Olanzapine Treatment of Adolescent Rats Alters Adult D2 Modulation of Cortical Inputs to the Ventral Striatum

Julie M. Brooks, PhD; Patricio O’Donnell, MD, PhD; Douglas O. Frost, PhD

Department of Anatomy and Neurobiology (Drs Brooks and O’Donnell), Department of Psychiatry (Drs O’Donnell and Frost), and Department of Pharmacology (Dr Frost), University of Maryland School of Medicine, University of Maryland, Baltimore, Maryland.

Correspondence: Patricio O’Donnell, MD, PhD, Neuroscience and Pain Research Unit, Pfizer, Inc., 610 Main St, Cambridge, MA 02139 (patricio.odonnell@pfizer.com).

Abstract

**Background:** The striatal dopamine system undergoes vast ontogenetic changes during adolescence, making the brain vulnerable to drug treatments that target this class of neurotransmitters. Atypical antipsychotic drugs are often prescribed to children and adolescents for off-label treatment of neuropsychiatric disorders, yet the long-term impact this treatment has on brain development remains largely unknown.

**Methods:** Adolescent male rats were treated with olanzapine or vehicle for 3 weeks (during postnatal day 28–49) using a dosing condition designed to approximate closely D2 receptor occupancies in the human therapeutic range. We assessed D2 receptor modulation of corticostriatal inputs onto medium spiny neurons in the adult ventral striatum using in vitro whole-cell current clamp recordings.

**Results:** The D2/D3 agonist quinpirole (5 µM) enhanced cortically driven medium spiny neuron synaptic responses in slices taken from adult rats treated with vehicle during adolescence, as in untreated adult rats. However, in slices from mature rats treated with olanzapine during adolescence, quinpirole reduced medium spiny neuron activation. The magnitude of decrease was similar to previous observations in untreated, prepubertal rats. These changes may reflect alterations in local inhibitory circuitry, as the GABA-A antagonist picrotoxin (100 µM) reversed the effects of quinpirole in vehicle-treated slices but had no impact on cortically evoked responses in olanzapine-treated slices.

**Conclusions:** These data suggest that adolescent atypical antipsychotic drug treatment leads to enduring changes in dopamine modulation of corticostriatal synaptic function.

Keywords: antipsychotic drugs, olanzapine, rat, adolescence, ventral striatum

Introduction

Adolescence is a critical developmental stage during which corticostriatal circuits and their modulation by dopamine (DA) are still maturing. The protracted developmental trajectory of dopaminergic projections in rodents (Tseng and O’Donnell, 2007; Benoit-Marand and O’Donnell, 2008; Huppe-Gourgues and O’Donnell, 2012) and humans (Casey et al., 2010; Galvan, 2010) makes these circuits especially vulnerable to the effects of psychotropic drug treatment during adolescence. Second-generation atypical antipsychotic drugs (AAPDs), such as olanzapine (OLA), are commonly prescribed for adolescent psychosis...
in accordance with protocols approved by The University of
Swartz, 2008; Olfson et al., 2010). Although studies of adolescent
antipsychotic treatment have increased over recent years, the
primary focus of this research has been on drug efficacy and
tolerance. While even time-limited AAPD therapy can alter
brain developmental trajectories, the long-term consequences
of AAPD treatment of children and adolescents remain largely
unknown.

Recent work using animal models has begun to shed light
on these issues. AAPDIs administered to adolescent rodents
at doses that produce D2 receptor occupancies in the human
therapeutic range result in a variety of long-term behavioral
and neurobiological effects. We (Milstein et al., 2013; Vinish et
al., 2013) and others (Llorente-Berzal et al., 2012) have shown that
adult rats treated with OLA at these doses during adolescence
exhibit abnormalities in learning tasks that are sensitive to DA
function. These rats also exhibit changes in DA transmission
(Milstein et al., 2013; Vinish et al., 2013), glutamate (GLU), and
GABA levels (Xu et al., 2015) and the organization of neuronal
networks (Milstein et al., 2013) in several brain regions that
are mediators of the affected behaviors, including the ventral
 striatum (VS). The VS, which includes the nucleus accumbens
(NAC) and the medial aspect of the dorsal striatum, is defined
by its connectivity with limbic regions, including the medial
prefrontal cortex, ventral hippocampus, and amygdala (Voorn
et al., 2004). DA transmission in the VS is believed to fine tune
the dynamic integration of the diverse GLU inputs that con-
verge upon this region (O’Donnell and Grace, 1994; O’Donnell,
2003; Brady and O’Donnell, 2004), and the nature of this modu-
lation changes dramatically during adolescence. In prepubertal
rats, D2 receptor activation attenuates excitatory postsynaptic
potentials (EPSPs) evoked in NAC medium spiny neurons (MSNs)
by stimulation of corticostriatal afferents. Instead, D2 ac-
tivation in adult rats potentiates cortically evoked EPSPs, and
this response involves recruitment of local GABAergic transmission
(Benoit-Marand and O’Donnell, 2008). We also reported peripu-
bertal changes in D2 receptor modulation of AMPA-driven MSN
These data provided evidence of a maturational change in the
activation of local GABA circuitry by D2 family receptors. Here,
we evaluated the long-term impact of adolescent OLA treatment
on D2 receptor modulation of GLU cortical afferents to the VS
with whole-cell recordings of corticostriatal synaptic responses
in MSNs of the VS of adult rats treated as adolescents with OLA
or vehicle (VEH).

Methods

Subjects

Adult (94–181 days old), male Long-Evans rats (350–450 g),
colony-bred (breeding stock from Charles River Laboratory:
Wilmington, MA) and treated during adolescence, served as
subjects for all experiments. On postnatal day (PD) 7, litters
were culled to 10 to 12 pups. On PD21, rats were weaned and
pair- or triple-housed with same-sex littermates. The rats were
maintained in a temperature- and humidity-controlled environ-
ment on a 12-hour-light/-dark cycle (lights on at 6:30 AM) with
food and water available ad libitum. A subset of rats was ini-
tially housed in a reversed light cycle (lights on at 6:30 PM) but
were given 2 weeks to habituate to the regular light cycle prior
to testing. Animal care and experimentation were performed
in accordance with protocols approved by The University of
Maryland School of Medicine Institutional Animal Care and Use
Committee and consistent with the NIH Guide for the Care and
Use of Laboratory Animals.

Adolescent Drug Treatment

Our drug administration protocols were previously described
(Milstein et al., 2013; Vinish et al., 2013). Briefly, on PD28 to 49,
drinking water was replaced with an aequous solution of OLA in
1 mM acetic acid or VEH. Each day the OLA solution was mixed
fresh at a concentration calculated to deliver a target dose of
7.5 mg/kg/d based on the weight and water consumption of the
rats during the previous 24 hours. This regimen achieved an
average of approximately 96% of the target dose. All subjects
were switched to normal drinking water on PD50. This protocol
was designed to approximate closely D2 receptor occupancies
in the human therapeutic range (Kapur et al., 2003).

Electrophysiology

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.)
and transcardially perfused prior to decapitation with oxygenated
ice-cold artificial cerebral spinal fluid (aCSF) containing (in
mM): NaCl, 125; NaHCO3, 25; glucose, 10; KCl, 3.5 mM; NaH2PO4,
1.25; CaCl2, 0.5 mM; MgCl2, 3 mM; pH 7.4, osmolality 295 mOsm,
constantly oxygenated with 95% O2 and 5% CO2. Parasagittal
slices (300 μm thick) cut at a 10° angle containing the VS and
corticostriatal fibers were sectioned using a Vibratome. Slices
were incubated in oxygenated aCSF warmed to approximately
34°C for at least 1 hour prior to recording. For each experiment,
slices were placed in a submersion-type recording chamber
superfused with oxygenated aCSF at a flow rate of 2 mL/min and
maintained at 33°C to 34°C. Recording aCSF formulations were
adjusted to include 2 mM CaCl2, and 1 mM MgCl2.

Whole-cell current clamp recordings were performed from
MSNs within the VS of brain slices obtained from adult rats
So treated as adolescents with either OLA or VEH. Ventral striatal
MSNs were identified using infrared differential interference
contrast video microscopy (Olympus BX50-WI) using a 40x
water-immersion objective. Visual guidance was obtained with
an IR-sensitive CCD camera (DAGE-MTI) connected to a moni-
tor. Patch pipettes (6–10 MΩ) were made from 1.5-mm O.D. boro-
silicate glass tubing (World Precision Instruments, Sarasota,
FL) and 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; osmolar-
ity 280 mOsm). Neurobiotin (0.125%) was added to the internal
recording solution for histological identification of recorded
cells. Whole-cell recordings were acquired with a computer-
controlled Multiclamp 700B amplifier (Axon Instruments, Foster
City, CA), digitized (Digidata, Axon Instruments), and sampled
with an Axoscope 9.0 (Axon Instruments) at a rate of 10 kHz.
Electrode potentials were adjusted to zero before recording
without correcting the liquid junction potential.

Synaptic responses were evoked in VS MSNs via electrical
stimulation of corticostriatal fiber tracts (0.2–0.9 mA, 0.5-ms
duration) every 15 seconds using a computer programmed Master
8 pulse generator (A.M.P.I., Jerusalem, Israel). EPSPs were evoked
using a bipolar electrode made from a twisted pair of Teflon-
coated tungsten wires (tips approximately 200 μm apart). The
stimulating electrode was placed in the forceps minor, approxi-
mately 500 μm from the recorded cell (Figure 1). The initial
5 min after the seal was broken was used to stabilize recordings.
This was followed by a full assessment of passive membrane
properties, including membrane potential and input resistance

(Baeza et al., 2014) and are often used off-label for a variety of
other neuropsychiatric and behavioral indications (Domino and
Swartz, 2008; Olfson et al., 2010). Although studies of adolescent
antipsychotic treatment have increased over recent years, the
primary focus of this research has been on drug efficacy and
tolerance. While even time-limited AAPD therapy can alter
brain developmental trajectories, the long-term consequences
of AAPD treatment of children and adolescents remain largely
unknown.
(measured using the slope of a current-voltage plot obtained with 500-ms hyperpolarizing and depolarizing pulses) before and after bath application of drugs. Neurons exhibiting a resting membrane potential more depolarized than -70 mV and/or an input resistance <80 MΩ were excluded from analysis. Once a steady baseline was established, activity was recorded for 10 minutes prior to drug application. Solutions containing drugs were superfused for 5 minutes followed by aCSF superfusion. Baseline values for all parameters were calculated by averaging EPSPs over a 2-minute period directly preceding drug application. Similarly, drug effects were calculated by averaging EPSP values during a 2-minute period starting 3 minutes after the onset of drug application to allow the stabilization of drug levels within the recording area. Drugs utilized include the D2 family agonist (-)-quinpirole, the GABA-A antagonist picrotoxin, and the competitive AMPA/kainate receptor blocker 6-cyano-7-nitroquinoxaline-2,3-dione (10 µM) nearly abolished the evoked responses, confirming their glutamatergic nature (Figure 2). To investigate the long-term impact of adolescent OLA treatment on D2 modulation of corticostriatal synaptic activity, we assessed EPSP amplitudes before and after bath application of quinpirole (5 µM). An initial comparison revealed that D2 receptor modulation of corticostriatal synaptic responses differed between VEH- and OLA-treated rats (F1,16=16.438, P =.001). In adult VEH-treated rats, quinpirole administration significantly increased EPSP amplitude by approximately 25% (from 6.9±2.3 mV to 8.6±3.2 mV; P =.018, n =8) (Figure 3a-b), consistent with previous observations in MSNs of untreated adult rats (Benoit-Marand and O’Donnell, 2008). In contrast, quinpirole administration significantly decreased EPSP amplitudes by approximately 18% (from 6.0±3.2 mV to 4.8±2.8 mV; P =.005, n =10) (Figure 3c-d) in MSNs of adult rats treated with OLA during adolescence. This decrease in synaptic response is similar to that obtained previously in MSNs of untreated, prepubertal rats (PD23-38) (Benoit-Marand and O’Donnell, 2008). These data suggest that D2 receptor antagonism during the maturation of the mesolimbic pathway causes long-term changes in DA modulation of corticostriatal synaptic activity, resulting in a prolonged adolescent profile of MSN responses to cortical afferent activation.

Adolescent OLA Treatment Evoked Alterations in D2 Modulation of Corticostriatal Activity Include a GABAergic Component

Previous data from our laboratory indicate that developmental changes in D2 family receptor modulation of corticostriatal synaptic responses in the VS are attributable to the recruitment of a local depolarizing GABAergic mechanism that emerges during adolescence (Benoit-Marand and O’Donnell, 2008). It is possible OLA treatment during this critical period of development inhibits that process, yielding adult MSNs that retain a D2-mediated attenuation of corticostriatal synaptic responses but lack the
Adolescent olanzapine (OLA) treatment alters D2 receptor modulation of medium spiny neuron (MSN) responses to cortical afferent stimulation. (a) Responses before (baseline) and during bath application of quinpirole (±5 μM) in a representative MSN from an adult rat treated with vehicle (VEH) as an adolescent. (b) Group data on excitatory postsynaptic potential (EPSP) amplitudes for individual MSNs from VEH-treated rats are shown in grey. Quinpirole administration increases EPSP amplitude (in this and subsequent figures, data from the cell whose averaged traces are illustrated are indicated by open black circles; group averages are indicated by closed black circles). (c) Average EPSPs in a representative MSN from an adult rat treated with olanzapine (OLA) as an adolescent. (d) Average EPSP amplitudes for individual MSNs from OLA-treated rats. Quinpirole administration decreases EPSP amplitude in adult MSN when the rats had been treated with OLA during adolescence.

Discussion

These experiments demonstrate that OLA treatment during adolescence alters adult D2 receptor modulation of corticostriatal responses. Quinpirole administration significantly enhanced synaptic responses in slices obtained from adult rats treated with VEH on PD28 to 49 but attenuated MSN activation in mature rats treated with OLA during the same epoch. The magnitude of suppression observed in adult OLA-treated rats was similar to that in normal prepubertal rats (Benoit-Marand and O’Donnell, 2008). In the presence of the GABA-A antagonist, picrotoxin, D2 receptor activation attenuated cortically evoked EPSPs in VEH-treated adult rats, as it does in untreated control rats both pre- and postpubertally. In OLA-treated adult rats, the combination of picrotoxin and quinpirole decreased EPSP magnitude, as for D2 receptor activation with quinpirole alone. These data suggest that adolescent OLA treatment alters the developmental trajectory of DA modulation of corticostriatal synaptic function in the VS.
Our data show that D2 receptor antagonism by OLA during this period disrupts the developmental trajectory of DA modulation of corticostriatal responses in MSNs. As no differences in basic membrane properties were observed between MSNs of adult rats treated with OLA and VEH during adolescence, it is unlikely that the impact is on MSN physiology. Indeed, recorded cells from both adolescent treatment groups exhibited resting membrane potentials between -70 mV and -80 mV and input resistances between 93 and 151 MΩ, values similar to those previously described in untreated adult rats (O’Donnell and Grace, 1993). Furthermore, quinpirole alone has no effect on MSN membrane potentials between -70 mV and -80 mV and input resistances between 93 and 151 MΩ, values similar to those previously described in untreated adult rats (O’Donnell and Grace, 1993). These data suggest that D2 receptor antagonism during the maturation of the mesolimbic pathway leads to a prolonged adolescent profile of MSN responses to cortical afferent activation. In the adult rat, D2 receptor activation is thought to enhance MSN synaptic responses to cortical stimulation by engaging fast-spiking, parvalbumin immunoreactive, GABAergic interneurons (Benoit-Marand and O’Donnell, 2008). This is evidenced by the emergence of GLU-independent inward currents in MSNs following quinpirole administration, an effect reversed only by GABA-A receptor blockade (Benoit-Marand et al., 2008). Recruitment of this neuronal population can have a depolarizing effect in target neurons with resting membrane potentials below the K+ equilibrium potential (Koos and Tepper, 1999). Parvalbumin positive interneurons can be activated by corticostriatal inputs (Mallet et al., 2005; Gruber et al., 2009a) and modulate responses in VS to diverse inputs (Calhoon and O’Donnell, 2013). This feed-forward mechanism is intact in VEH-treated control animals, as quinpirole administration enhanced corticostriatal EPSPs and the effect was reversed by the administration of the GABA-A open channel blocker picrotoxin. However, D2 receptor recruitment of depolarizing GABAergic responses was absent in OLA-treated rats as evidenced by quinpirole decreasing, rather than enhancing, EPSP amplitude in MSNs and the persistence of this effect in the presence of picrotoxin. This possibility is consistent with the reduced levels of GABA in the NAc of adult rats treated with OLA as adolescents (Xu et al., 2015). OLA treatment could reduce D2 receptor binding on interneurons or alter the coupling of those receptors to G-proteins. Alternatively, it could increase D2 receptor binding on presynaptic corticostriatal axon terminals that synapse on the interneurons, thus reducing glutamatergic
transmission at those synapses. This hypothesis is consistent with the reduction of GLU levels (Xu et al., 2015) and the overall increase in D2 receptor binding (Vinish et al., 2013) in the NAc of adult rats treated with OLA as adolescents. Thus, D2 receptor-mediated effects on MSN activation are likely to involve local interneuron activation, and adolescent OLA treatment may block the emergence of this mechanism. The result is a striatal circuit in which D2 direct modulation of MSN is not balanced by interneuron recruitment, and the net effect of DA via D2 receptors is to attenuate corticostriatal transmission.

The effect of adolescent OLA treatment on the modulation of corticostriatal synaptic responses by DA reported here is likely to drive a number of behavioral changes. Indeed, adult rats treated with OLA during adolescence exhibit deficits in several behavioral paradigms associated with corticostriatal afferent activation, including working memory and conditioned place preference (Milstein et al., 2013; Vinish et al., 2013). Furthermore, these behavioral changes are accompanied by reductions in DA release and in GABA and GLU levels in the NAc, and disrupted DA and GABA signaling in the PFC (Milstein et al., 2013; Vinish et al., 2013; Xu et al., 2015). Abnormal behavioral outcomes in OLA-treated rats were obtained at 3 to 8.5 months of age (Milstein et al., 2013; Vinish et al., 2013), an age range similar to that showing the electrophysiological changes we report here. The present and previous studies (Llorente-Berzal et al., 2012; Milstein et al., 2013; Vinish et al., 2013; Xu et al., 2015) show that adolescent OLA treatment induces a variety of enduring behavioral and neurobiological effects. Our data suggest that D2 receptor antagonism by adolescent OLA treatment has long-lasting consequences that affect the normal development of corticocumbens synapses, specifically their modulation by DA.

The neurobiological impact of antipsychotic drug therapy can vary depending on the homeostatic effect of the treatment, which can change over the protracted course of brain development. The current study focused specifically on the enduring changes that occur following antipsychotic treatment during adolescence, a period of dynamic change in the DA system. This is not to say that alterations in D2 modulation of corticostriatal synaptic physiology would not occur in rats treated as adults following a similar treatment protocol and posttreatment interval. While we are unaware of any data addressing this exact question, numerous studies have demonstrated that chronic AAPD treatment in adult rats significantly impacts striatal DA, GLU, and GABA neurotransmission (Lieberman et al., 2008). However, those data provide no insight as to the effects of D2 receptor antagonism on substrates particularly vulnerable to DA modulation, as would be the case during adolescence. We also emphasize that the effects of adolescent OLA treatment reported here and in other studies are present long after the cessation of a brief, subchronic (3-week) exposure (Milstein et al., 2013; Vinish et al., 2013; Xu et al., 2015). The enduring effects of APD therapy following time-limited treatment of adults have not, to our knowledge, been systematically investigated.

Corticostriatal interactions are critical for decision making and reward-based learning (Christakou et al., 2004; Cohen et al., 2009). The VS integrates input from multiple afferent pathways, including the prefrontal cortex, hippocampus, and amygdala (Brady and O’Donnell, 2004; Gruber et al., 2009b; Calhoon and O’Donnell, 2013), which convey emotional and contextual information. DA and GABA modulation of this process is critical for the normal function of the VS at maturity. Our data predict that modulation of DA function by adolescent AAPD therapy in humans carries a strong risk of impaired decision-making and reward processing at maturity. Thus, AAPD treatment of adolescents should be used only when absolutely necessary. This consideration underscores the importance of developing new therapeutic strategies that would mitigate these adverse effects.

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Statement of Interest

Patricio O’Donnell is an employee and shareholder of Pfizer, Inc. All work reported here was conducted prior to Dr. O’Donnell joining Pfizer.

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