to examine the effect of dopamine on the excitability of local excitatory circuits in rat PFC in vitro. Whole cell voltage-clamp recording from visually identified layer II/III pyramidal neurons in rat brain slices revealed that, in the presence of bicuculline (10 μM), bath-applied dopamine (30–60 μM) increased the amplitude of excitatory postsynaptic currents (EPSCs) evoked by weak intracortical stimuli. The effect was mimicked by the selective D1 receptor agonist SKF 81297 (1 μM). Increasing stimulation resulted in epileptiform discharges. SKF 81297 (1 μM) significantly lowered the threshold stimulus required for generating epileptiform discharges to 83% of control. In the imaging experiments, bath application of dopamine or SKF 81297 enhanced the spatiotemporal spread of activity in response to weak stimulation and previously subthreshold stimulation resulted in epileptiform activity that spread across the whole cortex. These effects could be blocked by the selective D1 receptor antagonist SCH 23390 (10 μM) but not by the D2 receptor antagonist eticlopride (5 μM). These results indicate that dopamine, by a D1 receptor–mediated mechanism, enhances spatiotemporal spread of synaptic activity and lowers the threshold for epileptiform activity in local excitatory circuits within PFC.

INTRODUCTION

The mammalian prefrontal cortex (PFC) receives a substantial dopaminergic innervation from the midbrain ventral tegmental area (VTA) (Bjorklund and Lindvall 1984). Dopamine is an endogenous neuromodulator in the cerebral cortex and is believed to be important for normal brain processes (Bjorklund and Lindvall 1984; Williams and Goldman-Rakic 1995). There is strong evidence that alterations in dopamine function may have multiple effects on PFC neurons. Both increases (Ceci et al. 1999; Gonzalez-Burgos et al. 2002; Gorelova and Yang 2000; Henze et al. 2000; Penit-Soria et al. 1987; Tseng and O’Donnell 2004; Wang and O’Donnell 2001; Yang and Sehmann 1996) and decreases (Geijo-Barrientos and Pastore 1995) in postsynaptic excitability of pyramidal neurons have been reported following D1 receptor activation. In addition, changes in excitability mediated by D2 receptors have been reported (Gulledge and Jaffe 1998; 2001; Tseng and O’Donnell 2004). The effects of dopamine on synaptic responses are also complex and may be layer- and species-specific. AMPA receptor mediated excitatory postsynaptic currents (EPSCs) in layer V pyramidal cells are depressed by a D1 receptor–mediated effect of dopamine (Law-Tho et al. 1994; Sehmann et al. 2001), whereas N-methyl-D-aspartate (NMDA) responses have been reported to be both enhanced (Sehmann et al. 2001) and depressed (Law-Tho et al. 1994). EPSCs in layers II/III are enhanced by dopamine in rats (Gonzalez-Islas and Hablitz 2003) but decreased in primates (Urban et al. 2002). Given this complexity and micro-heterogeneity, the net effect of dopamine modulation of local circuits in PFC is not easily predicted.

The cerebral cortex contains interconnected local and distant networks of excitatory and inhibitory neurons. Stability of activity in such networks depends on the balance between recurrent excitation and inhibition (Durstewitz et al. 2000a; Shu et al. 2003). A shift of the balance toward excitation may lead to the generation of epileptiform activity. The presence of massive recurrent excitatory connections that depend on inhibition for regulation has been implicated in the susceptibility of the neocortex and the hippocampus to develop epileptiform activity and seizures (McCormick and Conteras 2001). Modulatory influences strongly influence activity in thalamocortical (McCormick 1992; McCormick and Pape 1990) and neocortical circuits (McCormick et al. 1993).

Dopamine is known to modulate epileptiform discharges both in vivo (Alam and Starr 1992, 1993b, 1994a; George and Kulkarni 1997) and in vitro (Alam and Starr 1993b, 1994b; al. 1984; Pirot et al. 1992; Reader et al. 1979; Sesack and Bunney 1989; Thierry et al. 1992; Yang and Mogenson 1990). Dopamine may also favor long-lasting transitions of PFC neurons to a more excitable up state (Lewis and O’Donnell 2000). During short-term working memory processing, dopamine-dependent increases in firing of groups of PFC neurons have been observed (Fuster 1995; Goldman-Rakic 1995). In vitro electrophysiological experiments suggest that dopamine has multiple effects on PFC neurons. Both increases (Ceci et al. 1999; Gonzalez-Burgos et al. 2002; Gorelova and Yang 2000; Henze et al. 2000; Penit-Soria et al. 1987; Tseng and O’Donnell 2004; Wang and O’Donnell 2001; Yang and Sehmann 1996) and decreases (Geijo-Barrientos and Pastore 1995) in postsynaptic excitability of pyramidal neurons have been reported following D1 receptor activation. In addition, changes in excitability mediated by D2 receptors have been reported (Gulledge and Jaffe 1998; 2001; Tseng and O’Donnell 2004). The effects of dopamine on synaptic responses are also complex and may be layer- and species-specific. AMPA receptor mediated excitatory postsynaptic currents (EPSCs) in layer V pyramidal cells are depressed by a D1 receptor–mediated effect of dopamine (Law-Tho et al. 1994; Sehmann et al. 2001), whereas N-methyl-D-aspartate (NMDA) responses have been reported to be both enhanced (Sehmann et al. 2001) and depressed (Law-Tho et al. 1994). EPSCs in layers II/III are enhanced by dopamine in rats (Gonzalez-Islas and Hablitz 2003) but decreased in primates (Urban et al. 2002). Given this complexity and micro-heterogeneity, the net effect of dopamine modulation of local circuits in PFC is not easily predicted.

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Cepeda et al. 1999; Siniscalchi et al. 1997; Suppes et al. 1985). In vivo studies in different models of epilepsy have suggested that dopamine may have a pro-convulsant effect mediated by D1 receptors and an anti-convulsant effect via D2 receptors (see Starr 1996 for review). A dopamine-mediated recruitment of neurons in local excitatory circuits and synchronization of activity in these neurons may underlie these effects of dopamine in neocortex. Local excitatory neocortical networks are complexes of interconnected pyramidal neurons. Since dopamine has diverse effects on these neurons, the action of dopamine in such networks is difficult to predict based on recordings from individual pyramidal neurons or unitary excitatory connections. The aim of this work was to investigate dopamine’s ability to modulate activity in the local excitatory circuits in PFC. Using a combination of whole cell recording and voltage-sensitive dye imaging, we have shown that dopamine, via D1 receptors, enhances local spatiotemporal spread of synaptic activity and lowers the threshold for evoking epileptiform discharges in neocortex. A preliminary account of some of these findings has been published (Hablitz and Gonzalez-Islas 2002).

METHODS

Slice preparation

Neocortical slices were prepared from Sprague-Dawley rats (25–30 days old). Animals were handled and housed according to the National Institutes of Health Committee on Laboratory Animal Resources guidelines. All experimental protocols were approved by the UAB Institutional Animal Care and Use Committee. Every effort was made to minimize pain and discomfort. Rats were anesthetized with ketamine and decapitated. The brain was removed and quickly placed in ice-cold saline which contained (in mM) 125 NaCl, 3.5 KCl, 26 NaHCO3, 10 D-glucose, 3 MgCl2, and 1 CaCl2. Coronal slices (300 μm thick) were cut on a Vibratome (Ted Pella, Redding, CA) from a block of brain containing PFC. The anterior cingulate cortex and the shoulder or Fr2 region of the frontal cortex (Paxinos and Watson 1986) were used for recording. Slices were stored for 45 min at 37°C and kept at room temperature until recording. The storage solution contained (in mM) 125 NaCl, 3.5 KCl, 26 NaHCO3, 10 g-glucose, 2.5 CaCl2, and 1.3 MgCl2. The solution was bubbled with 95%O2-5%CO2 to maintain pH around 7.4. Individual slices were subsequently transferred to a recording chamber continuously perfused (3 ml/min) with oxygenated saline at 22–23°C. Excess dye was washed out for ≥30 min prior to recording. The same area of PFC used for whole cell recording was chosen for imaging. Similar bipolar electrodes were used for stimulation; the stimulation site and strength were also similar to those used in whole cell recordings. Frequency of stimulation was once every minute.

Activity-dependent changes in fluorescence were detected using a Neuroplex 464 diode array (Red Shirt Imaging, Fairfield, CT). A sampling rate of 1.6 KHz was employed allowing frames to be acquired at 0.6-ms intervals. To excite the dye, light from a 100-W halogen lamp was passed through a 535 ± 40-nm filter. A computer-controlled shutter was used to limit illumination and minimize toxic effects of the dye. The emitted light was focused on the diode array after passing through a 590-nm long-pass filter. The optical signals were amplified and stored on a computer. The resting light intensity measured for each detector was used to normalize all fluorescence measurements. Dye bleaching was corrected using measurements taken in the absence of stimulation. All optical signals are represented as percent changes in fluorescence (∆F/∆F0, where ∆F is the fluorescence light intensity of the stained slice during illumination without evoked activity and ∆F0 is the fluorescence change during neuronal activity). A decrease in fluorescence, associated with membrane depolarization, is plotted as an upward deflection in all figures. Data are displayed as pseudocolor images for visualizing spatiotemporal patterns of activity. Pseudocolor scaling was fixed for all frames in a given figure.

Drug application

Dopamine was used as the endogenous agonist for dopamine receptors. R(+)-6-chloro-7,8-dihydroxy-1-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrobromide (SKF 81297) and R(−)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride (SCH 23390) were used as a selective D1 agonist and antagonist, respectively. Quinpirole and S(−)-eticlopride hydrochloride were used as a selective D2 agonist and antagonist, respectively. All the drugs were applied in bath. After recording control responses, dopamine and dopaminergic agents were bath applied for 4 min prior to acquisition of experimental data; bath application of the drug was then continued for the duration of acquisition of experimental data. For experiments with the dopamine antagonists, the antagonist was present in both the control and agonist-containing solutions. Drugs were stored in frozen stock solution and dissolved in the saline prior to each experiment. Sodium metabisulfite (50 μM) was used to protect against oxidation (Sutor and Ten Bruggencate 1990). Dopamine, SKF 81297, and eticlopride were purchased from Sigma (St. Louis, MO), whereas SCH 23390 and quinpirole were purchased from Tocris Cookson (Ellisville, MO).
Data analysis and statistics

For analyzing data from imaging experiments, a region of interest (ROI) that included visually obvious activity along with a surrounding area with no apparent activity was chosen in the control recording. Peak signal amplitudes (peak ΔF/F, where F is the fluorescence light intensity at the diode without stimulation and ΔF is the fluorescence change after stimulation) of five diodes outside the ROI were averaged to obtain the baseline noise level. Diodes in the ROI that showed peak signal amplitudes above twice that of the baseline noise were selected for analysis. These same diodes were selected for analysis in the image taken after drug application in that experiment. The peak signal amplitudes of these diodes were summed to get the “peak activity.” Lateral spread of activity was estimated by calculating the distance between the two most extreme diodes in the ROI showing activity above twice the baseline noise. Duration of activity was calculated by estimating the time interval between the first and the last frames of an acquisition showing activity. These three parameters were estimated under control conditions and 10 min after drug application in all the imaging experiments.

Statistical comparisons were done using Student’s t-test. P < 0.05 was considered significant. Statistical calculations were done with the help of OriginPro 7.0 software. Data are expressed as mean ± SD.

RESULTS

Dopamine and EPSCs

Whole cell voltage-clamp recordings were obtained from layer II/III pyramidal neurons in the PFC. In the presence of bicuculline (10 μM) to block GABA_A receptor–mediated inhibition, weak intracortical stimulation evoked small amplitude EPSCs. The stimulation level employed did not evoke epileptiform activity under control conditions. Figure 1A shows superimposed traces of 15 EPSCs under control conditions. The initial EPSC was occasionally followed by multiple, smaller amplitude, presumably polysynaptic, EPSCs. Application of 1 μM SKF 81297 increased EPSC amplitude (Fig. 1B), as reported previously (Gonzalez-Islas and Hablitz 2003; Seamans et al. 2001). This was accompanied by the occurrence of late epileptiform discharges of variable latency and amplitude. The responses in Fig. 1, A and B, were averaged and are shown superimposed in Fig. 1C. Similar results were obtained in 7 of 10 cells tested with the D1 agonist SKF 81297 (1 μM) and 8 of 10 tested with 30–60 μM dopamine.

To study the effect of dopamine on threshold for evoking epileptiform discharges, the stimulus intensity was gradually increased during whole cell recording until a late epileptiform discharge appeared. Under control conditions, there was a gradual increase in EPSC amplitude until epileptiform discharges were evoked in an all-or-none fashion. The threshold for generation of epileptiform activity was significantly decreased by 1 μM SKF 81297 (83 ± 4.6% of control; n = 7; P = 0.00006).

Voltage-sensitive dye imaging

Epileptiform discharges are generally thought to represent the synchronous discharge of a local population of neurons (Ayala et al. 1970; Gutnick et al. 1982). An important factor for epileptogenesis is the degree to which a population of neurons becomes synchronized and whether this activity spreads or propagates through the brain. The factors that determine whether a discharge stays localized or spreads are poorly understood. We therefore used imaging techniques to determine if dopamine could influence the spatiotemporal pattern of activation in local neocortical circuits.

A hexagonal photodiode array with 464 diodes was used for imaging. Using a 10× objective, the hexagonal array imaged an area with a width and height of approximately 1.8 mm, covering from pia to white matter (Fig. 2A). With weak intracortical stimulation in the presence of 10 μM bicuculline, there was an initial activation of a small region in cortical layers above the stimulation site (Fig. 2C). Activity then spread to the adjacent cortex before returning to control levels. Following bath application of the D1 receptor agonist SKF 81297 (1 μM), response amplitudes were increased as seen by the appearance of increased number of red pixels (Fig. 2D). The increases were clearly evident in dye signals in representative diodes (Fig. 2B). The “peak activity” (the sum of signal peaks in diodes showing activity; see METHODS) significantly increased (Fig. 5) from 0.1561 ± 0.0631 in controls to 0.2114 ± 0.0920 after SKF 81297 (1 μM) application (n = 11, P = 0.0089). The spatiotemporal spread of activity also was enhanced (Fig. 5). Activity spread laterally to greater distances (589 ± 126 vs. 964 ± 170 μm; n = 11) and persisted for a longer period of time (55 ± 16 vs. 93 ± 35 ms; n = 11) after the application of the D1 receptor agonist (Fig. 5). Both the lateral spread (P = 0.0002) and duration (P = 0.0069) of activity were significantly increased over control. Similar results were obtained in 11 of 23 slices (from 12 animals) tested with SKF 81297 (1 μM). In four of six slices (from 4 animals),
bath application of 60 μM dopamine also produced significant increases in lateral spread, duration, and peak amplitude of evoked activity (Fig. 5). There was a 122% increase in lateral spread of activity (431 ± 128 vs. 956 ± 215 μm; n = 4; P = 0.0021), whereas duration of activity increased by 185% (94 ± 40 vs. 174 ± 61 ms; n = 4; P = 0.0072) after dopamine application; “peak activity” increased from 0.1325 ± 0.0353 to 0.1854 ± 0.0411 (n = 4; P = 0.0003). Increases in activity appeared 6–9 min following bath application of dopamine or SKF 81297. The observed changes were reversible on washing. These findings suggest that the dopamine-induced increases in EPSC amplitudes are associated with enhanced spread of activity in local cortical networks.

In other experiments, activation of dopamine receptors produced a more marked response enhancement resulting in the initiation of a late, large amplitude dye signal (7 of 23 slices tested with 1 μM SKF 81297). This signal presumably is associated with the late synaptic currents (epileptiform discharges) observed in the whole cell recordings (Fig. 1). Control responses in the presence of 10 μM bicuculline are shown in Fig. 3A. Panels are shown at 15-ms intervals. Weak intracortical stimulation, subthreshold for eliciting an epileptiform discharge, produced a small region of activation near the stimulation site. Activity persisted for a mean duration of 65 ± 41 ms. After D1 receptor activation, the same stimulus elicited a larger response that activated a greater area of cortex. This activity was more persistent and additional areas of cortex were recruited. A late, large amplitude, depolarization subsequently occurred and propagated over widespread regions of cortex (Fig. 3B). This activity persisted for a mean duration of 345 ± 181 ms, which is significantly longer (P = 0.0037) than control. The late epileptiform discharge is clearly evident in the signals from individual diodes shown in Fig. 3C. Similar epileptiform discharge after stimulation was observed in one of six slices tested with dopamine. In the presence of the D1 receptor antagonist SCH 23390 (10 μM) (n = 3), the D1 agonist SKF 81297 (1 μM) did not produce a significant change in the spatiotemporal pattern of activity (Fig. 4). Neither the lateral spread (525 ± 130 vs. 625 ± 173 μm; P = 0.3827) nor the duration (101 ± 33 vs. 92 ± 24 ms; P = 0.2254) of activity changed significantly; peak activity did not show a significant change (0.1775 ± 0.0811 vs. 0.1730 ± 0.0876; P = 0.4626) either (Fig. 5). These results indicate that dye signals are constant and reproducible over time and that D1 receptor activation is responsible for the increased excitability in local neocortical circuits.

Application of the D2 receptor agonist quinpirole (10 μM; n = 3 slices from 2 animals) did not significantly alter evoked activity (lateral spread: 400 ± 43 vs. 425 ± 115 μm; P = 0.7418; duration of activity: 71 ± 14 vs. 74 ± 16 ms; P = 0.6666; peak activity: 0.0923 ± 0.0210 vs. 0.0919 ± 0.0150; P = 0.9227), suggesting that D2 receptors are not involved in the enhancement of activity observed in this study (Fig. 5). To see whether D1 receptor activation alone is sufficient for this
enhancement of activity, we applied dopamine (60 μM) in presence of the D2 receptor antagonist eticlopride (5 μM). Significant enhancement of activity similar to that observed with dopamine or SKF 81297 alone was observed (n = 3 slices from 2 animals): lateral spread increased from 325 ± 43 to 875 ± 43 μm (P = 0.0020); activity persisted for a longer period of time (49 ± 5 vs. 101 ± 18 ms; P = 0.0421); peak activity increased from 0.0924 ± 0.0199 to 0.1504 ± 0.0360 (P = 0.0335; Fig. 5).

**DISCUSSION**

These voltage-clamp results show that dopamine enhances evoked EPSC amplitudes in rat PFC layer II/III pyramidal neurons, as described previously (Gonzalez-Islas and Hablitz 2003). In addition, this enhancement was associated with an increase in recurrent EPSPs, which could result in the triggering of epileptiform activity. Using voltage-sensitive dye imaging, we have shown that dopamine enhancement of EPSCs resulted in alterations in the spatiotemporal pattern of activity in local cortical circuits. Dopamine could either enhance activity locally or, if enhancement was strong enough, result in epileptiform events which propagated throughout the slice. Dopamine effects were blocked by SCH 23390 indicating D1 receptor involvement. These results indicate that, in the disinhibited rat neocortex, D1 receptors have significant proconvulsant activity.

*Dopamine and epilepsy*

In vivo studies have shown that dopamine can affect seizures in several different models of epilepsy (see Starr 1996 for review). The reported effects of dopamine receptor activation were variable across seizure models. Most of the early work was done with nonselective dopamine agonists and antagonists. The nonselective dopamine agonist apomorphine primarily had an anticonvulsant effect (Loscher and Czuczwar 1986; Ogren and Pakh 1993; Turski et al. 1988), whereas the nonselective dopamine antagonist haloperidol was proconvulsant in several seizure models (Sato et al. 1980; Turski et al. 1988; Warter et al. 1988). D1 selective agonists had a proconvulsant effect in the pilocarpine model of epilepsy (Al-Tajir et al. 1990; Barone et al. 1990; Burke et al. 1990; Starr and Starr 1993a,b; Turski et al. 1990). SKF 38393, a selective D1 agonist, increased the frequency, severity, and lethality of pilocarpine-induced seizures in rats, effects which were blocked by the selective D1 antagonist SCH 23390 (Al-Tajir et al. 1990; Barone et al. 1990; Turski et al. 1990). Intrahippocampal injection of SCH 23390 increased the threshold for pilocarpine-induced motor seizures and reduced their severity in rats, suggesting a role for hippocampal D1 receptors in lowering seizure threshold (Alam and Starr 1992). Our results are consistent with the findings of these in vivo studies and show that, in disinhibited neocortical slices, D1 receptor stimulation has a proconvulsant effect.

*Fig. 3.* D1 receptor agonist SKF 81297 may trigger epileptiform discharges in disinhibited PFC. Pseudocolor images and dye signals from a representative experiment are shown. Bicuculline (10 μM) was present in bath throughout the experiment. Same strength and duration of intracortical stimulation were used to evoke activity in control condition and after SKF 81297 application. A and B: spatiotemporal pattern of activity evoked by weak intracortical stimulation under control condition and after bath application of D1 agonist SKF 81297 (1 μM) for 10 min, respectively. Each hexagonal panel shows spatial map of activity at a particular time point; panels are separated in time by 15 ms. Warm colors represent higher dye signals and hence, larger amplitude of activity. Pial surface is up in each panel, and pseudocolor scaling is fixed for all panels. C: dye signals from 3 different diodes under control condition (blue trace) and after bath application of D1 agonist SKF 81297 (1 μM) for 10 min (red trace), respectively. Signals represent change in fluorescence over basal fluorescence (ΔF/F) as a function of time. Upward deflection denotes decrease in fluorescence due to depolarization thus reflecting an increase in activity.
Initial in vitro studies showed that low concentrations (1 μM) of dopamine slowed down the rhythmic paroxysmal activity induced in CA1 neurons by low calcium/high magnesium solutions, whereas high concentrations (100 μM) of dopamine accelerated them (Haas et al. 1984). Other studies have shown that bath application of the D1 agonist SKF 38393 decreases low calcium-induced spontaneous epileptiform discharges in CA1 neurons, an effect blocked by pretreatment with the D1 antagonist SCH 23390 (Smialowski 1990). Penicillin-induced epileptiform discharges were also decreased in frequency by dopamine (Suppes et al. 1985). In the neocortex, the duration and frequency of spontaneously occurring epileptiform discharges induced by 4-aminopyridine in magnesium-free external solution were also reduced in a reversible manner by dopamine (Siniscalchi et al. 1997). The dopamine receptor subtype involved was not determined. Similarly, dopamine and the D1 agonist SKF 38393 suppressed zero magnesium-induced paroxysmal discharges in rat cingulate cortex slices (Alam and Starr 1993a). However, dopamine, at low concentrations, was also reported to have a facilitatory effect on the number of secondary depolarizing afterpotentials following initial paroxysmal spikes (Alam and Starr 1994b). The variety in dopamine receptors and their effector mechanisms, coupled with regional heterogeneity in expression, complicates understanding of the dopamine system and may underlie the diversity of effects on epileptiform discharges. The facilitatory effects seen in this study are in agreement with studies on slices of cortical tissue from children undergoing epilepsy surgery where dopamine and the D1 agonist SKF 38393 enhanced NMDA excitatory postsynaptic potentials (EPSPs) and favored emergence of epileptic activity (Cepeda et al. 1999).

**Dopamine-induced changes in spatiotemporal patterns of activity**

Imaging of voltage-sensitive dye signals has emerged as a powerful technique for studying epileptiform activity in brain slices (Demir et al. 1998; Kita et al. 1999; Sutor et al. 1994). Using this technique, it has been shown that, under normal conditions, moderate intensity stimulation can generate “ensemble activity,” a polysynaptic, all-or-none population activity in a local neocortical network (Wu et al. 2001). Spatially restricted epileptiform activity was also observed in neocortex following partial blockade of GABA<sub>A</sub> receptors (Langenstroth et al. 1996). Kita et al. (1999) found that epileptiform responses evoked by subcortical white matter stimulation in the presence of bicuculline were modulated by dopamine agonists. Both D1 and D2 receptor agonists reduced the peak optical response but did not alter the shape or size of the area of cortex responding to stimulation. There recordings were from the ventral rostral aspect of the frontal cortex, and averaged responses were analyzed. In this study, we observed spatially restricted activity in the presence of bicuculline when weak stimulation was used. The spatially restricted activity originated near the site of stimulation and spread rapidly in both directions.
vertical and horizontal directions. Increasing the stimulus intensity evoked epileptiform activity with spatiotemporal characteristics resembling those described previously using voltage-sensitive dye imaging (Albowitz et al. 1998; Demir et al. 1998; Sutor et al. 1994; Wu et al. 2001). Epileptiform activity involved all layers of cortex with the largest amplitude signals occurring in layers II/III, as described previously in rat (Sutor et al. 1994), guinea pig (Albowitz et al. 1990), and human (Albowitz et al. 1998) neocortex.

The spatially restricted enhanced activity seen after D1 receptor activation is likely to reflect recurrent activation via local excitatory axon collaterals. In this study, increased recurrent EPSCs were prominent following application of dopamine or the D1 receptor agonist SKF 81297. Subthreshold depolarization of a population of cells has been suggested to underlie formation of "dynamic ensembles" (Wu et al. 1999). Such assemblies have persistent activation, as also observed here. This long-lasting activity may enhance synchronization, activation of adjacent neurons and initiation of epileptiform activity.

Modulation and epileptogenesis

Prerequisites for the generation of synchronized epileptiform activity observed in vitro include 1) an intrinsic ability of the neurons to generate bursts, 2) powerful excitatory inputs through recurrent collateral connections to other cells in the local circuit sufficient to allow spread and divergence of the burst activity, and 3) an adequate decrease in inhibition in the network of neurons involved (Wong et al. 1984, 1986). The synchronization increases and sharpens, and its latency decreases as the level of disinhibition increases (Traub et al. 1987). These results indicate that an additional important variable in this process may be input from neuromodulatory system and that dopamine can have significant proconvulsant actions, in part by increasing recurrent excitation and enhancing synchronization.

The mechanisms underlying transition from spatiotemporally restricted interictal discharge to a widespread and more prolonged ictal discharge are not clear. Hippocampal modeling studies (Traub et al. 1993a,b, 1994) have suggested that intrinsic membrane properties (rhythmic dendritic bursts mediated by dendritic calcium spikes) along with activation of both AMPA and NMDA receptors may govern the spatiotemporal pattern of epileptic discharges. Any intervention that facilitates one or more of these factors involved in synchronization could facilitate generation and/or spread of epileptiform activity in the network. Previous studies have shown that D1 dopamine receptors potentiate NMDA-mediated excitability increases in neocortical neurons (Wang and O’Donnell 2001). Our results suggest that dopamine has an overall facilitatory effect on local excitatory connections in upper cortical layers. We have shown previously that AMPA and NMDA receptor–mediated EPSCs are both enhanced by D1 receptor activation (Gonzalez-Islas and Hablitz 2003). These results indicate that recurrent EPSCs are also enhanced. The net result of these alterations is a decrease in threshold for stimulus evoked epileptiform discharges and an enhanced susceptibility for increased spatiotemporal spread of epileptiform activity.

Dopamine and local circuit activity

PFC neurons are known to encode working memory by sustaining firing in the absence of afferent input. This persistent firing may arise from recurrent excitation within the local PFC networks (Goldman-Rakic 1995). Dopamine, via D1 receptor–mediated mechanisms, facilitates working memory function by increasing the signal-to-noise ratio, which minimizes the background noise thereby allowing the network to sustain specific task-related activity in the face of distracting inputs (Durstewitz and Seamans 2002; Durstewitz et al. 2000a,b). While extensive horizontal excitatory connections in layers II/III in the PFC may support such recurrent excitation within the local network (Gonzalez-Burgos et al. 2000; Kritzer...
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and Goldman-Rakic 1995), a balance between excitation and inhibition appears to be crucial in initiating and maintaining stable periods of persistent activity (Fellous and Sejnowski 2003; Shu et al. 2003). In this study, when using weak excitation in dis inhibited slices, dopamine increased synchronization in the local excitatory neocortex. When inhibition is intact, such localized increase in activity may stay spatially and temporally restricted by recurrent inhibition (Compte et al. 2000; Constantinidis et al. 2002). Thus dopamine-mediated synchronization of local excitatory networks in the PFC may play a role in physiologic functions like working memory. As the level of excitation increased in this study, the degree of synchronization increased, eventually producing epileptiform activity. Therefore the same dopamine-mediated synchronization can give rise to a pathologic phenomenon when the level of excitation is high and inhibition is low.

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