European Journal of Pharmacology 812 (2017) 256–263

Contents lists available at ScienceDirect

European Journal of Pharmacology

journal homepage: www.elsevier.com/locate/ejphar

Full length article

Early-life risperidone enhances locomotor responses to amphetamine during adulthood

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ARTICLE INFO

Keywords:
Antipsychotic
Dopamine
Catecholamine
Development
Psychostimulant
Stereotypy
Forebrain
Circadian

ABSTRACT

Antipsychotic drug prescriptions for pediatric populations have increased over the past 20 years, particularly the use of atypical antipsychotic drugs such as risperidone. Most antipsychotic drugs target forebrain dopamine systems, and early-life antipsychotic drug exposure could conceivably reset forebrain neurotransmitter function in a permanent manner that persists into adulthood. This study determined whether chronic risperidone administration during development modified locomotor responses to the dopamine/norepinephrine agonist, D-amphetamine, in adult rats. Thirty-five male Long-Evans rats received an injection of one of four doses of risperidone (vehicle, .3, 1.0, 3.0 mg/kg) each day from postnatal day 14 through 42. Locomotor activity was measured for 1 h on postnatal days 46 and 47, and then for 24 h once a week over the next two weeks. Beginning on postnatal day 75, rats received one of four doses of amphetamine (saline, .3, 1.0, 3.0 mg/kg) once a week for four weeks. Locomotor activity was measured for 27 h after amphetamine injection. Rats administered risperidone early in life demonstrated increased activity during the 1 and 24 h test sessions conducted prior to postnatal day 75. Taking into account baseline group differences, these same rats exhibited significantly more locomotor activity in response to the moderate dose of amphetamine relative to controls. These results suggest that early-life treatment with atypical antipsychotic drugs, like risperidone, permanently alters forebrain catecholamine function and increases sensitivity to drugs that target such function.

1. Introduction

Antipsychotic drugs have been commonly used to treat psychotic disorders in adults for several decades (Olfson et al., 2012). More recently, a variety of disorders in children have been increasingly managed with prescriptions of newer, second-generation antipsychotic drugs (Lohr et al., 2015; Olfson et al., 2012, 2015). Over the last two decades throughout Europe and North America, the number of antipsychotic drug prescriptions to children under the age of 14 has increased at a greater rate than that reported for adults (Bachmann et al., 2014; Kalverdijk et al., 2008; Murphy et al., 2013; Olfson et al., 2012). At the same time, the average duration of antipsychotic drug treatment in children has become significantly longer (Kalverdijk et al., 2008).

The most commonly prescribed antipsychotic drug for children is risperidone, which predominantly targets serotonin 5-HT_{2A} and dopamine D_2 receptors, along with dopamine D_3, D_4, adrenergic α_1 and α_2, and histamine H_1 receptors (Mailman and Murthy, 2010). Animal studies have revealed alterations in neural and behavioral functions, especially those linked to dopamine, due to early-life risperidone administration. For example, daily risperidone administration for four weeks up-regulates dopamine D_2 and D_4 receptors in several forebrain regions in juvenile and adult rats but only elevates dopamine D_1 receptors in the nucleus accumbens and caudate putamen in the younger group (Moran-Gates et al., 2007). At a behavioral level, daily risperidone administration between postnatal days 14–42 leads to hyperactivity that lasts for several months after cessation of treatment (Bardgett et al., 2013). These findings raise concerns that early-life risperidone administration could lead to an enhancement in behavioral sensitivity to drugs that target dopamine synapses during adulthood.

This study evaluated the effects of early-life risperidone administration on the locomotor activity produced by the well-characterized psychostimulant, amphetamine, in young adult rats. At a neurochemical level, amphetamine increases dopamine release, blocks dopamine and norepinephrine reuptake, and inhibits monoamine oxidase from disintegrating dopamine in the synapse (see Iversen et al., 2008 for review). Amphetamine is believed to stimulate motor activity via its action in the nucleus accumbens and caudate putamen, with low doses elevating locomotion and higher doses eliciting stereotypy, due to drug effects in each respective brain region (Kelly et al., 1975). Additionally,

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http://dx.doi.org/10.1016/j.ejphar.2017.07.025
Received 13 March 2017; Received in revised form 12 July 2017; Accepted 13 July 2017
Available online 14 July 2017

White and White (2006), using moderate to high doses of amphetamine, revealed a time- and dose-dependent pattern of amphetamine-induced activity, with an increase in locomotor activity observed for 6 h post-administration, succeeded by a temporary hypoactivity that was most marked in their study at 20 h post-administration.

Since risperidone administration during postnatal development elevates forebrain dopamine receptor density (Moran-Gates et al., 2007), it was hypothesized that early-life risperidone administration would significantly augment both the hyper- and hypo-locomotor effects of amphetamine later in life. It was also expected that the administration of the higher doses of amphetamine would elicit stereotypy over the first few hours post-injection, and that this effect would be magnified in rats administered risperidone early in life.

2. Materials and methods

2.1. Subjects

Thirty-five Long-Evans male rats were used. Nine pregnant mothers were purchased from Harlan Bioproducts (Indianapolis, IN) and arrived in the animal facility on gestational day 14. On postnatal day 8, pups were identified by sex, and litters culled to four males. Rats were weaned on postnatal day 21. Upon weaning, rats were housed 2–3 per cage with continuous access to food and water. A schedule of all of the experimental events and corresponding postnatal ages is presented in Table 1. The lights in the housing room were on between 6:00 a.m. and 6:00 p.m. The Northern Kentucky University Institutional Animal Care and Use Committee approved all of the proposed procedures and animal care.

2.2. Drugs

Each of the four risperidone dose groups (vehicle, .3, 1.0, and 3.0 mg/kg of body weight; n = 9, 9, 9, and 8, respectively) contained one male rat from each of the nine litters (one of the rats from the 3.0 mg/kg dose group died at postnatal day 21 due to a failure to gain weight). The doses of risperidone were based on our previous behavioral work (Bardgett et al., 2013; Gannon et al., 2015; Stevens et al., 2016) and reports from others demonstrating the effects of early-life risperidone on neurotransmitter receptor levels (Choi et al., 2009, 2010; Moran-Gates et al., 2007). Beyond these precedents, the 1.0 mg/kg dose was selected because it acutely reduces amphetamine-induced hyperactivity by 50% (a powerful preclinical predictor of antipsychotic drug activity) (Arnt, 1995) and occupies 60–80% of dopamine D_2 receptors in rat forebrain – a degree of receptor blockade associated with antipsychotic drug efficacy in humans (Kapur et al., 2003). Nonetheless, this dose does not consistently produce drug blood levels in adult rats that approximate those observed in adult humans maintained on risperidone (Kapur et al., 2003). With this concern in mind and cognizant that some children undoubtedly receive doses above those recommended even for adults, a 3.0 mg/kg of risperidone was included for study.

Rats were weighed and administered subcutaneous injections of risperidone daily from postnatal day 14 through 42. This developmental period in the rat corresponds to the time between early childhood and late adolescence in humans (Andersen, 2005; Spear, 2000) – ages at which pediatric populations are likely to receive antipsychotic drug treatment (Constantine et al., 2011; Olfson et al., 2012). Given that many young children receive antipsychotic drugs continuously over long periods of time (Constantine et al., 2012; Kalverdijk et al., 2008), the approach used here in rats was intended to mimic prolonged antipsychotic drug exposure during development in humans.

Risperidone was dissolved in a small volume of 10% glacial acetic acid, brought to volume with .9% saline, and adjusted to a pH ~ 6.2 with 1 M sodium hydroxide. Injections were administered at a volume of 2.0 ml/kg of body weight. The National Institute of Mental Health's Chemical Synthesis and Drug Supply program kindly provided the risperidone.

D-amphetamine (Sigma) was dissolved in .9% saline. Amphetamine was injected subcutaneously once a week for four weeks as described below. Four doses of amphetamine were used (saline, .3, 1.0, and 3.0 mg/kg of body weight) at a volume of 1 ml/kg. These doses were based on the work of White and White (2006) that showed respective hyper- and hypo-activating effects of these doses over a 33-h session.

2.3. Locomotor activity

Locomotor activity was measured in a clear polypropylene cage (51 cm long × 26.5 cm wide × 32 cm high) with a wire top and inserted into a *SmartFrame* Cage Rack (Kinder Scientific, Poway, CA). Prior to testing, rats from the four treatment groups were equally divided into three testing squads. Locomotor activity was defined as the number of photobeam breaks recorded during each time bin, which varied from 5 min to one h depending on the experiment.

Locomotor activity was tabulated every 5 min over a 60-min period on postnatal days 46 and 47. Testing occurred between noon and 4:00 p.m. each day. These tests determined if rats administered risperidone early in life were more active several days after the end of the risperidone administration on postnatal day 42, as reported previously (Bardgett et al., 2013).

Over the next two weeks, rats were tested over 24 h to assess

Table 1
Experimental timetable.

Postnatal day	Activity	Description
8		Each litter culled to four males.
14		Begin daily subcutaneous injections with vehicle or risperidone (.3, 1.0 or 3.0 mg/kg). n = 9, 9, 9, or 8 rats per respective group.
21		Wean litters, house rats 2–3 per cage
42		Last injection of risperidone or vehicle
46 and 47	Spontaneous locomotion	Test locomotor activity in each rat for one h each day.
55, 56, or 57	Circadian locomotion	Test locomotor activity in each rat for 24 h on one of the three days listed. Same procedure is repeated one week later on day 62, 63, or 64.
62, 63, or 64	Circadian locomotion	
75, 77, or 79	Locomotor response to amphetamine	Test activity for 30 min prior to and 27 h after subcutaneous injection of saline or one of three doses (.3, 1.0, or 3.0 mg/kg) of amphetamine. During each of the four weeks, each rat tested on only one of the three days listed. On each of the four weeks, the same procedure is conducted except that each rat receives a different amphetamine dose than it did on any of the other weeks.
82, 84, or 86	Locomotor response to amphetamine	
89, 91, or 93	Locomotor response to amphetamine	
95, 97, or 99	Locomotor response to amphetamine	

circadian patterns of locomotor activity, as well as to habituate the rats to the testing environment prior to amphetamine administration. On either postnatal day 55, 56, or 57, each squad of 11–12 rats was tested once for 24 h. Testing began at 1:00 p.m. each day, and activity was tabulated every h. Lights in the locomotor testing room were automatically turned off and on at 6:00 p.m. and 6:00 a.m. respectively. One week later on either postnatal day 62, 63, or 64, each squad of rats was tested once again for 24 h.

Locomotor responses to amphetamine were tested separately in each of the three squads of rats beginning on either postnatal day 75, 77, or 79 (see Table 1). On one of those days and once a week for three weeks thereafter, rats were placed in the locomotor activity chambers for 30 min, and the number of photobeam breaks was recorded. Each rat was then removed from the chamber, received a subcutaneous injection of one of four amphetamine doses, and returned to the chamber for 27 h. By the end of the four weeks of testing, each rat had received all four doses of amphetamine. The order of the amphetamine doses across the four weeks was counterbalanced within each risperidone dose group, such that 2–3 rats from each risperidone group received each amphetamine dose during a given week. The 27 h test period was chosen since White and White (2006) used it to reveal delayed hypolocomotive effects of acute amphetamine administration. Testing began at 1:00 p.m. each day. Locomotor activity was tabulated every h during each 27 h session.

Stereotypy was also assessed for 2 h after amphetamine administration. Each rat was observed for one min every 30 min. The scale reported by Kelly et al. (1975) was used to grade the level of stereotypy observed during each one-min recording period:

0 — asleep or stationary
1 — active
2 — predominantly active with bursts of stereotyped sniffing or rearing
3 — stereotyped activity predominantly sniffing and rearing over a large area of the cage
4 — stereotyped behavior maintained in one location
5 — stereotyped behavior in one location with bursts of gnawing or licking
6 — continual gnawing or licking of the cage bars

The 2-h assessment session was chosen since White and White (2006) reported that stereotypy was most prevalent during the initial two h after administration of a high amphetamine dose.

2.4. Statistical analyses

The data collected over 1 h on postnatal days 46 and 47 were summed and analyzed using a one-way analysis of variance (ANOVA) with risperidone dose serving as a between-subjects factor. For the 24-h locomotor data generated between postnatal days 55–57 and 62–64, the number of photobeam breaks generated every h for the first 5 h was compared using a three-way ANOVA with risperidone dose serving as a between-subjects factor and week of testing and time as within-subjects factors. The locomotor activity generated during the dark phase (6–17 h) and remaining light phase (18–24 h) were summed respectively and the data compared using a two-way ANOVA with risperidone dose and week of testing as respective between- and within-subjects factors.

The total locomotor activity generated over the 30 min prior to amphetamine injection was analyzed using a two-way ANOVA with risperidone dose and week of testing as respective between- and within-subjects factors. The locomotor data gathered over the 27 h after amphetamine injection were first analyzed only in the rats administered vehicle using a two-way ANOVA with amphetamine dose and time serving as respective between- and within-subjects factors. As mentioned above, in an attempt to control for baseline differences in activity between the risperidone groups when analyzing responses to amphetamine, the activity observed in each rat over the first five h after saline injection was subtracted from the activity observed over the same period after injection of each amphetamine dose. These difference scores were compared separately for each amphetamine dose using a two-way ANOVA with risperidone dose and time as respective between- and within-subjects factors. Locomotor data gathered between 6 and 17 h, and 18 and 27 h post-amphetamine were summed over each time period and compared using a two-way ANOVA with risperidone and amphetamine dose as respective between- and within-subjects factors. The stereotypy data recorded after amphetamine injection were summed across the 2 h testing session and compared using a two-way ANOVA with risperidone and amphetamine dose as respective between- and within-subjects factors. All post-hoc testing was performed using Fishers Protected Least Significant Difference test (two-tailed). Significant differences were accepted for $P < .05$.

3. Results

3.1. Locomotor activity **on** postnatal days 46 and 47

After the cessation of risperidone administration on postnatal day 42, locomotor activity was recorded every 5 min for 1 h on postnatal days 46 and 47. On postnatal day 46, locomotor activity was significantly greater in rats administered the highest dose of risperidone, $F(3, 31) = 2.94$, $P = .05$ (Fig. 1). Rats administered the highest risperidone dose early in life demonstrated greater activity relative to rats administered vehicle or the lowest risperidone dose (Fishers Protected Least Significant Difference test used in this and all subsequent post-hoc analyses, $P = .01$ for each comparison).

3.2. Circadian locomotor activity

Locomotor activity was recorded for 24 h once for each rat between postnatal days 55–57 and once again between postnatal days 63–65 to assess whether early-life risperidone altered the circadian pattern of locomotor activity during adulthood. Each week, activity was analyzed as a function of three distinct periods across the 24-h test cycle – initial light phase (0–5 h), dark phase (6–17 h), and remaining light phase (18–24 h). This approach allowed for taking into account the effects of the initial novelty of the test cage on activity as well as for allowing for consistent comparisons with the 27-h amphetamine testing, which was analyzed as a function of the same three time periods.

During the first week of 24-h testing, there was a significant interaction between risperidone administration and time across the first five h of the session, $F(12, 124) = 2.62$, $P = .004$ (Fig. 2A). Post-hoc

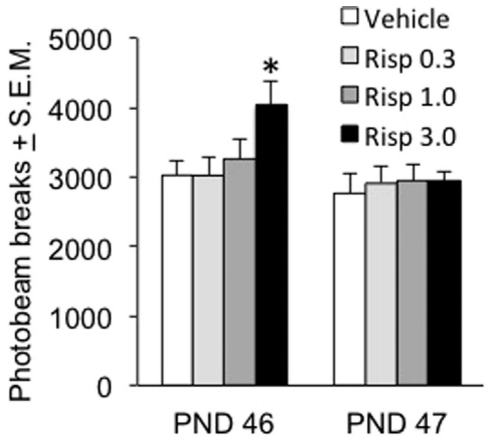

Fig. 1. Locomotor activity summed over 1 h on postnatal days (PND) 46 and 47. For PND 46, the differences between risperidone (Risp) 3.0 group relative to the vehicle and Risp .3 groups are indicated by *. n = 9 per group except Risp 3.0 where n = 8. Data represent means ± S.E.M.

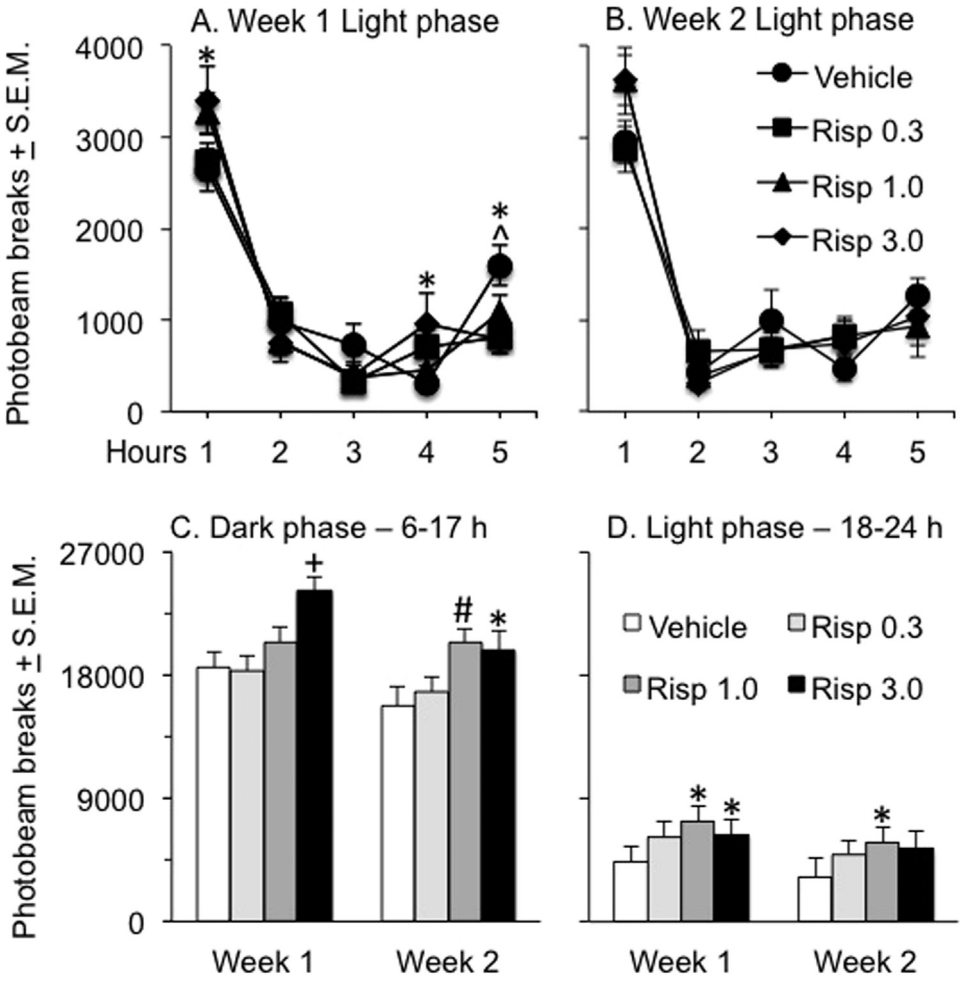

Fig. 2. Locomotor activity recorded during the first 5 h between postnatal days 55–57 (A.) and 62–64 (B.). Differences between vehicle and risperidone (Risp) .3 groups are indicated by ^ and between vehicle and risperidone 3.0 groups by *. Locomotor activity during the dark phase of testing between 6 and 17 h (C.) and remaining light phase between 18 and 24 h (D.). In C. and D., differences between indicated group and vehicle noted by *, between indicated group and vehicle and Risp .3 groups by #, and between Risp 3.0 group and all other groups by +. n = 9 per group except Risp 3.0 where n = 8. Data represent means ± S.E.M.

analyses revealed the risperidone 3.0 group was significantly more active than the vehicle group during the first and fourth h of testing (P = .05 and .04, respectively), while the latter group was significantly more active than the risperidone .3 and 3.0 groups during the fifth h (P = .006 for each post-hoc comparison). During the second week of testing, there was no interaction between risperidone administration and h, or main effect of risperidone administration, but there was a significant time effect, $F(4, 124) = 118.14$, $P < .0001$ (Fig. 2B).

During the dark phase of testing between six and 17 h, there was no significant interaction between risperidone administration and time found in either week's data. Therefore, the data were collapsed into total activity for each risperidone dose group as a function of week of testing. This data analysis revealed a significant group by week interaction, $F(3, 31) = 3.02$, P = .04 (Fig. 2C). In the dark phase during the first week, rats in the risperidone 3.0 group were significantly more active than rats in all other groups (P = .001, .0009, and .03, in comparison to the respective vehicle, risperidone .3, and risperidone 1.0 groups). During the second week of testing, rats in the risperidone 1.0 group were significantly more active than rats in the vehicle and risperidone .3 groups (P = .007 .03, respectively), and rats in the risperidone 3.0 group were more active than the vehicle rats (P = .02). The vehicle and risperidone 3.0 groups were significantly less active during the second week of testing as compared to the first week (P = .03 and .007, respectively).

In the remaining light phase (18–24 h) of the 24-h tests, there were no significant time by risperidone dose interactions – accordingly, the data were summed over time and compared between the risperidone groups. This approach revealed significant main effects of risperidone administration, $F(3, 31) = 3.29$, P = .03, and week, $F(1, 31) = 20.68$, $P < .0002$, but not a risperidone administration × week interaction (Fig. 2D). Rats in the risperidone 1.0 and 3.0 groups were significantly more active than the vehicle rats during the first week of testing (P = .004 and .05, for each respective comparison). In the second week, the risperidone 1.0 group was significantly more active than the vehicle group (P = .02), with a trend towards greater activity in the risperidone 3.0 group relative to vehicle group (P = .06). Rats in every group except the risperidone 3.0 group demonstrated significant declines in activity across the two weeks of testing (P = .01, .03, and .04 for the respective vehicle, risperidone .3, and risperidone 1.0 comparisons).

3.3. Locomotor responses to amphetamine

During the four subsequent weeks, locomotor activity was tested for 30 min prior to amphetamine injection. Analysis of these pre-injection data did not reveal main effects of week or risperidone administration, or an interaction between these two variables (Fig. 3). However, there was a statistical trend towards an effect of risperidone administration, $F(3, 31) = 2.51$, P = .08. Rats that received the higher doses of risperidone early in life had greater mean levels of locomotor activity than vehicle controls.

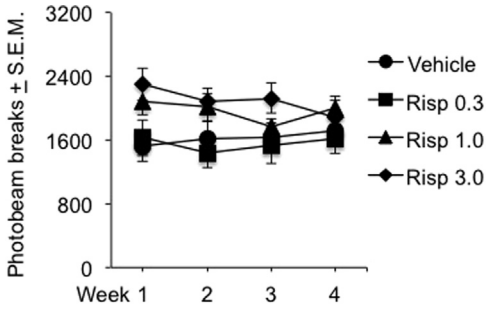

Fig. 3. Locomotor activity recorded for 30 min prior to amphetamine injections during each of the four weeks of amphetamine administration. Data represent photobeam breaks for each risperidone (Risp) group during each week ± S.E.M. There were no significant effects of risperidone or time. n = 9 per group except Risp 3.0 where n = 8.

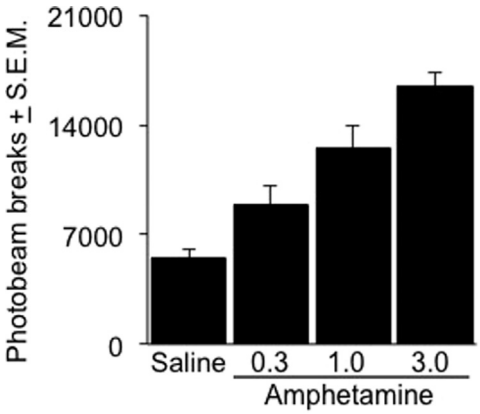

Fig. 4. Total locomotor activity observed in the vehicle rats for 5 h after injection of different doses (mg/kg) of amphetamine. The activity score represented by each bar is significantly different from all others. Data represent means ± S.E.M. n = 9 per group.

After this 30-min acclimation period, all rats received an injection of saline or one of three amphetamine doses (.3, 1.0, and 3.0 mg/kg) and were returned to the activity cages for 27 h. To gain a better sense of how amphetamine alone affected locomotor activity over the 27-h test period, the effects of each amphetamine dose were compared over time in the vehicle group only. This analysis revealed statistically significant main effects of amphetamine, $F(3, 32) = 4.17$, $P = .01$, and time, $F(26, 832) = 54.79$, $P < .0001$, and an amphetamine x time interaction, $F(78, 832) = 4.10$, $P < .0001$. The significant effects of amphetamine were mainly observed over the first five h post-injection (Fig. 4). Over this time period, amphetamine increased locomotor activity in a dose-dependent manner, with statistically significant differences found in the comparisons of the activity seen after each amphetamine dose ($P \leq .05$ for all post-hoc comparisons). Over the remaining 22 h, there were no dramatically different effects of amphetamine dose on activity.

Since the elevating effect of amphetamine on locomotor activity was greatest during the first 5 h post-injection, the analyses of the effects of early-life risperidone on amphetamine-induced hyperactivity focused mainly on this period. After saline injection, there were no significant effects of early-life risperidone on activity levels across the 5-h post-injection period, nor was there an interaction between risperidone administration and time (Fig. 5A). There was a significant time effect, $F(4, 124) = 65.96$, $P < .0001$, with animals in each group exhibiting much higher activity during the first post-injection h.

Being mindful that early-life risperidone led to increased activity in some of the previous testing, the locomotor responses to amphetamine were assessed by subtracting the hourly activity levels observed in each rat after saline injection from the hourly activity levels observed after each amphetamine dose. When analyzed in this manner, there were still significant effects of risperidone on the locomotor activity elicited by amphetamine. Main effects of risperidone on locomotor activity were not observed after injection of the .3 or 3.0 mg/kg doses of amphetamine (Fig. 5B and D), although there was a main effect of time observed after each injection, $F(4, 124) = 20.32$ and 2.87, $P \leq .0001$ and .03 for each respective dose. However, after injection of the 1.0 mg/kg dose of amphetamine, there was a significant interaction between risperidone administration and time, $F(12, 124) = 2.40$, $P = .008$ (Fig. 5C). One h after injection of this amphetamine dose, rats in the risperidone 3.0 group were significantly more active than rats in the other three groups ($P = .01$, .03, and .01 relative to vehicle, risperidone .3, and risperidone 1.0 respectively). At 2 h post-amphetamine injection, rats in the risperidone 1.0 and 3.0 groups were significantly more active than rats in the vehicle group ($P = .05$ and .002, respectively).

The data generated over the remaining 22 h of locomotor testing post-amphetamine injection were broken down into two periods corresponding to the dark phase (6–17 h) and the remaining light phase of testing (18–27 h). The analysis of total activity generated between 6 and 17 h by each risperidone group after each amphetamine dose indicated that there were significant effects of risperidone, $F(3, 31) = 8.41$, $P = .0003$, and amphetamine, $F(3, 93) = 9.52$, $P < .0001$, but no interaction between these variables (Fig. 6). Regardless of amphetamine dose, rats in the risperidone 3.0 group were significantly more active than rats in the vehicle group ($P = .0001$, .007, .003, and .0004 for comparisons between these groups after injections of saline, or .3, 1.0, and 3.0 mg/kg of amphetamine respectively) and rats in the risperidone .3 group ($P = .01$, .02, .03, and .003, respectively for each amphetamine dose). Similarly, rats in the risperidone 1.0 group were more active than vehicle rats ($P = .003$, .003, .03, and .01 for between group comparisons at each dose, respectively), and more active than those in risperidone .3 group after injection of the .3 mg/kg dose of amphetamine ($P = .02$). Finally, rats in the risperidone .3 group were more active after saline injection than those in the vehicle group ($P = .02$).

Amphetamine administration was associated with a dose-dependent decrease in activity between 6 and 17 h post-injection (Fig. 6). Collectively, rats administered the 1.0 mg/kg dose of amphetamine were less active than rats injected with saline ($P = .02$), but significantly more active that rats injected with the 3.0 mg/kg dose of amphetamine ($P = .02$). Rats in the latter group were also less active than rats injected with saline or .3 mg/kg of amphetamine ($P < .0001$ for each comparison).

Activity levels during the remaining light phase of testing (18–27 h) did not differ significantly between the groups that received different doses of amphetamine or different doses of risperidone early in life.

Stereotypy scores were recorded during the first two h after amphetamine injection. Amphetamine increased stereotypy in a dose-dependent manner, $F(3, 124) = 245.68$, $P < .0001$; all doses different from one another at $P < .0001$ (Fig. 7). However, there was no effect of risperidone alone or an interaction between risperidone administration and amphetamine on stereotypy.

4. Discussion

The main purpose of this study was to determine if early-life risperidone administration altered locomotor responses to the psychostimulant drug amphetamine during adulthood. As previously shown (Bardgett et al., 2013; Stevens et al., 2016), rats administered risperidone early in life displayed greater activity levels within the first week after cessation of daily risperidone injection. Over the following two weeks, locomotor activity remained elevated in rats administered risperidone early in life during the light and dark phases of two 24-h test sessions. Finally, rats administered risperidone early in life exhibited higher activity levels after amphetamine injection even when baseline activity was taken into account. These findings indicate that developmental exposure to risperidone alters the neural mechanisms that initiate and maintain locomotor activity, and increases their

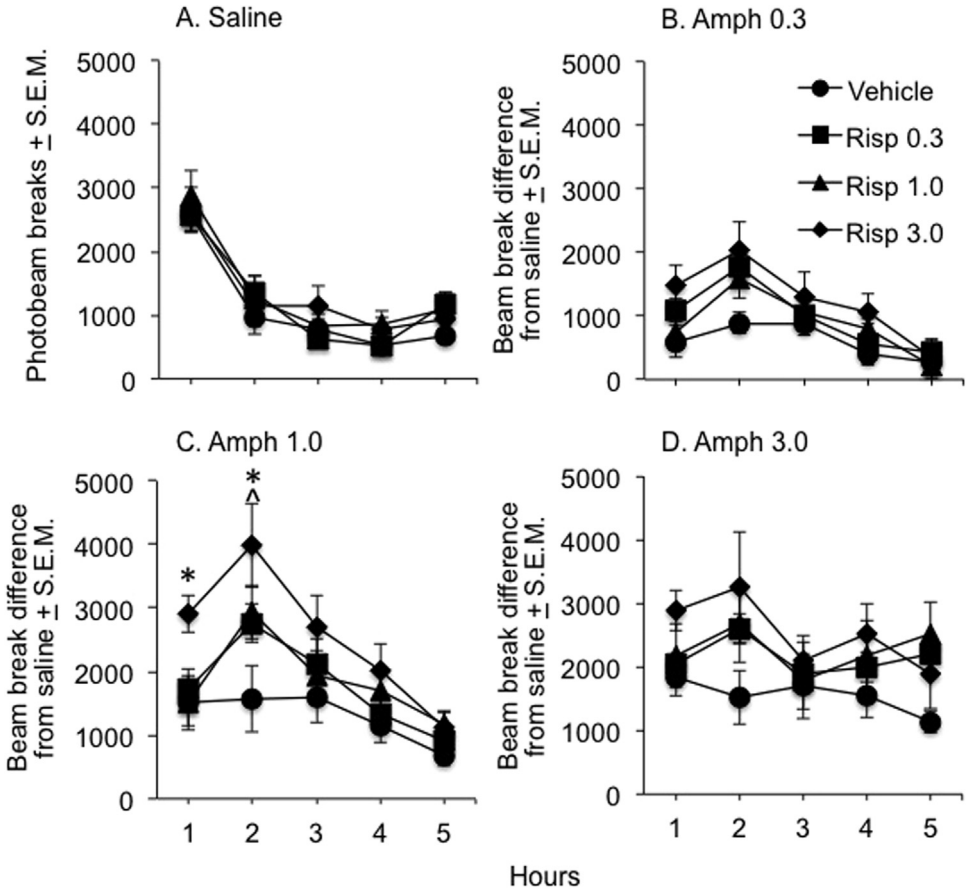

Fig. 5. Locomotor activity observed for 5 h after injection of different amphetamine (Amph) doses (in mg/kg). Data in A. represent mean photobeam breaks observed after saline injection ± S.E.M. Data in B., C., and D. represent the mean photobeam breaks (± S.E.M.) observed after injection of .3, 1.0, or 3.0 mg/kg of Amph respectively minus the photobeam breaks generated by each group after saline injection. Differences between vehicle and risperidone (Risp) 1.0 groups are indicated by ^, and between vehicle and Risp 3.0 groups by *. n = 9 per group except Risp 3.0 where n = 8.

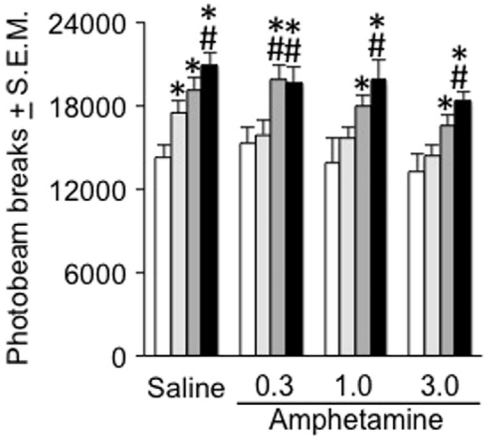

Fig. 6. Locomotor activity observed over the dark phase of testing between 6 and 17 h after injection of different amphetamine doses (in mg/kg). Differences between any risperidone (Risp) group and vehicle are indicated by *, and between any group and the Risp .3 rats by #. n = 9 per group except risperidone 3.0 where n = 8.

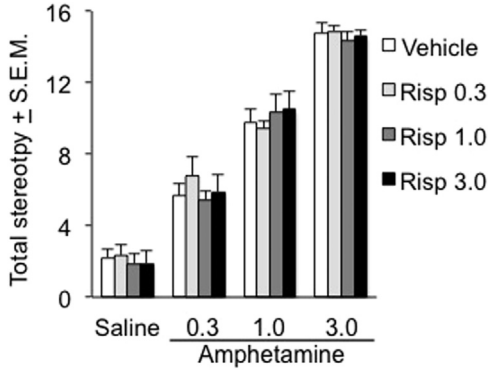

Fig. 7. Stereotypy recorded for 2 h after injection of different amphetamine doses (in mg/kg). Data represent mean total scores for each group generated over the 2-h post-amphetamine injection period ± S.E.M. The scores for each dose group were significantly different from one another. n = 9 per group except risperidone (Risp) 3.0 where n = 8.

sensitivity to amphetamine.

Past work has considered the effects of early-life antipsychotic drug administration on locomotor activity. Daily haloperidol injections across the first three weeks of development do not alter spontaneous activity in adulthood (Cuomo et al., 1981), whereas daily injections of somewhat larger doses of haloperidol between postnatal days 30–37 have been associated with higher activity levels (Soiza-Reilly and Azcurra, 2009). Others have reported that spontaneous locomotor activity during adulthood is not modified in rats that received the atypical antipsychotic drugs, clozapine, during the first three weeks of life (Cuomo et al., 1983a) or olanzapine from postnatal day 28–49 (Milstein et al., 2013). Consistent with the effects reported by Soiza-Reilly and Azcurra (2009), but contrary to the others, daily risperidone administration between postnatal days 14–42 elicited higher levels of locomotor activity during early adulthood. Specifically, when tested four days after the cessation of daily injections, rats that received risperidone during development were more active on the first day of testing. This effect may simply reflect withdrawal from the sedative effects of risperidone, but it should be noted that this effect is not seen

in adult rats that receive the same daily dose of risperidone for four weeks (Stevens et al., 2016). That the elevated activity is only observed on the first day of testing and not the second suggests an enhanced sensitivity to novelty (i.e., the locomotor testing cage) in the risperidone-treated rats, although some of the group differences seen later in the 24-h testing argue against novelty as the sole explanation for these effects. Since previous studies of early-life haloperidol, clozapine, or olanzapine (Cuomo et al., 1981, 1983a; Milstein et al., 2013) did not find differences in adult locomotor behavior, the effects of early-life risperidone reported here may be related to design and procedural differences, such as the timing and route of drug administration, rat strain, housing, or methods of monitoring locomotor behavior. But the difference may also stem from unique elements of risperidone's pharmacology relative to haloperidol, clozapine, and olanzapine, such as slightly greater affinities for adrenergic α_1 and $5HT_{1A}$, $5HT_{1D}$, or $5HT_{2A}$ receptors (Richelson and Souder, 2000). Perhaps, blockade of these receptors early in development ultimately modifies the tonic state of neural mechanisms that initiate and maintain locomotor activity in adulthood.

In all rats, amphetamine increased locomotor activity in a dose-dependent manner for five h after injection. Rats that received risperidone early in life were significantly more active over this same period after injection of the moderate dose of amphetamine. This effect could not be accounted for by baseline differences in locomotion since the hourly activity scores recorded for each rat after saline injection were subtracted from the activity scores recorded after injection of each amphetamine dose. This enhanced sensitivity to amphetamine is consistent with the potentiation of the rewarding effects of amphetamine in rats administered olanzapine between postnatal days 28–49 (Vinish et al., 2013).

On the other hand, rats exposed to the typical antipsychotic drug, haloperidol, during gestation and through nursing do not demonstrate dramatic differences in locomotor responses to amphetamine in comparison to controls (Spear et al., 1980), indicating that long-term consequences of early-life antipsychotic drug administration can depend on the type or timing of antipsychotic drug exposure. That early-life risperidone did not alter the ability of amphetamine to elevate stereotypy further suggests that only certain behaviors linked to amphetamine may be susceptible to modification by such administration. A final caveat is that, based on the present data, one can not be certain if chronic antipsychotic drug administration regardless of age would have produced the same effects as our developmental regimen of administration. But as mentioned above, we (Stevens et al., 2016) have recently shown that adult rats administered daily risperidone for four weeks, unlike similarly-treated periadolescent rats, do not demonstrate altered locomotor activity one week after the cessation of such treatment. This outcome could be viewed as evidence of a critical developmental period for risperidone's long-term effects on activity, including locomotor responses to amphetamine.

Our work does not provide direct insight into the neurobiological changes that might account for the increased sensitivity to amphetamine seen in rats administered risperidone early in life (or even the effects of early-life risperidone on baseline locomotor activity). It is well established that amphetamine reverses monoamine transporters, but it is not clear whether there are specific changes in noradrenergic or dopaminergic neurotransmission that account for the heightened sensitivity to amphetamine in the rats administered risperidone early in postnatal life. While little work has addressed how early-life antipsychotic drug administration affects noradrenergic neurotransmission, several studies have assessed how dopaminergic neurotransmission is altered by such exposure. Early work (Cuomo et al., 1981, 1983a, 1983b; Spear et al., 1980) found that rats exposed to haloperidol, but not clozapine, very early in postnatal life were more sensitive to the hypolocomotive effects of apomorphine and the cataleptogenic effects of haloperidol later in life. More germane to the present study, Moran-Gates and colleagues (Moran-Gates et al., 2007) reported that dopamine D_2 and D_4 receptors were increased in various forebrain regions of young adult rats that received three weeks of daily risperidone injections beginning on postnatal day 21, as was also found in rats administered risperidone chronically in adulthood. But unlike the latter group, rats administered risperidone early in life also demonstrated an increased density of dopamine D_1 receptors in the nucleus accumbens and caudate-putamen. Chronic oral olanzapine exposure during early adolescence in rats elevates dopamine D_2 receptors in the nucleus accumbens core and frontal cortex later in adulthood, but decreases dopamine D_1 receptors in the same areas (Milstein et al., 2013; Vinish et al., 2013), suggesting that the developmental effects of antipsychotic drugs on dopamine receptors may vary significantly according the specific drug or route of administration.

Locomotor activity was recorded for 27 h after amphetamine injection in order to capture any potential interactions between early-life risperidone and the delayed effects of amphetamine. Overall, amphetamine decreased activity between 6 and 17 h (dark phase) of testing, while there were no significant effects observed between 18 and 27 h. It is possible that the diminished activity observed in the dark phase of testing could reflect a type of withdrawal from the initial locomotor elevating effect of amphetamine – a response that has been reported by others (White and White, 2006) albeit at later (e.g., 20–22 h) time points post-amphetamine injection. Early-life risperidone did not significantly impact the delayed hypolocomotive effects of amphetamine, although if one compares the activity levels observed after saline injection to those observed after injection of the high dose of amphetamine (see Fig. 6), the decreases after the latter injection are greater in the .3 and 3.0 mg/kg risperidone groups. These subtle differences perhaps merit further empirical scrutiny.

In children under 6 years of age, antipsychotic drugs are most commonly prescribed for ADHD (Constantine et al., 2011; Cooper et al., 2004; Kuehn, 2009; Olfson et al., 2010) while in older children they are most commonly prescribed for disruptive behavioral disorder (Olfson et al., 2012). Children with these disorders are at increased risk for substance abuse and addiction during young adulthood (Harstad and Levy, 2014; Levy et al., 2014; Salvo et al., 2012; Zonnevylle-Bender et al., 2007), including abuse of psychostimulant medications used to treat ADHD symptoms (Faraone and Wilens, 2007; Wilens et al., 2008). The results from this study suggest that early-life antipsychotic drug exposure may raise sensitivity to some behavioral effects of psychostimulants later in life, eliciting concern regarding greater substance abuse potential in children treated with antipsychotic drugs. Future preclinical and clinical research should determine if developmental antipsychotic drug administration modifies the reinforcing (Vinish et al., 2013) and cognitive effects (Sherrill et al., 2013) of psychostimulants later in life, and whether early-life antipsychotic drug exposure alters behavioral and neural responses to other drugs of abuse.

Acknowledgements

This work was supported by the National Institute of Health (Grant nos. P20GM103436, R15DA041708 and R15MH094955). These funding sources had no other role in the research other than funding. All authors declare no conflict of interest.

References

Andersen, S.L., 2005. Stimulants and the developing brain. Trends Pharmacol. Sci. 26, 237–243.

Arnt, J., 1995. Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. Eur. J. Pharmacol. 5, 55–62.

Bardgett, M.E., Franks-Henry, J.M., Colemire, K.R., Juneau, K.R., Stevens, R.M., Marczinski, C.A., Griffith, M.S., 2013. Adult rats treated with risperidone during development are hyperactive. Exp. Clin. Psychopharmacol. 21, 259–267. http://

dx.doi.org/10.1037/a0031972.
Bachmann, C.J., Lempp, T., Glaeske, G., Hoffmann, F., 2014. Antipsychotic prescription in children and adolescents: an analysis of data from a German statutory health insurance company from 2005 to 2012. Dtsch. Arztebl. Int. 111, 25–34. http://dx.doi.org/10.3238/arztebl.2014.0025.
Choi, Y.K., Gardner, M.P., Tarazi, F.I., 2009. Effects of risperidone on glutamate receptor subtypes in developing rat brain. Eur. Neuropsychopharmacol. 19, 77–84. http://dx.doi.org/10.1016/j.euroneuro.2008.08.010.
Choi, Y.K., Moran-Gates, T., Gardner, M.P., Tarazi, F.I., 2010. Effects of repeated risperidone exposure on serotonin receptor subtypes in developing rats. Eur. Neuropsychopharmacol. 20, 187–194. http://dx.doi.org/10.1016/j.euroneuro.2009.09.002.
Constantine, R.J., Jentz, S., Bengtson, M., McPherson, M., Andel, R., Jones, M.B., 2012. Exposure to antipsychotic medications over a 4-year period among children who initiated antipsychotic treatment before their sixth birthday. Pharmacoepidemiol. Drug. Saf. 21, 152–160. http://dx.doi.org/10.1002/pds.2189.
Constantine, R.J., Tandon, R., McPherson, M., Andel, R., 2011. Early diagnoses and psychotherapeutic medication treatment experiences of a cohort of children under 6 years old who received antipsychotic treatment in Florida's Medicaid program. J. Child Adolesc. Psychopharmacol. 21, 79–84. http://dx.doi.org/10.1089/cap.2010.0068.
Cooper, W.O., Hickson, G.B., Fuchs, C., Arbogast, P.G., Ray, W.A., 2004. New users of antipsychotic medications among children enrolled in TennCare. Arch. Pediatr. Adolesc. Med. 158, 753–759.
Cuomo, V., Cagiano, R., Coen, E., Mocchetti, I., Cattabeni, F., Racagni, G., 1981. Enduring behavioural and biochemical effects in the adult rat after prolonged postnatal administration of haloperidol. Psychopharmacology 74, 166–169.
Cuomo, V., Cagiano, R., Mocchetti, I., Coen, E., Cattabeni, F., Racagni, G., 1983a. Behavioural and biochemical effects in the adult rat after prolonged postnatal administration of clozapine. Psychopharmacology 81, 239–243.
Cuomo, V., Cattabeni, F., Racagni, G., 1983b. The influence of early postnatal treatment with haloperidol on the effects induced by small and large doses of apomorphine on locomotion of adult rats. Neuropharmacology 22, 1137–1139.
Faraone, S.V., Wilens, T.E., 2007. Effect of stimulant medications for attention-deficit/hyperactivity disorder on later substance use and the potential for stimulant misuse, abuse, and diversion. J. Clin. Psychiatry 68, 15–22.
Gannon, M.A., Brown, C.J., Stevens, R.M., Griffith, M.S., Marczinski, C.A., Bardgett, M.E., 2015. Early-life risperidone administration alters maternal-offspring interactions and juvenile play fighting. Pharmacol. Biochem. Behav. 130, 90–96. http://dx.doi.org/10.1016/j.pbb.2015.01.007.
Harstad, E., Levy, S., 2014. Committee on Substance Abuse. Attention-deficit/hyperactivity disorder and substance abuse. Pediatrics 134, e293–e301. http://dx.doi.org/10.1542/peds.2014-0992.
Iversen, L.L., Iversen, S.D., Bloom, F.E., Roth, R.H., 2008. Introduction to Neuropsychopharmacology. Oxford University Press, Oxford.
Kalverdijk, L.J., Tobi, H., van den Berg, P.B., Buiskool, J., Wagenaar, L., Minderaa, R.B., de Jong-van den Berg, L.T., 2008. Use of antipsychotic drugs among Dutch youths between 1997 and 2005. Psychiatr. Serv. 59, 554–560. http://dx.doi.org/10.1176/appi.ps.59.5.554.
Kapur, S., VanderSpek, S.C., Brownlee, B.A., Nobrega, J.N., 2003. Antipsychotic dosing in preclinical models is often unrepresentative of the clinical condition: a suggested solution based on in vivo occupancy. J. Pharmacol. Exp. Ther. 305, 625–631.
Kelly, P.H., Seviour, P.W., Iversen, S.D., 1975. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res. 94, 507–522.
Kuehn, B.M., 2009. FDA panel OKs 3 antipsychotic drugs for pediatric use, cautions against overuse. JAMA 302, 833–834.
Levy, S., Katusic, S.K., Colligan, R.C., Weaver, A.L., Killian, J.M., Voigt, R.G., Barbaresi, W.J., 2014. Childhood ADHD and risk for substance dependence in adulthood: a longitudinal, population-based study. PLoS One 9, e105640. http://dx.doi.org/10.1371/journal.pone.0105640.
Lohr, W.D., Chowning, R.T., Stevenson, M.D., Williams, P.G., 2015. Trends in atypical antipsychotics prescribed to children six years of age or less on Medicaid in Kentucky. J. Child. Adolesc. Psychopharmacol. 25, 440–443. http://dx.doi.org/10.1089/cap.2014.0057.
Mailman, R.B., Murthy, V., 2010. Third generation antipsychotic drugs: partial agonism or receptor functional selectivity? Curr. Pharm. Des. 16, 488–501. http://dx.doi.org/10.2174/138161210790361461.
Milstein, J.A., Elnabawi, A., Vinish, M., Swanson, T., Enos, J.K., Bailey, A.M., Kolb, B., Frost, D.O., 2013. Olanzapine treatment of adolescent rats causes enduring specific memory impairments and alters cortical development and function. PLoS One 8, e57308. http://dx.doi.org/10.1371/journal.pone.0057308.
Moran-Gates, T., Grady, C., Park, Y.S., Baldessarini, R.J., Tarazi, F.I., 2007. Effects of risperidone on dopamine receptor subtypes in developing rat brain. Eur. Neuropsychopharmacol. 17, 448–455. http://dx.doi.org/10.1016/j.euroneuro.2006.10.004.
Murphy, A.L., Gardner, D.M., Cooke, C., Kisely, S., Hughes, J., Kutcher, S.P., 2013. Prescribing trends of antipsychotics in youth receiving income assistance: results from a retrospective population database study. BMC Psychiatry 13, 198. http://dx.doi.org/10.1186/1471-244X-13-198.
Olfson, M., Blanco, C., Liu, S., Wang, S., Correll, C.U., 2012. National trends in the office-based treatment of children, adolescents, and adults with antipsychotics. Arch. Gen. Psychiatry 69, 1247–1256. http://dx.doi.org/10.1001/archgenpsychiatry.2012.647.
Olfson, M., Crystal, S., Huang, C., Gerhard, T., 2010. Trends in antipsychotic drug use by very young, privately insured children. J. Am. Acad. Child Adolesc. Psychiatry 49, 13–23.
Olfson, M., King, M., Schoenbaum, M., 2015. Treatment of young people with antipsychotic medications in the United States. JAMA Psychiatry 72, 867–874. http://dx.doi.org/10.1001/jamapsychiatry.2015.0500.
Richelson, E., Souder, T., 2000. Binding of antipsychotic drugs to human brain receptors focus on newer generation compounds. Life Sci. 68, 29–39.
Salvo, N., Bennett, K., Cheung, A., Chen, Y., Rice, M., Rush, B., Bullock, H., Bowlby, A., 2012. Evidence on tap concurrent disorders collaborative team. prevention of substance use in children/adolescents with mental disorders: a systematic review. J. Can. Acad. Child Adolesc. Psychiatry 21, 245–252.
Sherrill, L.K., Stanis, J.J., Gulley, J.M., 2013. Age-dependent effects of repeated amphetamine exposure on working memory in rats. Behav. Brain Res. 242, 84–94. http://dx.doi.org/10.1016/j.bbr.2012.12.044.
Soiza-Reilly, M., Azcurra, J.M., 2009. Developmental striatal critical period of activity-dependent plasticity is also a window of susceptibility for haloperidol-induced adult motor alterations. Neurotoxicol. Teratol. 31, 191–197. http://dx.doi.org/10.1016/j.ntt.2009.03.001.
Spear, L.P., 2000. The adolescent brain and age-related behavioral manifestations. Neurosci. Biobehav. Rev. 24, 417–463.
Spear, L.P., Shalaby, I.A., Brick, J., 1980. Chronic administration of haloperidol during development: behavioral and psychopharmacological effects. Psychopharmacology 70, 47–58.
Stevens, R.M., Gannon, M.A., Griffith, M.S., Bardgett, M.E., 2016. Delayed yet persistent effects of daily risperidone on activity in developing rats. Behav. Pharmacol. 27, 460–469.
Vinish, M., Elnabawi, A., Milstein, J.A., Burke, J.S., Kallevang, J.K., Turek, K.C., Lansink, C.S., Merchenthaler, I., Bailey, A.M., Kolb, B., Cheer, J.F., Frost, D.O., 2013. Olanzapine treatment of adolescent rats alters adult reward behaviour and nucleus accumbens function. Int. J. Neuropsychopharmacol. 16, 1599–1609. http://dx.doi.org/10.1017/S1461145712001642.
White, W., White, I.M., 2006. An activity indicator of acute withdrawal depends on amphetamine dose in rats. Physiol. Behav. 87, 368–376. http://dx.doi.org/10.1016/j.physbeh.2005.10.009.
Wilens, T.E., Adler, L.A., Adams, J., Sgambati, S., Rotrosen, J., Sawtelle, R., Utzinger, L., Fusillo, S., 2008. Misuse and diversion of stimulants prescribed for ADHD: a systematic review of the literature. J. Am. Acad. Child Adolesc. Psychiatry 47, 21–31. http://dx.doi.org/10.1097/chi.0b013e31815a56f1.
Zonnevylle-Bender, M.J., Matthys, W., van de Wiel, N.M., Lochman, J.E., 2007. Preventive effects of treatment of disruptive behavior disorder in middle childhood on substance use and delinquent behavior. J. Am. Acad. Child Adolesc. Psychiatry 46, 33–39.