

D₂ dopamine modulation of corticoaccumbens synaptic responses changes during adolescence

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Abstract

Dopaminergic afferents from the ventral tegmental area (VTA) modulate information processing in the nucleus accumbens (NA), a brain region critical for motivation and reward mechanisms. In NA medium spiny neurons (MSNs) from young rats, D₂ agonists have been shown to decrease the amplitude of corticoaccumbens synaptic responses. As several dopamine-related functions change during adolescence, we assessed the D₂ modulation of cortical inputs with whole-cell recordings in slices obtained from adult and preadolescent rats. The D₂ agonist quinpirole (5 μM) decreased synaptic response of NA MSNs to electrical cortical stimulation in slices from preadolescent rats. In slices from adult rats, however, quinpirole increased both the amplitude of evoked synaptic responses and the frequency of spontaneous synaptic events. These effects were blocked by the GABA-A antagonist picrotoxin (50 μM), revealing a D₂-mediated decrease. These results suggest that D₂ receptors modulate NA neurons differently in young and adult rats, due to the emergence of a D₂-facilitated GABA component in corticoaccumbens responses during adolescence.

Introduction

The nucleus accumbens (NA) is a key brain region in which cortical and limbic inputs are integrated (Sesack & Pickel, 1990; O'Donnell *et al.*, 1999). The NA receives glutamatergic afferents from the prefrontal cortex (PFC), hippocampus and amygdala (Kelley & Domesick, 1982; Brog *et al.*, 1993; Groenewegen *et al.*, 1999), and these inputs converge on individual medium spiny neurons (MSNs; O'Donnell & Grace, 1995; French & Totterdell, 2002, 2003, 2004). In addition to its glutamatergic afferents, the NA receives a dense dopamine (DA) innervation originating in the ventral tegmental area (VTA; Groenewegen *et al.*, 1980) with terminals in close apposition to glutamatergic afferents (Sesack & Pickel, 1990). The interactions among all these inputs may provide a substrate for the 'linking motivation to action' role attributed to the NA (Mogenson *et al.*, 1980).

DA has a complex set of postsynaptic effects on NA neurons. These include depolarization and changes in cell excitability (Akaïke *et al.*, 1987; Uchimura *et al.*, 1989; O'Donnell & Grace, 1996), as well as modulation of glutamatergic afferents. DA consistently reduces the amplitude of corticoaccumbens excitatory postsynaptic potentials (EPSPs), both in slices (O'Donnell & Grace, 1994; Harvey & Lacey, 1996; Nicola *et al.*, 1996; Brady & O'Donnell, 2004) and *in vivo* (Brady & O'Donnell, 2004). Our *in vivo* study revealed that a D₂, not D₁, antagonist blocked the attenuation of corticoaccumbens EPSPs by VTA stimulation in adult animals, but this was evident only during MSN up-states (Brady & O'Donnell, 2004). This suggests that at the negative membrane potential typically observed *in vitro* (equivalent to

the *in vivo* down-state in which no effect of VTA stimulation was observed), D₂ modulation would not be expected in slices from adult animals. Also, as previous *in vitro* studies have been conducted in preadolescent animals, it is possible that the D₂ modulation of corticoaccumbens responses changes during adolescence. Indeed, the NA is not completely mature before five or more weeks of age in rats. Electrophysiological properties of striatal MSNs become stable by postnatal day (PD) 40 in rats (Tepper *et al.*, 1998), and the levels of D₁ and D₂ receptors determined with binding assays increase until PD 28 in the NA (Teicher *et al.*, 1995; Tarazi *et al.*, 1998). Recordings from the PFC have shown that cortical DA actions continue to mature during adolescence (Tseng & O'Donnell, 2005). As puberty in rats typically occurs at PD 35–40 and the subsequent couple of weeks have been defined as adolescence (Spear, 2000), we sought to explore the nature of D₂ modulation of corticoaccumbens synaptic responses before and after this age range. We performed whole-cell recordings from NA MSNs in brain slices obtained from preadolescent (PD 23–38) and developmentally mature (PD 50–63) rats, examining the D₂ modulation of MSN corticoaccumbens synaptic responses.

Materials and methods

All experimental procedures were performed according to the USPHS *Guide for Care and Use of Laboratory Animals* and were approved by the Albany Medical College and the University of Maryland School of Medicine Institutional Animal Care and Use Committees. Young adult (PD 50–63) and preadolescent (PD 23–38) male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were maintained on a 12-h light–dark cycle with food and water available *ad libitum* until the time of recording. Rats were deeply anaesthetized with chloral hydrate (400 mg/kg, i.p.) before decapitation. Brains were

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removed into ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl, 125; NaHCO₃, 25; glucose, 10; KCl, 3.5; NaH₂PO₄, 1.25; CaCl₂, 0.5; and MgCl₂, 3; pH, 7.45; osmolarity 295 mOsm. Parasagittal slices (350 μm thick) with a near 10° angle that preserves corticoaccumbens fibers (O'Donnell & Grace, 1994) were cut on a Vibratome in ice-cold ACSF. Slices were transferred and incubated in warm (35 °C) ACSF solution constantly oxygenated with 95% O₂ and 5% CO₂ for at least 1 h before recording. Slices were then transferred to a submersion-type recording chamber maintained at 33–35 °C and superfused with oxygenated ACSF at a flow rate of 2 mL/min. In the recording ACSF, CaCl₂ was increased to 2 mM and MgCl₂ was reduced to 1 mM.

Whole-cell current- and voltage-clamp recordings were performed from MSNs within the core region of the NA in adult and preadolescent rat brain slices. Patch pipettes (5–10 MΩ) were filled with (in mM): K-gluconate, 115; HEPES, 10; MgCl₂, 2; KCl, 20; Mg-ATP, 2; Na₂-ATP, 2; and GTP, 0.3; pH 7.3, 280 mOsm. Neurobiotin (0.125%) was added to the internal solution for labelling of the recorded cell. All drugs were mixed into oxygenated ACSF and applied to the recording solution in known concentrations. Both control and drug-containing ACSF were oxygenated continuously throughout the experiments. Quinpirole, eticlopride, picrotoxin and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were purchased from Sigma (St Louis, MO, USA).

Nucleus accumbens MSNs were identified under visual guidance using infrared differential interference contrast (IR-DIC) video microscopy (Olympus BX50-WI). The image was detected with an IR-sensitive CCD camera (DAGE-MTI) and displayed on a monitor. Whole-cell current-clamp and voltage-clamp signals were acquired with an Axoclamp-2A amplifier (Axon Instruments, Foster city, CA, USA), digitized with an A/D converter (Digidata, Axon Instruments) and sampled with Axoscope 8.1 (Axon Instruments) at a rate of 10 kHz. Electrode potentials were adjusted to zero before recording without correcting the liquid junction potential (estimated at 10 mV). Postsynaptic potentials were evoked in MSNs by electrical stimulation of corticoaccumbens fibers in the white matter between the rostral PFC and the NA with 0.2–0.9 mA, 0.5 ms current pulses.

Membrane potential, input resistance (measured with the slope of a current–voltage (*I/V*) plot obtained with 500-ms-duration depolarizing and hyperpolarizing pulses) and synaptic potentials evoked by cortical stimulation were measured before and after applying drugs to the bath. Neurons that exhibited a resting membrane potential more depolarized than –70 mV or an input resistance <60 MΩ were excluded from the study. Typically, baseline activity was recorded for 10 min before drug application. Drug-containing solutions were superfused for 5 min. Baseline data were calculated from the average values in the 3-min period preceding drug superfusion and, for the analysis of drug effects, average values were calculated from a 3-min period starting 3 min after administration onset, to allow stabilization of drug levels. Data are expressed as mean ± SD. The statistical significance of the results was assessed by using a Wilcoxon test or a Mann–Whitney test (for paired and unpaired observations, respectively) and repeated-measures ANOVA.

At the end of each experiment, the slices were placed overnight in 4% paraformaldehyde, washed in 0.1 M PBS (pH 7.4), incubated with 0.3% Triton X-100 in PBS, and then incubated with 0.3% hydrogen peroxide for 15 min to block endogenous peroxidases. The avidin–biotin complex method was used to detect Neurobiotin-injected cells (ABC peroxidase kit; Vector Laboratories, Burlingame, CA, USA) and the reaction product was visualized with 3,3'-diaminobenzidine tetrachloride (DAB; Sigma). To visualize tyrosine hydroxylase-containing structures, some slices were re-sectioned to 30 μm using

a freezing microtome. Sections were also washed in 0.1 M PBS (pH 7.4) and incubated with 0.3% hydrogen peroxide for 15 min. After washing in PBS, sections were incubated for 1 h in 10% normal horse serum and 0.3% Triton X-100 in PBS, then incubated overnight at 4 °C in mouse monoclonal antityrosine hydroxylase (1 : 10 000; Sigma-T1299) with 1% normal horse serum. After washing in PBS, sections were incubated with the secondary antibody (biotinylated horse antimouse IgG, 1 : 800; Vector Laboratories, Burlingame, CA) at room temperature for 1 h. The avidin–biotin complex method was used to detect the secondary antibody (ABC peroxidase kit, Vector Laboratories) and the reaction product was visualized with DAB.

Results

Passive membrane properties of MSNs in the NA of preadolescent and adult rats

Ninety-three MSNs from preadolescent rats and 129 MSNs from adult rats were recorded in NA slices. The resting membrane potential was -76.7 ± 3.8 mV (mean ± SD) in preadolescent rats and -76.3 ± 4.0 mV in adult rats; input resistance was 97.1 ± 31 MΩ in preadolescent rats and 99.9 ± 45 MΩ in adult rats (Fig. 1A and B). No evident statistical differences between adult and preadolescent animals could be found for basic membrane properties.

Action potential firing in the NA of adult and preadolescent rats

Firing properties were assessed with 500-ms depolarizing current pulses. In both preadolescent and adult rats, the first action potential (AP) was preceded by a ramp depolarization (Fig. 1A and B), as previously reported (O'Donnell & Grace, 1993). The thresholds for AP firing were -36.9 ± 3.9 and -37.9 ± 4.0 mV in preadolescent and adult rats, respectively. AP amplitude (measured from threshold) was 76.2 ± 6.6 mV in preadolescent and 75.8 ± 5.9 mV in adult rats. AP half-widths were 1.7 ± 0.4 and 1.6 ± 0.4 ms in preadolescent and adult rats, respectively. As the amplitude of depolarizing current injections increased, more APs were evoked. Firing-frequency adaptation was observed as increased interspike intervals between consecutive APs. Instantaneous frequency was calculated from each interspike interval and plotted against the amplitude of depolarizing current injected. As previously described in young rats (O'Donnell & Grace, 1993), firing frequency adaptation was proportional to the current injected in both preadolescent and adult rats (data not shown). Thus, firing properties of NA MSNs did not differ between preadolescent and adult rats.

Synaptic responses of NA MSNs to cortical stimulation in preadolescent and adult rats

EPSPs were evoked by electrical stimulation of the white matter adjacent to the NA and carrying cortical fibers (Fig. 2A). The amplitude of evoked EPSPs ranged from 5.7 to 35.5 mV and, when more depolarizing than the action potential threshold, APs were evoked in both preadolescent and adult rats. The mean maximal EPSP amplitude was not different between young (14.6 ± 6.6 mV) and adult (14.3 ± 6.9 mV) rats. EPSP duration was calculated by measuring the time to decay to half-amplitude. EPSP half-decay was longer in preadolescent rats than in adult rats (20.4 ± 7.9 ms, $n = 44$, in preadolescent rats; 16.4 ± 3.9 ms, $n = 29$, in adult rats; $P = 0.013$). Bath application of the AMPA antagonist CNQX (5 μM) nearly

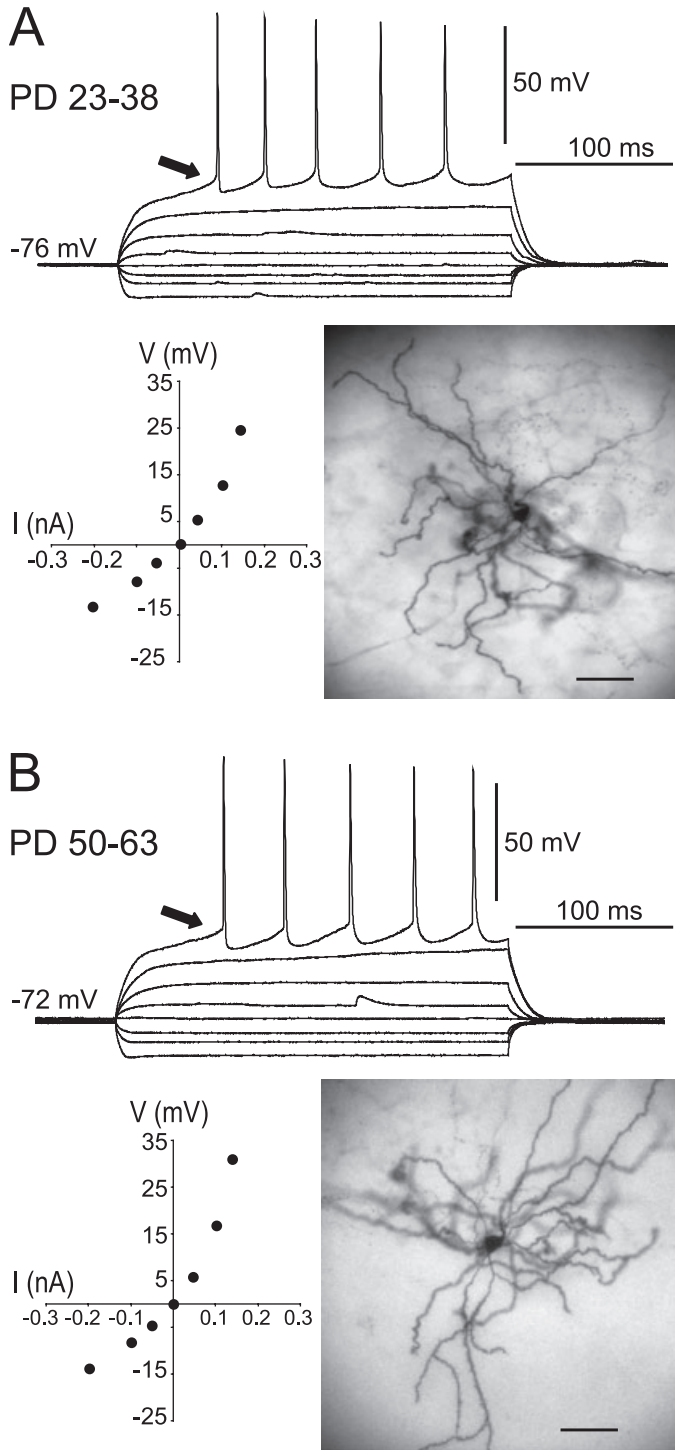


FIG. 1. Basic membrane properties of preadolescent and adult MSNs recorded in the NA. Traces show overlays of whole-cell current-clamp responses to depolarizing and hyperpolarizing current pulses (50-pA steps from -200 to +300 pA, 500 ms duration) in (A) preadolescent and (B) adult rats. Arrows point to the ramp depolarization preceding spikes in both age groups. Bottom left on both panels: I/V plots revealing an inward rectification with depolarizing current pulses, which corresponds to the ramp depolarization indicated by the arrow. Bottom right on both panels: DAB staining of representative MSNs filled with Neurobiotin during recording. Calibration bars, 50 μ m.

abolished EPSPs in both preadolescent and adult rats ($P < 0.001$, repeated-measures ANOVA, $n = 4$ preadolescent and $n = 6$ adult; Fig. 2B and C). These observations show that cortical inputs to NA

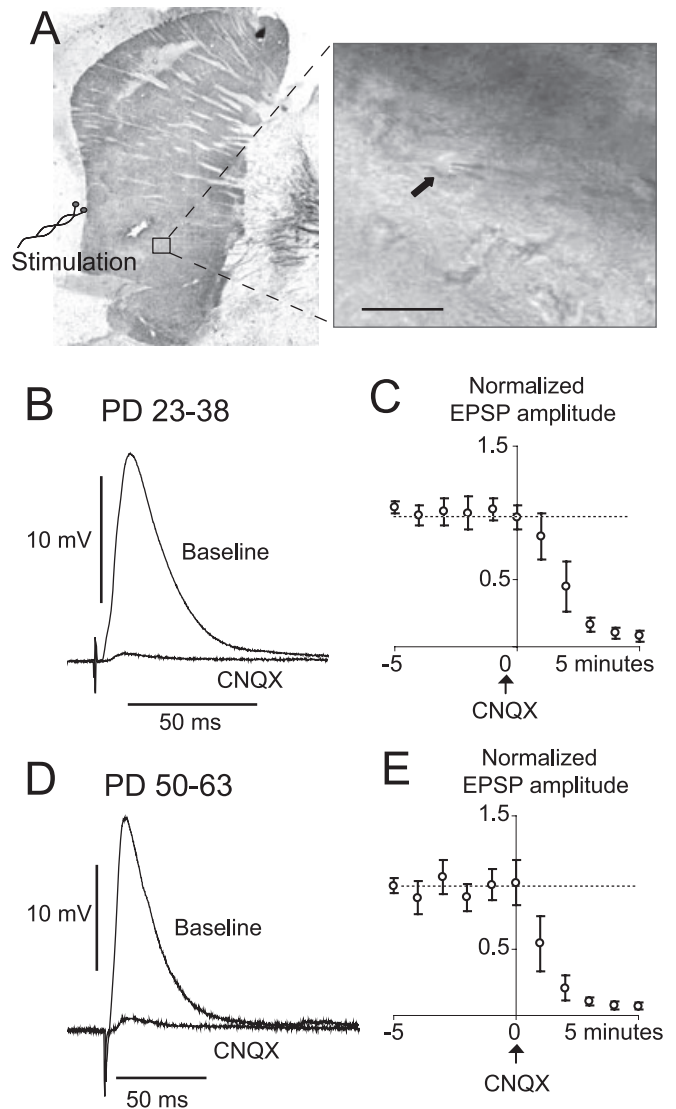


FIG. 2. EPSPs evoked by electrical stimulation of the white matter adjacent to the NA and carrying cortical fibers in preadolescent and adult rats. (A) Tyrosine hydroxylase-stained slice illustrating the position of recording and stimulating electrodes. Inset shows an IR-DIC image of a MSN in the NA (arrow). (B) Representative corticoaccumbens EPSP in a slice from a preadolescent rat showing an overlay of EPSP evoked before (baseline) and after CNQX (10 μ M). Traces are average of five repetitions. (C) Population data of EPSP amplitude normalized to baseline levels. Values are averages per minute. (D) Overlay of representative corticoaccumbens EPSPs in a slice from an adult rat showing blockade with CNQX. (E) Time course of normalized EPSP amplitudes for all the neurons tested. Calibration bar, 25 μ m.

neurons evoked an AMPA response at negative membrane potentials in this preparation in both preadolescent and adult rats. For the rest of the study, stimuli were delivered at an intensity that evoked EPSPs with half of the maximal amplitude, allowing us to observe either increases or decreases in synaptic responses by DA treatment.

D₂ modulation of synaptic responses in preadolescent and adult rats

The effect of D₂ receptor activation on corticoaccumbens EPSPs was determined by measuring EPSP amplitude before and

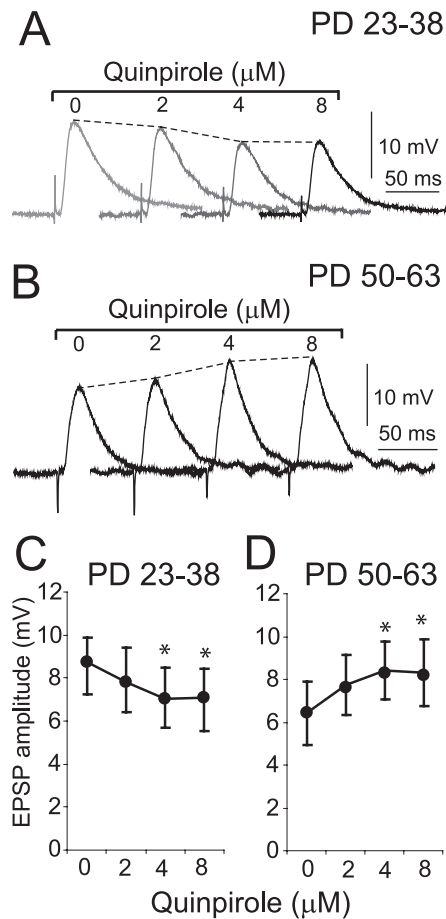


FIG. 3. Dose-dependent effects of a D₂ agonist on corticoaccumbens responses are different in young and adult slices. (A) Examples of corticoaccumbens EPSPs following exposure to different concentrations of quinpirole at 10-min intervals in a representative MSN from a preadolescent rat. Each trace is an average of five repetitions. (B) Representative EPSP with increasing quinpirole concentrations in an MSN from an adult rat. (C) Plot indicating average EPSP amplitude for each concentration of quinpirole in preadolescent rats (mean \pm SD). (D) Plot of EPSP amplitude after quinpirole in adult rats. * $P < 0.02$.

after bath application of quinpirole at 2, 4 and 8 μM in both preadolescent and adult rats. Quinpirole had a significant effect on EPSP amplitude at the two higher concentrations in both groups, with a decrease observed in slices from preadolescent rats (Fig. 3A and C) and an increase in slices from adult rats (Fig. 3B and D). For the rest of the study quinpirole was delivered at 5 μM . In preadolescent rats, 5 μM quinpirole significantly decreased EPSP amplitude from 9.8 ± 1.3 to 8.4 ± 0.9 mV ($P = 0.004$, $n = 5$; Fig. 4A). This decrease was observed in most of the neurons recorded and did not affect the resting membrane potential or input resistance. Switching the bath to ACSF solution did not affect corticoaccumbens EPSPs (Fig. 4A). In adult rats, however, the D₂ agonist increased EPSP amplitude from 6.3 ± 1.2 to 7.9 ± 1.8 mV ($P = 0.005$, $n = 7$; Fig. 4B) without changing input resistance or resting membrane potential, and this effect was blocked by the selective D₂ antagonist eticlopride (20 μM ; Fig. 4B). Thus, D₂ receptors attenuated corticoaccumbens synaptic responses in slices from young rats, as described previously (O'Donnell & Grace, 1994), but in adult rats this modulation changed to an enhancement of EPSP amplitude.

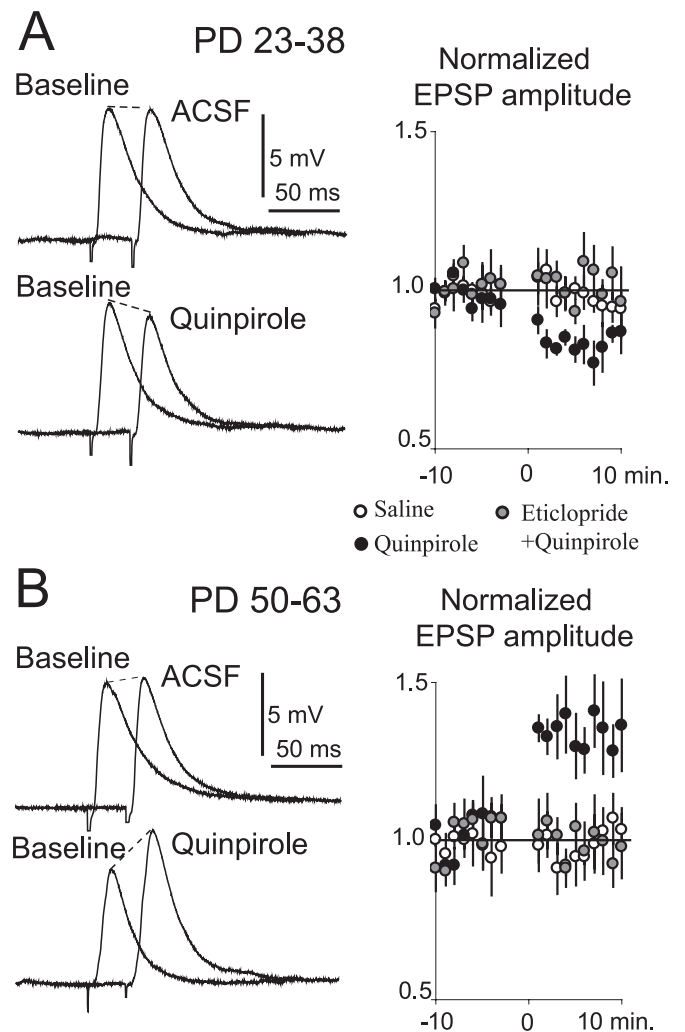


FIG. 4. D₂ modulation of EPSPs. (A) Representative traces of cortico-NA EPSPs before (baseline) and after changing the bath to ACSF (top) or quinpirole (bottom) in slices from preadolescent rats. Each trace is the average of 10 repetitions. The graph to the right plots normalized EPSP amplitude after changing the bath to ACSF (open circles), quinpirole (black circles) or a cocktail of the selective D₂ antagonist eticlopride and quinpirole (grey circles). Values are averages per minute. (B) Representative traces of cortico-NA EPSPs and normalized EPSP amplitude plot in slices from adult rats.

D₂ modulation of adult NA MSNs required GABA

The larger EPSP amplitude observed with D₂ activation in slices from adult compared to preadolescent rats could be due to a change in D₂ effects on NA MSNs or to the recruitment of an additional depolarizing component. Cortical afferents can activate striatal fast-spiking interneurons (Mallet *et al.*, 2005), and a similar interneuron population in the PFC acquires a modulation by D₂ receptors during adolescence (Tseng & O'Donnell, 2006). Thus, it is possible that a late maturation of D₂ actions exists in NA interneurons, yielding a potential contribution of GABA (which at the negative membrane potential recorded in slices would be depolarizing) to the D₂ modulation of corticoaccumbens responses. To investigate the possible role of GABA transmission in the D₂ effects on adult NA MSNs, we performed recordings before and after blocking GABA-A receptors with picrotoxin. EPSP amplitude did not change after bath application of picrotoxin (50 μM) in adult or preadolescent rats (Fig. 5A and B). The

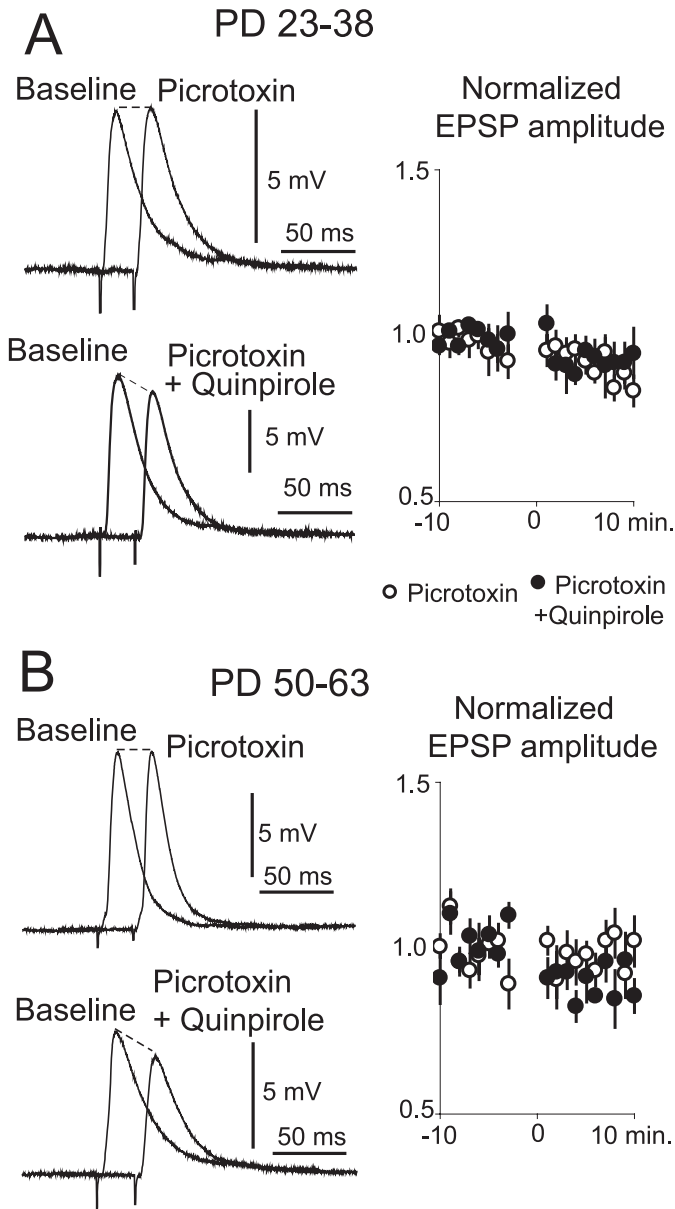


FIG. 5. GABA-A component in the D_2 modulation of corticoaccumbens EPSPs. (A) Representative traces of corticoaccumbens EPSPs before and after changing the bath to picrotoxin (top) or picrotoxin + quinpirole (bottom) in slices from preadolescent rats. Each trace is an average of 10 repetitions. The graph to the right shows normalized EPSP amplitude for each treatment. Values are averages per minute. (B) Similar display of EPSP amplitude and normalized plots obtained with slices from adult rats.

GABA-A antagonist did not affect the D_2 attenuation of EPSPs in slices from preadolescent rats (Fig. 5A). In slices from adult rats in which GABA-A receptors were blocked, quinpirole (5 μ M) decreased EPSP amplitude from 7.4 ± 2.9 to 6.5 ± 2.8 mV ($P = 0.002$, $n = 7$; Fig. 5B), instead of the increase observed without GABA blockade (see Fig. 4B). This decrease was smaller than that elicited by quinpirole in preadolescent rats and was observed in most neurons recorded without changes in resting membrane potential or input resistance. These results suggest that D_2 receptor activation can attenuate corticoaccumbens transmission in both adult and preadolescent rats, but in the adult rat this effect is over-compensated by a D_2 facilitation of a cortically evoked GABA component, which is depolarizing at the negative resting membrane potential of MSNs.

D_2 modulation of spontaneous synaptic events in preadolescent and adult rats

If the D_2 enhancement of corticoaccumbens EPSPs in slices from adult rats involves a GABA-A component, D_2 agonists should enhance GABA synaptic responses in MSNs. We tested this possibility by assessing the effect of quinpirole on spontaneous synaptic events using current-clamp and voltage-clamp recordings. First, the frequency of spontaneous depolarizing postsynaptic potentials (dPSPs) was analysed before and after quinpirole bath application in brain slices from both age groups. Spontaneous depolarizing events were recorded at membrane potential values similar to those observed with the drug treatments by using current clamp, to obtain insight into changes in spontaneous synaptic events that could be compared to the modulation of evoked responses. Baseline frequency of dPSP was similar in adult and preadolescent rats, 7.8 ± 3.5 and 9.1 ± 3.2 Hz, respectively. In slices from adult rats, quinpirole significantly increased dPSP frequency from 7.3 ± 1.5 to 9.8 ± 2.2 Hz ($P = 0.005$, $n = 6$; Fig. 6). Second, we assessed spontaneous synaptic currents recorded while holding the neuron at -70 mV in voltage-clamp conditions. The frequency of spontaneous inward current events was not different between adult and preadolescent rats (4.7 ± 1.4 and 3.8 ± 1.8 Hz, respectively; Fig. 7A and B). The AMPA antagonist CNQX (10 μ M) decreased the frequency of inward synaptic currents from 3.8 ± 1.8 to 0.3 ± 0.2 Hz in preadolescent rats and from 4.7 ± 1.4 to 0.5 ± 0.4 Hz in adult rats (Fig. 7C). After blockade of AMPA currents, bath application of a cocktail of CNQX and quinpirole had no effect in preadolescent slices (Fig. 7A and B), but increased the frequency of spontaneous synaptic currents in slices from adult rats (from 0.5 ± 0.4 to 2.2 ± 1.1 Hz; $P = 0.02$, $n = 5$; Fig. 7B and C). These CNQX-resistant (i.e. nonglutamatergic) and quinpirole-enhanced synaptic currents were abolished by the GABA-A receptor antagonist picrotoxin ($P = 0.03$, $n = 5$; Fig. 7D). Thus, at negative membrane potentials and with AMPA receptors blocked, a D_2 agonist was able to increase the occurrence of GABA-A synaptic currents in MSNs, indicating that D_2 receptors may have recruited local inhibitory synapses in adult rats.

Discussion

We examined basic membrane properties and D_2 responses of NA MSNs in slices obtained from preadolescent and adult rats. Although MSNs recorded before and after adolescence presented similar membrane properties and synaptic responses, there were differences in the effects of a D_2 agonist. Quinpirole attenuated synaptic responses in slices from preadolescent rats but enhanced evoked synaptic responses to cortical stimulation in MSNs from adult rats. Moreover, D_2 activation attenuated corticoaccumbens responses in adult rats in the presence of a GABA antagonist. Glutamate-independent spontaneous inward currents recorded at -70 mV were more frequently observed in the presence of the D_2 agonist, and were blocked by a GABA-A antagonist, but only in slices from adult rats. These results suggest that D_2 receptors modulate MSNs differently in young and adult rats, due to the recruitment of a depolarizing GABA component after puberty.

Basal NA electrophysiological properties

Passive and active membrane properties recorded from NA MSNs were similar across the age groups tested here. MSNs had a resting membrane potential between -70 and -80 mV, displayed a depolarizing ramp preceding action potential in response to current injection, and exhibited inward rectification when hyperpolarized from the

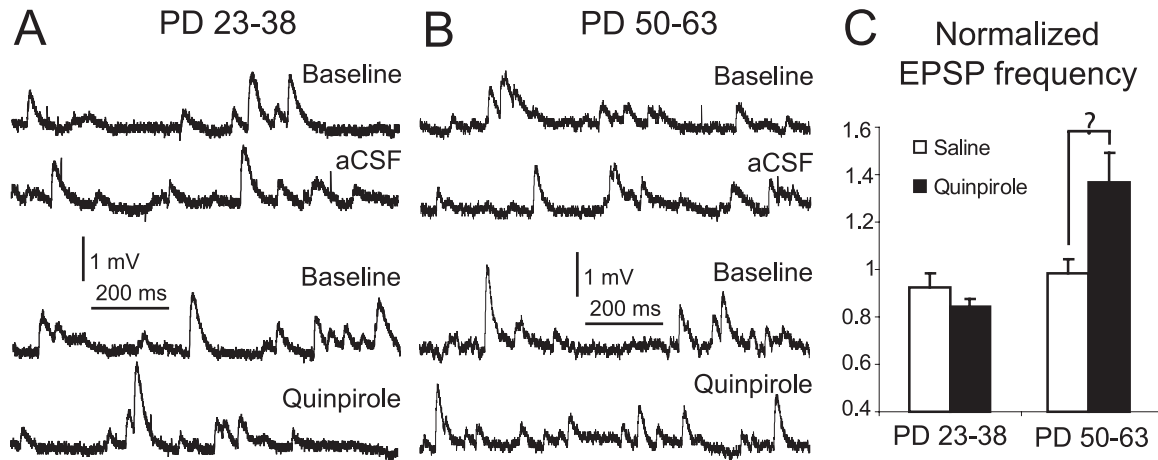


FIG. 6. Spontaneous depolarizing potentials in slices from adult and preadolescent rats. (A) Representative traces of spontaneous dPSPs before and after changing the bath to ACSF (top) or quinpirole (bottom) in slices from preadolescent rats. (B) Similar display of representative traces obtained in slices from adult rats. (C) Bar graph showing normalized frequency of spontaneous dPSP in adult and preadolescent rats after ACSF or quinpirole treatment.

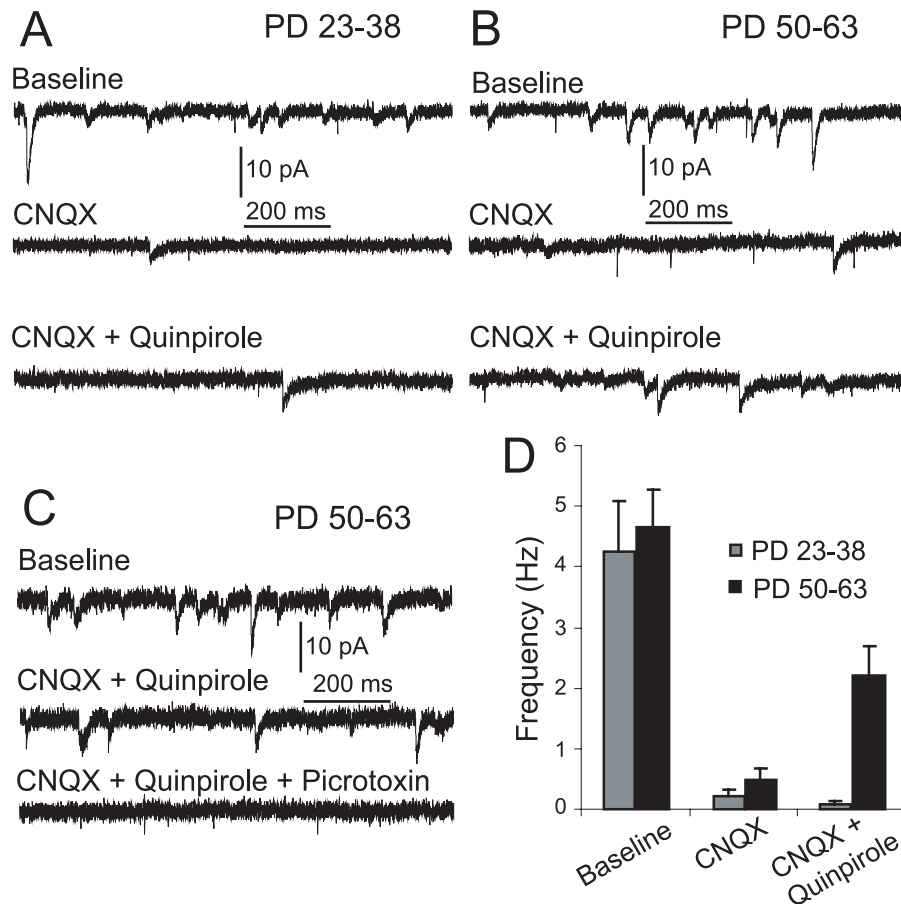


FIG. 7. Effect of D₂ activation on spontaneous inward currents recorded in voltage-clamp at -70 mV. (A) Representative traces of spontaneous inward current events before and after changing the bath to ACSF (top), CNQX (middle) and CNQX + quinpirole (bottom) in slices from preadolescent rats. (B) Representative traces of spontaneous inward currents with similar treatments in slices from adult rats. (C) Representative traces of spontaneous inward currents after changing the bath to ACSF (top), CNQX + quinpirole (middle) and CNQX + quinpirole + picrotoxin (bottom) in slices from adult rats. (D) Bar graph depicting the frequency of spontaneous inward current events in slices from preadolescent and adult rats for each treatment.

resting membrane potential, as described previously in young rats (Yim & Mogenson, 1982, 1988; Yang & Mogenson, 1984; Chang & Kitai, 1986; Kawaguchi *et al.*, 1989, 1995; Uchimura *et al.*, 1989; Kawaguchi, 1992, 1993; Pennartz *et al.*, 1992; O'Donnell & Grace,

1993). The similarity we observed between preadolescent and adult rats regarding MSN membrane properties is consistent with previous studies showing that those properties are acquired during the first three postnatal weeks (Tepper *et al.*, 1998; Belleau & Warren, 2000).

Electrical stimulation in the white matter located between the rostral PFC and NA activated corticoaccumbens fibers, evoking EPSPs that were blocked by the AMPA antagonist CNQX. The recording sites were located ~ 1 mm away from the stimulating site, making it unlikely that current spread directly activated the recorded neuron or other cells in the vicinity. Instead, the small angle of the parasagittal slice allowed preservation of corticoaccumbens fibers. Stimulating and recording electrodes could therefore be separated by long distances and still evoke large-amplitude EPSPs, which exhibited the same amplitude in both groups of animals but faster decay in slices from adult rats. As passive membrane properties were not different between neurons from the two age groups, it is likely that other synaptically evoked factors contributed to the difference. Candidates include a different profile of AMPA and/or NMDA receptor activation in these synapses, with a higher presence of long-lasting NMDA responses in juvenile slices, and differences in voltage-gated channels during development. These results suggest that NA MSNs already exhibit mature membrane properties during puberty but there may be differences in corticoaccumbens synaptic responses.

Differential effect of D₂ receptor activation before and after puberty

In vivo and *in vitro* recordings have shown that DA decreases EPSP amplitude in dorsal and ventral striatal MSNs (Cepeda *et al.*, 1993; O'Donnell & Grace, 1994; Harvey & Lacey, 1996; Levine *et al.*, 1996; Nicola & Malenka, 1997). The receptor subtype involved has been a matter of controversy. Although some studies identified a D₁-like modulation of corticoaccumbens responses (Harvey & Lacey, 1996; Nicola & Malenka, 1997; Brady & O'Donnell, 2004), others revealed a D₂ modulation (O'Donnell & Grace, 1994; West & Grace, 2002; Charara & Grace, 2003; Brady & O'Donnell, 2004). Here, the D₂ modulation of synaptic responses was different between preadolescent and adult rats. Consistent with previous *in vitro* studies conducted in the NA (O'Donnell & Grace, 1994) and striatum (Hsu *et al.*, 1995) from young rats (Sprague–Dawley rats weighing < 300 g are not yet adults), quinpirole decreased the amplitude of cortically evoked EPSPs in preadolescent rats. In slices from adult rats, however, NA synaptic responses to cortical stimulation were enhanced by D₂ activation. Thus, the nature of DA–glutamate interactions in the NA changes during adolescence.

D₂ effects on corticoaccumbens synaptic responses in adult rats involved a GABA component

The D₂ agonist reduced corticoaccumbens EPSPs in slices from young animals but increased EPSP amplitude in slices from adult animals. As basic membrane properties were not affected by quinpirole in any age group, it is possible that this effect is due to a direct modulation of corticoaccumbens responses by D₂ receptors. The pre- or postsynaptic nature of the receptors involved cannot be ascertained with the techniques used here, but there is strong evidence of D₂ heterosynaptic receptors being present in corticostriatal terminals and attenuating glutamate release (Bamford *et al.*, 2004).

The change in D₂ modulation of synaptic responses after adolescence could be attributed to a modification of the corticoaccumbens network. In addition to their glutamatergic afferents, MSNs receive considerable GABA inputs that could also be modulated by DA, contributing to the effects observed in corticoaccumbens EPSPs. We tested this possibility by repeating the experiments in the presence of the GABA-A antagonist picrotoxin. Before adolescence, picrotoxin did not affect the D₂ attenuation of corticoaccumbens EPSPs.

However, GABA blockade in adult rats rendered the D₂ actions similar to those of preadolescent rats, i.e. decreasing EPSP amplitude instead of enhancing it. This suggests that the recruitment of depolarizing GABA responses by D₂ receptors emerges during adolescence, changing the manner in which DA modulates cortical information in the NA. This hypothesis is supported by the finding that spontaneous synaptic events recorded while rendering NMDA receptors ineffective by holding the neuron at -70 mV were reduced but not completely eliminated by blocking AMPA receptors. Furthermore, administration of quinpirole increased the frequency of those glutamate-independent depolarizing events. The inward current events that were AMPA antagonist-resistant and enhanced by quinpirole were GABA-A antagonist-sensitive. The frequency of those events was not increased by quinpirole in preadolescent slices, suggesting that intracortical GABA-A currents can be increased by D₂ activation, but only after adolescence. In addition, the amplitude of cortically evoked EPSPs decayed faster in slices from adult than in slices from preadolescent rats, further suggesting that components other than glutamate are activated in the adult slice. GABA fibers contacting NA neurons could originate from local collaterals from other MSNs or from local inhibitory interneurons (Meredith, 1999). In the dorsal striatum, MSNs send local collaterals that could provide some degree of feedback inhibition when MSNs are activated (Czubayko & Plenz, 2002; Tepper *et al.*, 2004). It is unlikely that local collaterals of neighbouring MSNs are involved in the effects reported here, as they have minimal impact on somatic membrane potential of other MSNs (Tepper *et al.*, 2004). On the other hand, it has been recently shown that cortical stimulation activates dorsal striatal GABA interneurons with a shorter latency than MSNs (Mallet *et al.*, 2005). This neuronal population may be activated in the NA by our cortical stimulation protocol combined with the D₂ agonist, and is likely to more effectively affect somatic membrane potential of MSNs. Thus, D₂ receptors may have a dual action in adult NA networks: on the one hand they directly attenuate corticoaccumbens EPSPs, and on the other hand they enhance feed-forward mechanisms by recruiting GABA interneurons that exert a depolarizing effect onto neighbouring MSNs with negative membrane potentials, but may effectively shunt responses to excitatory inputs when depolarized.

In the slice preparation we used, D₂ receptors may enhance corticoaccumbens EPSPs after puberty by engaging GABA transmission. However, we have also recently reported that D₂ receptors decrease the amplitude of corticostriatal synaptic responses *in vivo* in adult rats (Brady & O'Donnell, 2004). This discrepancy can be resolved if one takes into consideration the membrane potential at which this modulation is tested. Synaptic responses can be attenuated by endogenous DA *in vivo* (via D₂ receptors) only during the depolarized up-state (Brady & O'Donnell, 2004). In our preparation, EPSPs were tested at resting membrane potential (equivalent to the *in vivo* down-state), which is below the chloride reversal potential, causing GABA responses to be depolarizing. *In vivo*, activation of GABA receptors is likely to exert a depolarizing effect during the down-state as well, but this effect would be minimal during up-states that are closer to the chloride reversal potential. Thus, during up-states or under GABA-A blockade in our preparation, the direct D₂ receptor reduction in EPSP amplitude will be the dominant component.

Functional implications

The dopaminergic control of corticoaccumbens activity is critical for a variety of functions. DA cells are known to fire bursts of APs in the presence of reward or reward-predicting stimuli (Schultz, 1998) and may encode reward prediction errors (Tobler *et al.*, 2003). Based on

in vivo recordings, we had proposed that DA bursting sustains ongoing up-states in NA MSNs (bringing the membrane potential closer to threshold) and yet reduces the impact of weak cortical inputs (O'Donnell, 2003; Brady & O'Donnell, 2004). Indeed, VTA stimulation with a train of pulses resembling DA cell bursting depolarizes MSNs to a value close to their up-state, and such depolarization could be sustained for a few hundred milliseconds via D₁ or D₂ receptors (Goto & O'Donnell, 2001). During this depolarization, MSNs rarely fire and incoming PFC inputs are attenuated. This was discussed as a mechanism for filtering irrelevant inputs (Brady & O'Donnell, 2004). The results presented here add another dimension to this network of mesocortical projections. The ability of D₂ receptors to recruit a GABA response in the adult PFC (Tseng & O'Donnell, 2007) and NA (data presented here) contribute to further reducing the impact of irrelevant information in the integration of corticolimbic inputs. Its acquisition during adolescence implies a refinement in this circuitry, and could be a mechanism by which adolescents become better at selecting appropriate behavioural responses to a given context. Conversely, a failure in the periadolescent maturation of interneurons reported here could contribute to the delayed onset of neuropsychiatric conditions in which a dysfunction in GABA transmission has been implicated, such as schizophrenia.

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Abbreviations

ACSF, artificial cerebrospinal fluid; AP, action potential; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; dPSP, depolarizing postsynaptic potential; DA, dopamine; EPSP, excitatory postsynaptic potential; MSN, medium spiny neuron; NA, nucleus accumbens; PD, postnatal day; PFC, prefrontal cortex; VTA, ventral tegmental area.

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