Age-dependent actions of dopamine on inhibitory synaptic transmission in superficial layers of mouse prefrontal cortex

Kush Paul and Charles L. Cox

Department of Molecular and Integrative Physiology, Department of Pharmacology, Beckman Institute for Advanced Science and Technology, University of Illinois, Urbana, Illinois

Submitted 30 August 2012; accepted in final form 4 December 2012

Paul K, Cox CL. Age-dependent actions of dopamine on inhibitory synaptic transmission in superficial layers of mouse prefrontal cortex. J Neurophysiol 109: 1323–1332, 2013. First published December 5, 2012; doi:10.1152/jn.00756.2012.—Numerous developmental changes in the nervous system occur during the first several weeks of the rodent lifespan. Therefore, many characteristics of neuronal function described at the cellular level from in vitro slice experiments conducted during this early time period may not generalize to adult ages. We investigated the effect of dopamine (DA) on inhibitory synaptic transmission in superficial layers of the medial prefrontal cortex (PFC) in prepubertal [postnatal age (P; days) 12–20], periadolescent (P30–48), and adult (P70–100) mice. The PFC is associated with higher-level cognitive functions, such as working memory, and is associated with initiation, planning, and execution of actions, as well as motivation and cognition. It is innervated by DA-releasing fibers that arise from the ventral tegmental area. In slices from prepubertal mice, DA produced a biphasic modulation of inhibitory postsynaptic currents (IPSCs) recorded in layer II/III pyramidal neurons. Activation of D2-like receptors leads to an early suppression of the evoked IPSC, which was followed by a longer-lasting facilitation of the IPSC mediated by D1-like DA receptors. In periadolescent mice, the D2 receptor-mediated early suppression was significantly smaller compared with the prepubertal animals and absent in adult animals. Furthermore, we found significant differences in the DA-mediated lasting enhancement of the inhibitory response among the developmental groups. Our findings suggest that behavioral paradigms that elicit dopaminergic release in the PFC differentially modulate inhibition of excitatory pyramidal neuron output in prepuperty compared with periadolescence and adulthood in the superficial layers (II/III) of the cortex.

patch-clamp; pyramidal neuron; layer II/III

NÚMEROS DEVELOPMENTAL CHANGES in the nervous system occur during the first several weeks in the rodent lifespan (Andersen et al. 2000; McCutcheon and Marinelli 2009; Tseng and O’Donnell 2007a, b). Considering that most in vitro slice experiments that are used to characterize a variety of intrinsic and synaptic properties of neurons are conducted during this early time period, generalizations put forth from using the younger tissue may not persist in adult tissue. With respect to dopamine (DA)-dependent actions, a limited number of studies have indicated that there are developmental changes in the rat prefrontal cortex (PFC) (McCutcheon and Marinelli 2009). For example, DA, acting via D1 receptors, depolarizes fast-spiking (FS) interneurons in the rat medial PFC (mPFC) from both prepubertal and postadolescent animals. In contrast, D2 receptor activation produces a similar excitatory action only in postadolescent animals (Gorelova et al. 2002; McCutcheon and Marinelli 2009; Tseng and O’Donnell 2007b). The magnitude of D2 receptor-mediated attenuation of excitatory glutamatergic synaptic transmission is greater in adult animals (Tseng and O’Donnell 2007b).

The PFC is typically associated with higher-level cognitive functions, such as working memory and executive decisions (O’Grada and Dinan 2007; Seamans and Yang 2004). This region appears to play a role in the initiation, planning, and execution of various actions, motivation, and cognition (Egan and Weinberger 1997; Goldman-Rakic et al. 1989; Seamans and Yang 2004). Impairment of the PFC has been associated with a number of neurological and psychiatric disorders. The PFC is richly innervated by DA-containing fibers arising from the ventral tegmental area. In this study, we investigated the influence of DA on inhibitory synaptic transmission in mPFC supragranular pyramidal neurons (layer III/IV) from three distinct ages: prepubertal [postnatal age (P; days) 12–20], periadolescent (P30–48), and adult (P70–100). The PFC, like other cortical areas, contains multiple subtypes of GABAergic inhibitory neurons that send their projections to different layers within the cortex and may provide functionally distinct forms of inhibition (Kawaguchi and Kondo 2002; Xu and Callaway 2009). Therefore, we tested if DA indiscriminately modulated inhibitory processes via distinct pathways. In this study, we stimulated distinct inhibitory inputs to the pyramidal neuron by selectively stimulating layer I fibers as well as local inhibitory inputs (Cauler and Connors 1994; Gabbott and Somogyi 1986).

Our investigations found that an early suppression of inhibitory synaptic currents due to D2 receptor activation occurred in prepubertal animals but not in periadolescent and adult animals. In addition, the magnitude of a late-onset facilitation of inhibition due to D1 receptor activation was significantly greater in the prepubertal group. We also found significant differences in the magnitude of DA-mediated actions depending on the pathway of stimulation. These findings indicate that DA produces complex biphasic modulation of inhibitory synaptic transmission in layer III/IV pyramidal neurons of PFC that is modified over development from prepuberty to adolescence.

METHODS

All experimental procedures were carried out in accordance with the National Institutes of Health guidelines and approved by the University of Illinois Animal Care and Use Committee. Mice [FVB; P12–20 (prepubertal), P30–48 (periadolescent), and P70–100 (adult)] were deeply anesthetized with sodium pentobarbital (50 mg/kg) and decapitated. The brain was quickly removed and placed into a cold, oxygenated slicing medium containing (in mM): 2.5 KCl, 10.0...
MgCl₂, 0.5 CaCl₂, 1.25 NaH₂PO₄, 26.0 NaHCO₃, 11.0 glucose, and 234.0 sucrose. Tissue slices (300 μm thickness) were cut in the coronal plane using a vibrating tissue slicer, transferred to a holding chamber, and incubated for at least 1 h before recording. Individual slices were transferred to a submission-type recording chamber on a modified microscope stage and continuously superfused with oxygenated physiological saline at 32°C. A low-power objective (5×) was used to identify layer III/III of the mPFC, and a high-power water-immersion objective (63×) was used to visualize individual pyramidal neurons. The physiological solution used in the experiments contained (in mM): 126.0 NaCl, 2.5 KCl, 1.25 MgCl₂, 2.0 CaCl₂, 1.25 NaH₂PO₄, 26.0 NaHCO₃, and 10.0 glucose. This solution was gassed with 95% O₂/5% CO₂ to a final pH of 7.4.

Whole-cell recordings were obtained with the visual aid of a modified Axioskop 2FS microscope equipped with infrared differential interference contrast optics (Zeiss Instruments, Thornwood, NY). Recording pipettes had tip resistances of 3–6 MΩ when filled with an intracellular solution containing (in mM): 117 K-glucurate, 13 KCl, 1.0 MgCl₂, 0.07 CaCl₂, 0.1 EGTA, 10.0 HEPES, 2.0 Na₂-ATP, 0.4 Na-GTP, and 0.3% biocytin for evoked inhibitory responses. For recordings of spontaneous and miniature inhibitory currents, Cs⁺ was substituted for K⁺ in the recording pipette. The pH was adjusted to 7.3 using CsOH (or KOH), and the osmolarity was adjusted to 290–300 mosM. The initial access resistance ranged from 10 to 25 MΩ and remained stable during the recordings included for analyses.

A Multiclamp 700 amplifier (Molecular Devices, Foster City, CA) was used in voltage-clamp mode for current recordings. Voltage and current protocols were generated using pCLAMP software (Molecular Devices), and data were stored on a computer. In current-clamp recordings, an active bridge circuit was continuously adjusted to balance the drop in potential produced by passing current through the recording electrode. The apparent input resistance was calculated from the linear slope of the voltage–current relationship near rest, obtained by applying constant current pulses ranging from −100 pA to +40 pA (500 ms duration).

Stimulating electrodes were placed in layer I (S1) and deep layer (layer V/VI; S2), and synaptic responses were evoked with constant current pulses (50–400 μA, 100 μs). All evoked and spontaneous inhibitory postsynaptic currents (IPSCs) were recorded in the presence of N-methyl-d-aspartic acid (NMDA) and non-NMDA glutamate receptor antagonists, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP; 10 μM) and DNQX (20 μM), respectively. To obtain isolated layer I stimulation, a scalpel was used to make an incision perpendicular to the pial surface just below the border through the underlying white matter (Caulder and Connors 1994). The stimulating electrode was placed in layer I on the opposite side of the transection from the recording electrode. This preparation, in conjunction with glutamate receptor antagonists, ensures that a monosynaptic, inhibitory pathway is obtained between the stimulating electrode and the recording electrode via layer I. Stimuli to layer I (S1) or deep layers (S2) were delivered alternatively at 10-s intervals.

Agonists were applied by injecting a bolus into the input line of the chamber using a motorized syringe pump. Based on the rates of drug injection and chamber perfusion, the final bath concentration of the agonist was estimated to be one-fourth of the concentration introduced into the flow line (Cox et al. 1995). All concentrations in the manuscript refer to final bath concentration. Control injections of physiological saline did not alter the membrane potential or input resistance, indicating that the temporary increase in flow rate during bolus injection had no effect on the recordings. DA was prepared with 0.08% ascorbic acid to prevent oxidation, and this mix was made fresh every 2–3 h during the course of the experiment. All antagonists were bath applied. All chemicals were obtained from Tocris (Ellisville, MO).

Biocytin (0.3%) was included in the recording pipette in a subpopulation of recordings to confirm the morphology of the recorded. Following these recordings, slices were fixed in 4% paraformaldehyde and incubated overnight in an avidin-biotycin-horseradish peroxidase complex (ABC Elite; Vector Labs, Burlingame, CA) and reacted with dianinobenzidine using established procedures to visualize recorded neurons (Cox and Sherman 2000; Horikawa and Armstrong 1988). Slices were then mounted with Permount, and neurons were examined and photographed using bright-field microscopy.

Data Analyses

For analyses involving evoked synaptic responses, the average of five consecutive responses (evoked at 20-s intervals) was calculated, and different conditions were then compared: pre-drug, drug (DA or agonist), and wash. Statistical analyses of drug effect vs. pre-drug control were done using a paired t-test. For comparison across age groups, the evoked responses were compared between the prepubertal (P12–20) and periadolescent (P30–48) groups within an “early” time period (7–15 min) and “late” time period (19–28 min). Differences between the different age groups during the distinct early or late time periods were assessed using a two-way ANOVA with Tukey-Kramer multiple comparisons test. All data are presented as mean ± SE unless noted otherwise. P values <0.05 were considered statistically significant in all cases.

RESULTS

Whole-cell recordings were obtained from 86 layer III/III pyramidal neurons in mPFC, which included both prelimbic and cingulate regions. Biocytin was included in the recording pipette of a subpopulation of recordings to confirm that neurons had characteristic pyramidal cell morphology. The evoked IPSC recordings were obtained from neurons in tissue slices from prepubertal (P12–20), periadolescent (P30–48), and adult (P70–100) mice. The resting membrane potential of the prepubertal and periadolescent groups was −80.2 ± 0.6 mV and −82.4 ± 3.4 mV, respectively, and did not differ significantly from each other (P > 0.05, t-test). The apparent input resistances of neurons from these two groups were 262.4 ± 11.0 MΩ and 114.2 ± 10.0 MΩ, respectively, and differed significantly from each other (P < 0.05, t-test). The corresponding values for resting membrane potential and apparent input resistance for the adult group were −78.0 ± 1.8 mV and 186.0 ± 32.2 MΩ, respectively.

Differential Actions of DA in Prepubertal and Adolescent Mice

IPSCs were evoked by superficial layer I (S1) or deep-layer/white-matter (S2) stimulation. These different stimulation sites were used to investigate possible differences in DA modulation of inhibitory inputs that arise from different sources. Evoked IPSCs were pharmacologically isolated by carrying out all experiments in the presence of NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid glutamate receptor antagonists CPP (10 μM) and DNQX (20 μM), respectively.

Layer I stimulation. In a representative pyramidal neuron recorded from a prepubertal animal (P13), bath application of DA (50 μM, 4 min) produced an initial suppression of the IPSC evoked by S1 stimulation that recovered within 5 min (Fig. 1A, i and ii). In contrast, in a pyramidal neuron from an older animal (P45), DA produced minimal alteration of the IPSC amplitude during the initial 7–15 min (early; 72.3 ± 4.2% control; n = 9; P < 0.05, paired t-test). In neurons from the P30–48 group, DA also significantly reduced the IPSC amplitude during the early period (7–15 min

J Neurophysiol • doi:10.1152/jn.00756.2012 • www.jn.org

Downloaded from http://jn.physiology.org/ by 10.220.33.3 on May 24, 2017
post-DA: 90.9 ± 5.7% control; n = 9; P < 0.05, paired t-test). The DA-mediated suppression of IPSC in the P12–20 group was significantly greater than that in the P30–48 group (n = 18; F1,7 = 27.76; P < 0.0005, two-way ANOVA). During the late period (19–28 min post-DA), IPSC amplitudes were not altered significantly in either age group (P12–20: 108.0 ± 7.2% control; P30–48: 102.4 ± 8.9% control; P > 0.1, paired t-tests).

A paired-pulse stimulus paradigm was used to test if the alteration in synaptic response is consistent with a presynaptic mechanism. With the use of a 150-ms interstimulus interval, there was a small degree of paired-pulse depression in the baseline condition. In the P12–20 group, the paired-pulse ratio (IPSC2/IPSC1) was unaffected by DA (early phase, pre-drug: 0.92 ± 0.02; DA: 0.90 ± 0.03; n = 7; P > 0.1, paired t-test). Similarly, we found that during the late period (19–28 min), the paired-pulse ratios remained unchanged (pre-drug: 0.83 ± 0.02; DA: 0.88 ± 0.03; n = 7; P > 0.1, paired t-test). In the P30–48 group, the paired-pulse ratio was unchanged during the early period (pre-drug: 0.92 ± 0.02; DA: 0.94 ± 0.05; n = 6; P > 0.1, paired t-test) and late period (pre-drug: 0.92 ± 0.02; DA: 0.90 ± 0.08; n = 6; P > 0.1, paired t-test) following DA application. These results are consistent with a postsynaptic site of action by DA on the IPSC.

Deep-layer stimulation. A second stimulating electrode was placed in the subgranular layers near underlying white matter (S2). As shown in Fig. 1Bi, in a neuron from a P13 animal, DA produced an initial suppression, followed by a longer-lasting facilitation of the IPSC. In a representative neuron from the P30–48 group, DA only produced a lasting facilitation of the IPSC (Fig. 1B, i and ii). In the P12–20 group, DA significantly suppressed the IPSC during the early phase to 83.6 ± 5.3% control (n = 9; P < 0.05, paired t-test), which was followed by a significant, longer-lasting facilitation of the IPSC (123.2 ± 8.9% control; n = 9; P < 0.05, paired t-test). In neurons from the P30–48 group, the IPSC was unchanged during the early phase (100.9 ± 4.3% control; n = 9; P > 0.1, paired t-test) but facilitated significantly during the late phase (114.0 ± 6.0% control; n = 9; P < 0.05, paired t-test). Between age groups, the effects of DA during the early phase differed significantly (Fig. 1Bii; F1,8 = 79.4; P < 0.05, two-way ANOVA); however, the facilitation of the IPSC during the late phase did not differ significantly (F1,8 = 0.09; P > 0.1, two-way ANOVA).

As observed with S1 stimulation, the DA-mediated suppression of the IPSC did not alter paired-pulse depression, which is consistent with a postsynaptic site of action. In neurons from younger animals, paired-pulse depression was unaltered during the early phase (pre-drug: 0.78 ± 0.03; DA: 0.79 ± 0.04; n = 7; P > 0.1, paired t-test) and late phase (pre-drug: 0.78 ± 0.03; DA: 0.75 ± 0.01; n = 7; P > 0.1, paired t-test) of the DA-mediated response. Similarly, in the P30–48 group, the paired-pulse ratio was unaffected by DA (early phase, pre-drug: 0.74 ± 0.03; DA: 0.70 ± 0.05; n = 6; P > 0.1, paired t-test; and late phase, pre-drug: 0.74 ± 0.03; DA: 0.77 ± 0.04; n = 6; P > 0.1, paired t-test).

S1 vs. S2 stimulation. We next analyzed if the magnitude and/or direction of the DA-mediated effect on the IPSC differed depending on the stimulation site—superficial (S1) vs. subgranular (S2) stimulation. In the P12–20 group, the DA-mediated early suppression of the IPSC evoked by S1 and S2

---

**Fig. 1.** Dopamine (DA) modulation of evoked inhibitory postsynaptic currents (IPSCs) is greater in prepubertal mice. **A:** average of 5 consecutive traces in control (gray), 50 μM DA (black), and recovery/late DA phase with superﬁcial layer I (S1) in a cell from a postnatal age 13 days; P13; left) and a P45 (right) animal. **Aii:** timeline response to DA for these neurons. **Aiii:** population data indicate that the IPSC amplitude (Amp.) was signiﬁcantly attenuated in the early phase in the P12–20 group (n = 9) and the P30–48 group (n = 9). The suppression of the IPSC was signiﬁcantly greater in the P12–20 group compared with the P30–48 group. During the “late” phase, the IPSC was enhanced slightly from control levels in both groups but was not signiﬁcantly different from control levels. ***P < 0.0005. **B:** with S2 stimulation in the same 2 neurons, DA produced a decrease in the IPSC response in the P13 animal but had no effect on the P45 animal. **Bii:** overall DA produced a signiﬁcant attenuation of the IPSC during the “early” phase in the P12–20 group (n = 9) but no change from control levels in the P30–48 group (n = 9). This effect was signiﬁcantly greater in the P12–20 group compared with the P30–48 group. During the late phase, DA signiﬁcantly enhanced the IPSC in both age groups, but the respective facilitations were not signiﬁcantly different from each other. ***P < 0.0005.
stimulation did not differ significantly from each other ($F_{1,8} = 3.61; P > 0.05$, two-way ANOVA). In contrast, the D2-mediated facilitation of the IPSC during the late phase differed significantly between stimulation sites ($F_{1,8} = 4.55; P < 0.05$, two-way ANOVA). In the P30–48 group, both early-phase attenuation ($F_{1,8} = 17.43; P < 0.05$, two-way ANOVA) and late-phase enhancement ($F_{1,8} = 4.8; P < 0.05$, two-way ANOVA) of the IPSC by DA differed significantly between stimulation sites.

**D2 Receptor-Mediated Suppression of Inhibition**

**S1 stimulation.** As illustrated by a neuron from a prepubertal animal (P17), the selective D2 receptor agonist quinpirole (10 μM) produced a reversible suppression of the IPSC (Fig. 2A, i and ii). In a neuron from a P43 animal, quinpirole (10 μM) produced a smaller initial suppression of the IPSC (Fig. 2A, i and ii). In the overall population, quinpirole significantly attenuated the IPSC to 89.5 ± 1.7% control ($n = 12; P < 0.05$, paired t-test), but there was no corresponding significant reduction in the P30–48 group (Fig. 2Aiii). During the early phase, there was a significant difference between the P12–20 and P30–48 groups (Fig. 2Aiii; $F_{1,7} = 20.77; P < 0.05$, two-way ANOVA). Whereas the reduction in the IPSC was long lasting and returned slowly to control levels, we found no significant difference between the quinpirole responses and control during the late phase (Fig. 2Aiii).

**S2 stimulation.** In the same neuron from the P17 animal above, quinpirole produced a similar attenuation in the S2-evoked IPSC amplitude that eventually returned to control levels (Fig. 2B, i and ii). In the neuron from the P43 animal, the IPSC was unaltered by quinpirole (2B, i and ii). Overall, quinpirole (10 μM) significantly reduced the IPSC amplitude to 94.3 ± 1.7% control ($n = 12; P < 0.05$, paired t-test) in the P12–20 group, but there was no significant change in the P30–48 group. As predicted, during the early and late phase, attenuation of the IPSC produced by quinpirole in the P12–20 group was significantly different from that in the P30–48 group (Fig. 2Bi; 7–15 min: $F_{1,7} = 34, P < 0.05$; 19–28 min: $F_{1,7} = 8.31, P < 0.05$, two-way ANOVA).

**S1 vs. S2 stimulation.** We next compared the effect of stimulus location on quinpirole response within the two groups. In the P12–20 group, S1 stimulation produced a significantly greater attenuation of the IPSC during the early phase (7–15 min) compared with S2 stimulation ($n = 12; F_{1,7} = 4.38; P < 0.05$, two-way ANOVA). Whereas the peak reductions are similar during the early phase, the suppression onset occurs earlier with S2 stimulation.

**D1 Receptor-Mediated Facilitation of Inhibition**

**S1 stimulation.** We next tested the influence of the selective D1 agonist SKF38393 on the evoked IPSCs. In a neuron from a P16 animal, SKF38393 (10 μM) produced a long-latency enhancement of the IPSC that reached peak magnitude, ~25 min post-DA (Fig. 3A, i and ii). In the population data, SKF38393 produced a facilitation of the IPSC significantly greater than control levels during the late phase (138.4 ± 9.0% control; $n = 7; P < 0.05$, paired t-test) but not during the early phase (Fig. 3Aiii; 103.3 ± 4.6% control; $n = 7; P > 0.1$, paired t-test). In a representative neuron from an older animal (P36), SKF38393 produced a small enhancement of the IPSC (Fig. 3A, i and ii). The small enhancement was significantly greater than baseline during the early phase (114.9 ± 3.9% control; $n = 7; P < 0.05$, paired t-test) but not during the late phase. In the P12–20 group, the paired-pulse ratios were unchanged by SKF38393 application during both the early and late phases.

Fig. 2. Activation of D2 receptors attenuates IPSCs in medial prefrontal cortex neurons. A, i and ii: quinpirole (Quin; 10 μM) produces an attenuation of the IPSC evoked by S1 stimulation in a P17 animal (left) but not in a P43 animal (right). iii: in the overall population, quinpirole significantly attenuated the peak onset IPSC response in the P12–20 group ($n = 12; P < 0.05$, paired t-test) but no significant attenuation in the P30–48 group. The average attenuation in the early phase was significantly greater in the P12–20 group compared with the P30–48 group. ***$P < 0.001$. B, i and ii: in the same 2 neurons as in A, quinpirole similarly attenuated the IPSCs evoked by S2 stimulation in the P17 animal but not the P43 animal. iii: overall, quinpirole significantly attenuated the IPSC in the P12–20 group ($n = 12; P < 0.05$, paired t-test) but not in the P30–48 group. ***$P < 0.001$. J Neurophysiol • doi:10.1152/jn.00756.2012 • www.jn.org
Fig. 3. D1 receptor stimulation produces facilitation of inhibitory responses in prepubertal and periadolescent animals with different timeline dynamics. A, i and ii (left): with S1 stimulation, SKF38393 (10 μM) produced a slow enhancement of the IPSC in a neuron from a P16 mouse. A, i and ii (right): in a neuron from a P36 animal, SKF38393 produced a small, transient increase in IPSC amplitude. Aiii: in the overall population, SKF38393 produced a gradual enhancement of the evoked IPSC in the P12–20 group that was significantly greater than control levels during the late phase. In the P30–48 group (open squares), the IPSC amplitude was significantly greater than the control levels in the early phase. During the early phase, facilitation was significantly greater in the P30–48 group (F1,7 = 9.42; P < 0.005), but no difference was observed in the P12–20 group. During the late phase, the facilitation of IPSC by SKF38393 in the P12–20 group was significantly larger than the P30–48 group (F1,7 = 29.57; P < 0.005). B, i and ii: IPSCs evoked by S2 stimulation in 2 different neurons from P16 and P36 animals. Biii: in the overall population, SKF38393 produced facilitation of the IPSC during the early and later time periods in both age groups. During the early phase, the P30–48 group was significantly larger than the P12–20 group (F1,7 = 112.1; P < 0.005); however, during the late phase, the facilitation in the P12–20 group was significantly larger than the P30–48 group (F1,7 = 135.4; P < 0.005). *P < 0.05; **P < 0.005.

Phase (pre-drug: 0.91 ± 0.04; SKF38393: 0.91 ± 0.02; n = 7; P > 0.1, paired t-test) and late phase (pre-drug: 0.91 ± 0.04; SKF38393: 0.85 ± 0.02; n = 7; P > 0.1, paired t-test). Similarly, in the P30–48 group, the paired-pulse ratios were unaltered by SKF38393 in both the early phase (pre-drug: 0.79 ± 0.04; SKF38393: 0.79 ± 0.07; n = 7; P > 0.1, paired t-test) and late phase (pre-drug: 0.79 ± 0.04; SKF38393: 0.80 ± 0.04; n = 7; P > 0.1, paired t-test).

During the early phase, the facilitation of the IPSC by SKF38393 was significantly greater in P30–48 animals than in P12–20 animals (Fig. 3Aiii; F1,7 = 7.32; P < 0.05, two-way ANOVA), whereas during the late phase, the facilitation of IPSC by SKF38393 was significantly greater in the P12–20 group (F1,7 = 29.57; P < 0.005). *P < 0.05, two-way ANOVA.

S2 stimulation. Similar to the S1 stimulation, SKF38393 produced a long-latency facilitation of the IPSC in the P12–20 group, whereas in the P30–48 group, there was a smaller magnitude, early enhancement of the IPSC, which remained stable throughout the recording (Fig. 3B, i and ii). In the P12–20 group, SKF38393 produced a significant facilitation of the IPSC magnitude during the early phase (Fig. 3Biii, 112.1 ± 3.7% control; n = 7; P < 0.05, paired t-test), which was enhanced further during the late phase (135.4 ± 7.5% control; n = 7; P < 0.05, paired t-test). In the P30–48 group, SKF38393 produced a significant enhancement during the early phase (119.8 ± 6.1% control; n = 5; P < 0.05, paired t-test), but unlike the P12–20 group, this enhancement remained stable during the late phase, (120.0 ± 7.2% control; n = 5; P < 0.05, paired t-test).

In the P12–20 group, the paired-pulse ratios were reduced by SKF38393 in both early phase (pre-drug: 0.82 ± 0.02; SKF38393: 0.76 ± 0.02; n = 7; P < 0.05, paired t-test) and late phase (pre-drug: 0.82 ± 0.02; SKF38393: 0.73 ± 0.03; n = 7; P < 0.05, paired t-test). However, in the P30–48 group, the paired-pulse ratios were unaltered by SKF38393 in both the early phase (control: 0.85 ± 0.12; SKF38393: 0.83 ± 0.16; n = 7; P > 0.1, paired t-test) and late phase (control: 0.85 ± 0.12; SKF38393: 0.85 ± 0.13; n = 7; P > 0.1, paired t-test).

During the early phase, the magnitude of the DA-mediated facilitation of the IPSC in the P30–48 group was significantly greater than the P12–20 group (F1,7 = 9.42; P < 0.05, two-way ANOVA). During the late phase, the facilitation of the IPSC in the P12–20 group was significantly greater than the P30–48 group (n = 7; F1,7 = 8.91; P < 0.05, two-way ANOVA).

S1 vs. S2 stimulation. When comparing the effect of SKF38393 on the IPSCs evoked by S1 and S2 stimulation, we found that in the early phase, the P30–48 group showed significant differences between the two different stimuli (F1,7 = 5.4; P < 0.02, two-way ANOVA), but no difference was observed in the P12–20 group. Similarly, in the late phase, the P30–48 group showed a significant difference in responses evoked by the different stimulus locations (n = 5; F1,7 = 9.8; P < 0.05, two-way ANOVA) but was not significant in the P12–20 group.

Actions of DA in adult mice. We further recorded evoked IPSCs in nine cells from adult mice (P70–100). In four of nine cells, DA produced no effect on the evoked IPSC in either the early or late phase. In the other five neurons, DA produced no...
change during the early phase but a significant enhancement of the IPSC during the late phase. In a representative pyramidal neuron (P93), DA (50 μM, 4 min) produced negligible initial suppression during the early phase with S1 stimulation, followed by a small increase in IPSC amplitude during the late phase (Fig. 4, A and B). Overall, in the P70–100 group, DA did not alter the evoked IPSC in the early phase (Fig. 4C; 98.0 ± 5.4% control; n = 5; P > 0.05, paired t-test). During the late period, the IPSC amplitude was significantly facilitated to 112.0 ± 5.0% control (n = 5; P < 0.05, paired t-test). With deep-layer (S2) stimulation, we observed a similar pattern as with S1 stimulation (Fig. 4, A and B). DA did not alter the IPSC amplitudes during the early phase (Fig. 4C; 100.5 ± 1.7% control; n = 5; P > 0.05, paired t-test), but IPSC amplitude was enhanced significantly during the late phase (112.1 ± 2.7%; n = 5; P < 0.05, paired t-test). These results are similar to that obtained in periadolescent mice (P30–48) and show that the reduction of the D2 receptor-mediated inhibition seen in periadolescent mice is also seen in adult mice.

**DA Enhances Miniature IPSC Activity**

In our experiments with evoked stimuli, we found that the early-phase reduction of evoked IPSC in response to DA application seen in prepubertal animals was attenuated in periadolescent animals. The early suppression could then be followed by a longer-lasting facilitation of the IPSC, which was more evident with S2 stimulation. Our experiments using paired-pulse stimuli revealed that DA did not alter paired-pulse ratios during the suppression, suggesting that this effect does not involve a presynaptic component. As an additional approach to investigate potential pre- and/or postsynaptic contributions to the observed DA-mediated actions, we monitored alterations in miniature IPSCs (mIPSCs) in response to DA agonists.

mIPSCs were recorded from prepubertal (P12–20) animals in the presence of TTX (1 μM), CPP (10 μM), and DNQX (20 μM). Under these conditions, the mIPSC frequency averaged 9.5 ± 0.5 Hz (n = 9) with a mean amplitude of 20 ± 0.4 pA (n = 9). DA (100 μM, 4 min) produced an increase in mIPSC frequency and amplitude that peaked ~12 min post-DA application and returned to baseline within 30 min (Fig. 5, A and B). In this particular neuron, mIPSC frequency was increased from 8.4 Hz to 10.9 Hz and mIPSC amplitude from 22.8 pA to 26.3 pA (Fig. 5B). In the overall population, DA significantly increased mIPSC frequency from 10.8 ± 1.0 Hz to 13.8 ± 1.5 Hz [n = 8; P < 0.0001, Kolmogorov-Smirnov (K-S) test] and mIPSC amplitude from 21.2 ± 1.2 pA to 22.8 ± 1.3 pA (n = 8; P < 0.0001, K-S test). These data suggest that DA increases GABA release from presynaptic axon terminals in the absence of TTX, CPP, and DNQX.

**Fig. 4. DA-mediated suppression of IPSC is absent in adult mice.** A: average of 5 consecutive traces in control (gray), 50 μM DA (black), and recovery/late DA phase with superficial layer I stimulation (S1; left) and deep-layer stimulation (S2; right) from a P73 animal and a P93 animal, respectively. B: time course illustrating the lack of suppression of the IPSC in the presence of DA. C: population data for the P70–100 group (n = 5) shows that DA does not alter the evoked IPSC response during the early phase but significantly enhances the IPSC amplitude during the late phase.

**Fig. 5. DA increases miniature IPSC (mIPSC) frequency (Freq.) and amplitude.** A: current traces from a layer II pyramidal neuron in control and in DA (100 μM). B: time course of DA effect on mIPSC frequency (i) and amplitude (ii). C: time course of population data illustrates the robust effect of DA on mIPSC frequency (i) and amplitude (ii; n = 8). D: DA significantly increased mIPSC frequency by 29 ± 5% (*P = 0.0001, paired t-test; i) and amplitude by 7.4 ± 1% (*P = 0.05, paired t-test; ii).
of presynaptic interneuron activity. However, this appears to be separate from the mechanism underlying the DA-mediated suppression of the IPSC during the early phase, since we did not observe a reduction of mIPSC frequency or amplitude. In a representative neuron, the D1-like receptor agonist SKF38393 (20 μM) significantly increased the mIPSC frequency from 7.4 to 9.1 Hz (Fig. 6A; \( P < 0.05 \), K-S test) and mIPSC amplitude from 20.8 to 22.9 pA (\( P < 0.05 \), K-S test). Overall, SKF38393 significantly increased the mIPSC frequency to 120.6 ± 2.8% control (control: 9.2 ± 1.3 Hz; SKF38393: 11.0 ± 1.4 Hz; \( n = 7 \), \( P < 0.0005 \), K-S test) and mIPSC amplitude to 104.1 ± 1.2% control (control: 19.2 ± 0.7 pA; SKF38393: 20.1 ± 0.8 pA; \( n = 7 \), \( P < 0.005 \), K-S test; Fig. 6D). These data suggest that the D1-like receptors are involved in the enhancement of mIPSC activity in mPFC pyramidal neurons. In contrast, the D2-like receptor agonist quinpirole (20 μM) did not alter the mIPSC activity in mPFC pyramidal neurons (\( n = 3 \)).

Considering SKF38393 enhanced mIPSC activity, we next tested the sensitivity of the DA-mediated changes to the selective D1 antagonist SCH23390. In the presence of SCH23390 (3 μM), DA (100 μM) did not alter mIPSC activity (Fig. 7, A and B). In the population data (Fig. 7D, i and ii), the frequency and amplitude of mIPSCs remained unchanged from control levels (frequency: \( n = 5 \), \( P = 0.6 \), K-S test; amplitude: \( n = 5 \), \( P = 0.3 \), K-S test). This is in striking contrast to the robust increase in mIPSC activity produced by DA (cf. Fig. 5). These data indicate that the increase in mIPSC activity is mediated by D1 receptors. Our data suggest that the presynaptic modulation of GABA by DA in these neurons is due to alterations in D1-mediated release and is separate from the mechanism that produces a decrease in evoked IPSC due to D2 receptor action in prepubertal mice.

**DISCUSSION**

In this study, we observed age-related alterations in dopaminergic modulation of synaptic transmission in layer II/III...
mPFC pyramidal neurons of mice. We also investigated potential differential actions of DA modulation on IPSCs due to distinct afferent inhibitory pathways on the pyramidal neurons. Our results show that in prepubertal animals (P12–20), DA produces an early suppression of the IPSC via activation of D2 receptors. This suppressive action is minimal or absent in periadolescent mice (P30–48). In recordings from adult animals (P70–100), the suppressive action of DA was absent, similar to that observed in periadolescent animals. Furthermore, DA produces a slow onset, long-lasting facilitation of afferent inhibitory activity via the activation of D1 receptors (Kroener and Lavin 2010; Seamans et al. 2001; Seamans and Yang 2004; Trantham-Davidson et al. 2004), and our data show that the magnitude of the facilitatory action is significantly greater in prepubertal animals compared with periadolescent and adult animals. In addition, we found differences in the influence of DA on IPSCs, depending on the site of stimulation, namely, layer I stimulation vs. columnar, white-matter stimulation. Overall, our data indicate not only that DA produces complex actions on inhibitory activity on mPFC neurons but also that through development, these actions change as well.

**D2-Mediated Suppression of IPSC**

A primary finding of this study is that there are significant differences in DA-mediated modulation of inhibitory synaptic neurotransmission between prepubertal and periadolescent mice in layer II/III pyramidal neurons of the mPFC. As is with most in vitro brain slice studies (McCutcheon and Marinelli 2009), cellular studies involving inhibitory neurotransmission in PFC have been conducted predominantly on young rats spanning P15–30 (Gulledge and Jaffe 1998, 2001; Lawtho et al. 1994; Penitsoia et al. 1987; Seamans et al. 2001; Wang et al. 2002). In these neurons, micromolar concentrations of DA produce a complex biphasic effect on inhibitory synaptic responses with an initial decrease in amplitude followed by subsequent faciliation (Seamans et al. 2001). The initial suppression is due to D2 receptor activation, and the latter facilitation is mediated by D1 receptors (Seamans et al. 2001; Seamans and Yang 2004; Trantham-Davidson et al. 2004). In our recordings in layer II/III pyramidal neurons in mice, we observed a similar biphasic response in the prepubertal group. However, in recordings from periadolescent and adult mice, we observed a negligible D2 receptor-mediated suppression of the IPSC amplitude. Our results clearly indicate that suppressive actions of DA are present during younger ages but do not persist through maturation in layer II/III pyramidal neurons. The original findings of the complex biphasic action of DA on evoked IPSC were observed in deep-layer neurons in rat PFC (Seamans et al. 2001; Trantham-Davidson et al. 2004), and this was further confirmed in deep-layer neurons in adult rats (Kroener and Lavin 2010). Therefore, our results suggest that the lack of suppressive action of DA in adolescent and adult mice may be specific to superficial layer pyramidal neurons. The developmental differences in D2 receptor-mediated inhibition that we observe compared with previous studies may be due to either lamina differences (i.e., layer II/III vs. layer V neurons) or perhaps species differences (i.e., mice vs. rats).

Age-related alterations in DA modulation of prefrontal activity have been investigated in a limited number of studies (McCutcheon and Marinelli 2009; Tseng and O’Donnell 2005, 2007b). In one study, in pyramidal neurons from older rats, D2 receptor activation produced a greater suppression of excitatory postsynaptic potential (EPSP) in older animals compared with younger animals (Tseng and O’Donnell 2007a), and this was attributed to increased excitability of interneurons in the older rats (Tseng and O’Donnell 2007b). In our experiments, we found that the DA/D2-mediated suppression of IPSC is significantly less in older mice compared with that in the prepubertal group. Since excitatory and inhibitory inputs provide opposing actions on the membrane potential of pyramidal neurons, our finding suggests that reduced suppression of inhibitory responses due to DA in older animals would promote stronger initial inhibitory actions on the pyramidal neurons. In contrast, in younger animals, greater suppression of inhibitory synaptic inputs in younger animals due to DA action may facilitate concomitant disinhibition of excitatory inputs arriving at the same time. Additional recordings from adult animals (P70–100) also showed a similar lack of suppression-evoked IPSC in the early phase, suggesting that the attenuation of D2-mediated suppression of IPSC lasts through adulthood.

Age-related declines in D2 receptor density have been widely reported in a number of species from rodents to humans in the striatum that implicate both molecular and cellular mechanisms (Antonini and Leenders 1993; Henry and Roth 1984; Iyo and Yamashiki 1993; Rinne et al. 1993; Seemann et al. 1984; Iyo and Yamasaki 1993; Rinne et al. 1993; Seeman et al. 1993). Limited studies have also reported an age-related decline in D2 receptors in the frontal cortex of rats and humans (Kaasinen et al. 2000; Tajuddin and Druse 1996; Valerio et al. 1994). Our results demonstrate that the reduced D2 receptor-mediated reduction in inhibition with development is consistent with declining D2 receptor levels layer II/III pyramidal neurons in the PFC.

**D1-Mediated Enhancement of IPSC**

The activation of D1 receptors produced a lasting facilitation of the IPSC, and there were significant differences between the two age groups: a significantly greater increase in IPSC amplitude in the periadolescent group compared with the prepubertal group during the early phase following D1 agonist application. Our data indicate an increased presynaptic excitability by the increase in mIPSC frequency stimulated by SKF38393, and earlier studies indicate an excitatory action of SKF38393 in cortical interneurons (Seamans and Yang 2004; Tseng and O’Donnell 2007b). Surprisingly, we observed a reversal in the relative evoked, inhibitory amplitudes between prepubertal and periadolescent groups during the late phase that occurs ~20 min post-drug application. During this phase, the inhibition in the prepubertal group was significantly greater than the periadolescent group. Previous studies show that D1 receptor stimulation increases both FS and non-FS interneuron activity in periadolescent animals but only FS activity in the prepubertal group (McCutcheon and Marinelli 2009; Tseng and O’Donnell 2007b). We would predict that due to a prolonged D1 receptor-mediated enhancement of IPSC, the excitatory synaptic responses recorded from pyramidal neurons would exhibit a greater suppressive effect on amplitude in prepubertal animals compared with periadolescent animals in response to the D1 receptor agonist. Furthermore, considering that the larger facilitation of the inhibitory postsynaptic poten-
tials/currents in prepubertal animals could attenuate NMDA receptor-mediated EPSP/Cs, this mechanism may significantly alter NMDA receptor-dependent mechanisms, such as long-term potentiation in prepubertal animals. In other words, the stable and smaller amplitude of IPSC over a time period of tens of minutes in periadolescent animals may facilitate a larger NMDA EPSP/C, which is critical for working memory function. Thus the working memory capacity or duration will be reduced in younger animals.

Modulation of mIPSCs by DA

DA receptors are located both postsynaptically on pyramidal neurons as well as on the axons of GABAergic interneurons in the mPFC (Bergson et al. 1995) and are therefore capable of altering both spontaneous GABA release as well as postsynaptic GABA-mediated IPSCs. The increase in mIPSC frequency by DA, which we observed, appears contradictory with our data, indicating that DA (or SKF38393) did not alter the paired-pulse ratio of evoked IPSCs. At this point, it is difficult to resolve, and the simplest explanation is that there are potentially multiple mechanisms underlying the D1 receptor-mediated facilitation of evoked IPSCs. Based on the lack of alteration in paired-pulse depression, we speculate that the D1-mediated action is postsynaptic; however, we also observed that D1 agonists produced a significant increase in mIPSC frequency. One potential explanation is that DA could increase the excitability of presynaptic terminals without depleting the releasable pool, thereby leading to facilitated IPSCs in response to both paired stimuli. Alternately, the presynaptic change could influence a subpopulation of GABA-containing terminals that were not significantly engaged by the electrical stimulation. It is interesting to note that the only alteration in paired-pulse ratios occurred with S2 (deep-layer) stimulation and SKF38393 application. Although our studies do not point clearly to a single mechanism, we consistently observed a robust facilitation of the evoked IPSC in response to D1 receptor activation that persisted across all ages tested.

Immunohistochemical studies indicate that DA terminals along with glutamatergic or GABAergic terminals form synaptic triadic arrangement on the dendritic spines of layer II pyramidal neurons in the primate mPFC (Goldman-Rakic et al. 1989). In layer III/IV pyramidal neurons in rat mPFC, Zhou and Hablitz (1999) showed that DA increased the frequency and amplitude of spontaneous IPSCs but did not alter mIPSCs. Their data would support the conclusion that there may be no functionally detectable, presynaptic DA receptors on GABA terminals, therefore negating the synaptic triad model proposed by Goldman-Rakic et al. (1989). However, in our experiments, we found that DA significantly increased mIPSC frequency and amplitude in layer III/IV pyramidal neurons in mouse mPFC. Therefore, our data suggest functional presynaptic DA receptors on GABAergic interneuron terminals and support the possibility of the synaptic triad arrangement proposed by Goldman-Rakic et al. (1989). Perhaps there are functional differences between rats and mice. Our results are consistent with the synaptic triad arrangement described in primates. The functional significance of this triadic arrangement is that local modulation of inhibition by DA at distal dendritic sites could occur independent of somatic-driven interneuron activity. This may serve to alter the overall PFC excitability or bias to incoming inputs.

The primary finding in this study shows that DA produces complex biphasic action on inhibitory synaptic transmission that changes through development from prepuberty to adolescence and adulthood in superficial layer III/III pyramidal neurons. Dopaminergic transmission in the PFC is important in a wide range of higher cognitive function and behaviors, such as working memory, executive control, and impulsivity, as well as in pathological conditions, such as schizophrenia, obsessive-compulsive disorder, and autism. The phenotype of these various behaviors and conditions is sculpted by the underlying cellular and synaptic activity in the PFC and the effect of neuromodulatory agents, such as DA, on them. Our results suggest that conclusions from experiments performed only in young animals—although relevant and essential due to developmental aspects of pathological conditions—may not accurately reflect the role of DA on synaptic transmission in all cortical layers (i.e., layer III/IV). In addition, we have shown that the pathway of synaptic input is also relevant for dopaminergic modulation of inhibition.

GRANTS

Support for this research was provided by the National Eye Institute (EY014024), National Institute of Mental Health (MH085324), and National Institute of Child Health and Human Development (HD002274).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: K.P. and C.L.C. conception and design of research; K.P. performed experiments; K.P. and C.L.C. interpreted results of experiments; K.P. and C.L.C. prepared figures; K.P. and C.L.C. drafted manuscript; K.P. and C.L.C. edited and revised manuscript; K.P. and C.L.C. approved final version of manuscript.

REFERENCES


