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Delayed Yet Persistent Effects of Daily Risperidone on Activity in Developing Rats

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Abstract

Early-life administration of risperidone, the most widely used antipsychotic drug in children, leads to persistently elevated locomotor activity in adult rats. This study determined if and when elevated locomotor activity emerges during developmental risperidone administration. Developing and adult rats were given daily injections of risperidone (1.0 and 3.0 mg/kg) or vehicle for four weeks beginning at postnatal day (PND) 14 and 74, respectively. Starting with the first injection and every seven days thereafter, locomotor activity was measured immediately after the injection and 20 minutes prior to the next day's injection. Activity was also recorded one week after the final injection. Risperidone profoundly decreased locomotor activity in developing and adult rats immediately after injection. Within 24 h after their first injection, adult rats administered risperidone demonstrated greater activity levels. In contrast, developing rats did not demonstrate compensatory hyperactivity until the beginning of the fourth week of risperidone administration. One week after the final risperidone injection, there was no evidence of hyperactivity in the adult rats maintained on risperidone, but developing rats administered risperidone, especially females, exhibited greater activity levels relative to vehicle-administered controls. In comparison to adult rats, the emergence of compensatory hyperactivity during long-term APD administration is delayed in developing rats but persists after treatment cessation.

Keywords

antipsychotic; development; forebrain; dopamine; postnatal; withdrawal; rat

Introduction

Antipsychotic drug (APD) use in children has escalated in many Western countries over the last two decades (Kalverdijk et al., 2008; Vitello et al., 2009; Olfson et al., 2012; Ronsley et al., 2013; Bachmann et al., 2014). In the United States, this trend has been particularly evident among children under the age of 6 (see Constantine et al., 2011, for review). Not only are more children receiving APDs, but the duration of APD treatment in children has also lengthened (Kalverdijk et al., 2008). APDs are most commonly prescribed to children

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for non-approved indications such as ADHD and disruptive behavioral disorders (Olfson et al., 2010, 2012; Constantine et al., 2011). The increased use of APDs among children reflects a rise in the prescription of second-generation or atypical APDs, primarily risperidone; boys are more likely to receive APDs than girls (Domino & Swartz, 2008; Olfson et al., 2012). Most reports of APD use in children lament the lack of data on the long-term effects of APDs on brain and behavioral development.

To address this concern, research using rats as a model of mammalian brain development have assessed the effects of early-life APD administration on later neural and behavioral function. Older work, concerned with the use of typical APDs, such as haloperidol, during pregnancy and nursing, revealed that rat pups exposed to APDs during the first three weeks of life exhibited behavioral control deficits, such as hyperactivity, later in life (Shalaby & Spear, 1980; Cuomo et al., 1981; Scalzo & Spear, 1985). More recently, we studied young male and female rats administered risperidone daily during a period of development (postnatal days (PNDs) 14–42) more akin to the time when children are more likely to receive APDs, and found that such treatment yields elevated locomotor activity during adulthood (Bardgett et al., 2013).

APD administration to adult rats produces dopaminergic supersensitivity, a compensatory set of biochemical and behavioral changes spawned by the blockade of dopamine D₂ receptors by APDs. For example, adult rats administered continuous haloperidol through a subcutaneous minipump for several weeks demonstrate increases in spontaneous locomotion, greater motor responses to dopamine agonists, and elevated densities of forebrain dopamine D₂ receptors (Samaha et al., 2007; Tadokoro et al., 2012). Earlier studies reported similar changes in adult rats after only a single APD injection, and the duration of dopaminergic supersensitivity typically mirrors the duration of drug exposure (see Muller & Seeman 1979 for review). Varela and colleagues (2014) generated comparable data in younger rats by showing that haloperidol or aripiprazole injected daily between PNDs 10–20 increased amphetamine-induced sniffing and elevated striatal D₂ densities between PNDs 21–28. Despite this work, it remains unclear as to if and when the locomotor hyperactivity produced by early-life risperidone administration (Bardgett et al., 2013) emerges during the course of administration, and if it evolves in a manner consistent with its emergence in adults (Muller & Seeman, 1979).

The purpose of this study was to measure the locomotor effects of risperidone across a four-week period of administration in developing (PNDs 14–42) and adult (PNDs 74–102) male and female rats. Once a week for five weeks beginning on the first day of injections, locomotor activity was recorded immediately following and 23.6 hours after a subcutaneous injection of risperidone (1.0 and 3.0 mg/kg) or vehicle. Locomotor activity was recorded immediately after drug injection to assess the level of locomotor suppression effected by risperidone in younger and adult rats. As a means of gauging if and when the hyperactivity observed after the cessation of risperidone administration actually emerged during the course of daily treatment, locomotor activity was also recorded at 23 hours post-injection and thereafter once a week at the same post-injection time point, in developing and adult rats. It is unlikely that locomotor activity at this time point is influenced by residual risperidone in plasma since plasma and brain half-lives of risperidone in adult rats are

approximately one and 3.5 hours respectively (van Beijsterveldt et al., 1994; Olsen et al., 2008), and our own work has demonstrated that risperidone is undetectable in the plasma of developing rats at 23 hours post-injection (Gannon et al., 2015). Finally, locomotor activity was measured one week after the cessation of daily risperidone administration, in order to determine if such administration led to enduring hyperactivity in younger and older rats.

Method

Subjects and housing

The experimental procedures were performed in accordance with the current Guide for the Care and Use of Laboratory Animals (National Research Council, 2010) under a protocol approved by the Institutional Animal Care and Use Committee at Northern Kentucky University. For the study of developing rats, six Long-Evans rat dams and litters (Harlan Laboratories, Indianapolis, IN) arrived in the Northern Kentucky University Department of Psychological Science animal facility on PND 7. Upon arrival, litters were culled to five female and five male pups per dam. Only six pups from each litter (one per each sex and each drug dose group) were used in this study, for a total of 36 rats. All pups were paw-tagged for identification purposes on PND 10 with subdermal ink injections into the footpad. On PND 21, the pups were weaned from their mothers, and housed three per cage. In the study of adult rats, 30 Long-Evans (15 female and 15 male) rats were used. These rats were shipped from Harlan Laboratories and arrived in the NKU Department of Psychological Science Animal Facility on postnatal day 62. They were housed three per cage. In all studies, lighting in the housing room was maintained on a 12 hour light-dark schedule with lights on at 07:00h. Except during the testing sessions, animals had free access to food and water. Drug treatments and testing were performed between 08:00 and 16:00h.

Risperidone Administration

Animals were assigned to one of three treatment groups: vehicle, risperidone 1.0 mg/kg, or risperidone 3.0 mg/kg. In the developmental study, each of the three dose groups contained 6 males and 6 females, and in the adult study, each group contained 5 males and 5 females. In the former study, rats were assigned to the treatment groups such that no litter had more than two pups of the same sex receiving the same drug dose. After PND 21 in the developmental study and throughout the adult study, the three rats housed within a single cage each received a different risperidone dose. Rats received daily subcutaneous injections of their assigned risperidone dose or vehicle solution from PNDs 14–42 in the development study and from PNDs 74–102 in the adult study. The injections were performed in a room just outside of the housing room. Weights were recorded daily just prior to injections.

Careful consideration was given to selecting the risperidone doses. The 1.0 mg/kg dose reportedly occupies 60–80% of D₂ receptors in the rat forebrain (Kapur et al., 2003) – a degree of receptor blockade associated with APD efficacy in humans (Kapur et al., 2003). The 1.0 mg/kg dose also decreases amphetamine-induced hyperactivity in rats by 50% (Arnt, 1995). However, because this dose of risperidone does not consistently produce drug blood levels in adult rats that are near those reported in adult humans (Kapur et al., 2003), a higher dose (3.0 mg/kg) of risperidone was also studied. This latter dose was also considered

because it is possible that some children receive risperidone doses that are at or above those recommended for adults. Finally, these doses were the same as those used in our recent behavioral studies of early-life risperidone administration (Bardgett et al, 2013; Gannon et al., 2015) as well as in studies demonstrating significant changes in forebrain receptor binding after early-life administration (Moran-Gates et al., 2007; Choi et al., 2009; 2010).

The National Institute of Mental Health's Chemical Synthesis and Drug Supply program provided the risperidone. Risperidone was first dissolved in ~1 ml of 10% glacial acetic acid; the resulting solution was brought to volume with saline, and the pH adjusted to ~6.2 with 6M NaOH. New solutions were made once a week. Solutions were injected at a volume of 2.0 ml/kg of body weight.

Locomotor Activity

The testing equipment consisted of 12 clear polypropylene cages (51 cm long×26.5 cm wide×32 cm high) each situated within a Kinder Scientific (Kinder Scientific, Poway, CA) Smart-Frame, photocell-based activity monitor. The activity monitor consisted of a stainless steel frame that contained 22 photobeams, located 2.5 cm apart along the length and width of the frame, and situated 3.5 cm above the bottom of the cage. Data were collected from the activity frames using MotorMonitor software (Kinder Scientific, Poway, CA). Testing was conducted between 08:00 and 14:00 in a dark room that was located near the animal housing room.

In the developmental and adult studies, locomotor activity was recorded for 40 minutes immediately after the first injection of risperidone. The number of photobeam breaks was tabulated every five minutes over this period of testing. Rats were tested again 20 minutes prior to the next day's injection, and the same measures were recorded. This regimen of locomotor testing was repeated once a week for four more weeks including the last day of injections. One week after the cessation of drug administration, the rats were tested for one hour as described above.

Data Analysis

In the studies of the immediate and delayed effects of risperidone, the total number of photobeam breaks recorded during each daily test was used as a dependent measure. These data were compared as a function of drug dose and sex (between subjects) and week (within subjects) using a three-way analysis of variance (ANOVA). For the assessment of locomotor activity observed one week following the cessation of drug administration, the total number of photobeam breaks was compared as a function of drug dose and sex (between subjects), and time (within subjects) using a three-way ANOVA. Fishers PLSD was used for all post-hoc testing and significant differences were accepted if $p < .05$ (two-tailed). The data analyses for the developing rats and adult rats were conducted separately since the two sets of rats were tested separately.

The within-subjects data were also subjected to Mauchly's Test of Sphericity to determine if the homogeneity of variance was the same at each time point, and in each test of the interaction between time x drug dose, time x gender, or time x drug dose x gender. If sphericity was violated at $p < .05$, a Huyhn-Feldt statistic was used to adjust the degrees of

freedom and p value for the analysis in question. Where sphericity was violated for a given set of repeated-measures data, the result of the Mauchly's Test is noted below after the F statistic from the analysis of the corresponding data.

Results

Locomotor activity immediately after risperidone administration

Locomotor activity was profoundly reduced immediately after risperidone administration in the developing rats (Figure 1a). There was a significant interaction between drug administration and week $F(5.47, 106.06) = 9.94, p < .001$ (Mauchly's Test of Sphericity, $\chi^2(9) = 35.48, p < .001$). During each weekly test, locomotor activity was markedly suppressed in rats administered either dose of risperidone in comparison to the activity observed in the rats administered the vehicle solution, although a spurious yet significantly greater level of activity was seen after risperidone administration on PND 21 in comparison to the other test days (Fishers PLSD, $p = .001-.02$, PND 21 risperidone groups compared to risperidone groups on all other test days). Additionally, rats administered vehicle were significantly more active on PND 14 in comparison to any other day of testing (Fishers PLSD, $p < .001$). A review of the number of photobeam breaks generated every five minutes across the 40-minute test period indicated that, unlike rats at any other age (including adults), activity levels in the PND 14 pups did not decrease across the 40 min test period (data not shown). There was no significant effect of sex or a sex x drug administration interaction.

In the adult rats, there were significant effects of drug administration $F(2, 24) = 297.26, p < .001$ and sex $F(1, 24) = 12.72, p < .002$ (Figure 1b). Risperidone reduced locomotor activity during each week of testing. Unlike the developing rats, there was no interaction between drug administration and test week, but there was some evidence of a dose response in the risperidone groups. When comparing overall activity between the three dose groups regardless of test week, activity levels were slightly yet significantly higher in the rats administered 1.0 mg/kg of risperidone than the rats that received 3.0 mg/kg of risperidone (Fishers PLSD, $p = .05$). A comparison of activity levels between the risperidone groups within weeks suggested that the rats administered the 1.0 mg/kg dose of risperidone were significantly more active than the rats administered the 3.0 mg/kg dose of risperidone after the first injection of risperidone (Fishers PLSD, $p = .05$). Similar comparisons within the remaining four weeks of testing did not reveal statistically significant differences between the groups administered risperidone. Overall, male rats were more active than female rats $F(1, 24) = 12.7, p < .002$.

Locomotor activity 23 hours after risperidone administration

Analysis of the locomotor activity observed 23.6 hours after drug injection in the developing rats indicated that there was a trend towards a significant interaction between drug administration and test week $F(5.95, 89.31) = 2.15, p = .06$ (Mauchly's Test of sphericity $\chi^2(9) = 37.38, p < .001$) and a significant main effect of drug administration $F(2, 30) = 4.15, p < .05$ (Figure 2a). There were no significant differences between the three dose groups during the first three weeks of testing. However, the rats administered risperidone were

significantly more active than the rats administered vehicle on PND 36 (Fishers PLSD, $p = .01$ & $.002$ for respective comparisons of the 1.0 and 3.0 mg/kg dose groups to vehicle) and PND 43 (Fishers PLSD, $p = .007$ & $.001$ for respective comparisons of the 1.0 and 3.0 mg/kg dose groups to vehicle). As was observed on PND 14 immediately after injection for the vehicle group, all groups were more active on PND 15 in comparison to all other test weeks, owing to a lack of habituation over the 20 minute test period (data not shown). There was no significant effect of sex and no significant sex x drug administration interaction.

Adult rats administered risperidone were more active 23.6 hours after their first injection and thereafter during each weekly test at the same time point $F(2, 24) = 6.3$, $p < .01$ (Figure 2b). When tested 23.6 hours after the first injection, adult rats administered the high risperidone dose were more active in comparison to vehicle controls (Fishers PLSD, $p = .02$). When tested during each subsequent week, rats administered either risperidone dose were more active than rats administered vehicle 23.6 hours after injection (Fishers PLSD, $p = .003 - .04$ for comparisons between the 1.0 mg/kg or the 3.0 mg/kg groups and vehicle group within each test day). There was also a significant effect of week $F(4, 96) = 10.9$, $p < .001$ on locomotor activity at 23.6 hours after injection. Overall, the rats were less active during the last fourth week of testing in comparison to the other four weeks of testing (Fishers PLSD, $p = .03 - .007$).

Locomotor activity one week after the cessation of risperidone administration

One week after the cessation of daily risperidone administration, locomotor activity was recorded in all rats for one hour. When tested at PND 49, there were significant main effects of drug administration $F(2, 30) = 8.8$, $p < .001$, sex $F(1, 30) = 5.8$, $p = .02$, and time $F(11.00, 330.00) = 85.6$, $p < .001$ (Mauchly's Test of Sphericity, $\chi^2(65) = 85.03$, $p = .06$), as well as a significant drug administration x sex interaction $F(2, 30) = 3.3$, $p < .05$ (Figure 3a & b). Locomotor activity was significantly greater in female rats administered risperidone early in life when compared to male rats. Male rats in the high dose risperidone group were significantly more active than male rats in the vehicle control group at 10 and 15 minutes (Fishers PLSD, $p = .009$ & $.006$, respectively)(Figure 3a). In contrast, female rats in the high dose risperidone group were more active than female rats in the vehicle control group at every time point (Fishers PLSD, $p < .0001 - .03$) (Figure 3b). Female rats in the low dose risperidone group were also more active than controls at 10, 15, and 30 minutes of testing (Fishers PLSD, $p = .01$, $.008$, & $.04$, respectively).

Analysis of the locomotor activity recorded on PND 109 in adult rats administered risperidone from PND 74–102 indicated that there were no significant effect of drug administration or interactions between drug administration and time or sex (Figure 3c & d). However, there were significant main effects of time $F(7.30, 174.46) = 130.1$, $p < .001$, and a significant time x sex interaction $F(7.30, 174.46) = 3.5$, $p < .001$ (Mauchly's Test of Sphericity, $\chi^2(65) = 120.11$, $p < .001$). Male rats were more active than females during the first five minutes of testing whereas females were slightly more active than males at 50 minutes (Fishers PLSD, $p = .07$ at each time point).

Weight gain during risperidone administration

Developing rats administered vehicle between PNDs 14–42 gained more weight than rats administered risperidone (Figure 4a). There was a significant drug administration x weeks interaction $F(3.26, 48.90) = 5.4, p < .002$ (Mauchly's Test of Sphericity, $\chi^2(9) = 145.32, p < .001$). Overall, rats that received vehicle weighed significantly more than those administered 3.0 mg/kg of risperidone on PNDs 21, 28, and 35 (Fishers PLSD, $p = .001, .001, \& .003$ respectively), or those administered 1.0 mg/kg of risperidone on PND 21 (Fishers PLSD, $p = .01$). There was also a significant sex x weeks interaction $F(1.63, 48.90) = 88.0, p < .001$ (Mauchly's Test of Sphericity, $\chi^2(9) = 145.32, p < .001$). Male rats weighed more than female rats beginning on PND 28 and thereafter (Fishers PLSD, $p = .01, .001, \& .001$ at PNDs 28, 35, & 42, respectively).

There was a significant drug administration x sex x weeks interaction of testing in the weight data recorded in the adult rat study $F(6.71, 80.52) = 8.5, p < .001$ (Mauchly's Test of Sphericity, $\chi^2(9) = 28.20, p = .001$). Risperidone reduced weight gain during the four weeks of daily administration but only in the adult male rats (Figure 4b). Male rats maintained on the 3.0 mg/kg risperidone dose weighed significantly less than male rats maintained on the 1.0 mg/kg risperidone dose or vehicle on PNDs 81, 88, 95, and 102 (Fishers PLSD, $p = .001-.03$ range for comparisons). Adult female rats gained weight over four-week period, and risperidone did not affect their rate of weight gain.

Discussion

Risperidone administration profoundly reduced locomotor activity in young and adult rats immediately following injection. Beyond this common effect, the ensuing compensatory behavioral changes were quite different in the two age groups. Early-life risperidone administration produced a form of compensatory, heightened locomotor activity that was slow to develop but more enduring. This was in contrast to the adult rats for which the compensatory changes in activity emerged after the first risperidone injection but were less persistent after the end of daily drug administration. Taken together, the data suggest that early-life risperidone administration resets the trajectory of brain development in such a way that the set point for ongoing locomotor activity is permanently raised.

One purpose of the present study was to provide reference data regarding the immediate effects of risperidone on locomotor activity in young rats. Numerous studies have described the locomotor suppressive effects of risperidone in adult rats (see Bardgett, 2004 for review), and the present study confirmed these effects in adult female and male rats. We also found that young rats between PNDs 14 and 42 were just as sensitive to the suppressive action of risperidone. Admittedly, the doses used here were higher than those typically administered to adult rats in order to characterize the more nuanced effects of risperidone on locomotor activity (Arnt, 1995; Bardgett et al., 2006). However, the selected doses were chosen based on their clinical relevance (Kapur et al., 2003) and their consistency with recent work on the behavioral and biochemical effects of early-life risperidone (Moran-Gates et al., 2007; Choi et al., 2009; 2010; Bardgett et al., 2013; Gannon et al., 2015). The reduced activity observed in developing rats administered risperidone is consistent with the effects of haloperidol or clozapine on activity in rats between the ages of PND 21 and adulthood (Wiley, 2008).

Activity immediately after risperidone administration was greater on PND 21 as compared other developmental time points for reasons that are not obvious – it is possible that the stress of weaning, which occurred at that time, altered the activity on this day. With the exception of the PND 21 data, there is some evidence in the present work that supports the idea that younger rats may be more sensitive to the suppressive effects of APDs (Wiley, 2008), because the locomotor response of adult rats to risperidone was dose-dependent whereas each risperidone dose was equally suppressive in the younger rats.

Christensen and colleagues (1976) were among the first to report that a single APD injection causes a compensatory behavioral hypersensitivity to dopamine agonists that appears within 24 h of injection and can be observed for 1–3 days post-injection depending on the specific APD and dose. To our knowledge, no one has since determined whether spontaneous locomotor activity, absent any drug challenge, is altered within 24 h of a single APD injection, whether it persists in a consistent manner across the course of APD administration, and how it is expressed as a function of age. Our data show that adult male and female rats administered a high dose of risperidone are more active 23.6 h after a single risperidone injection. Moreover, the elevated activity observed at this post-injection time point is consistent across weeks of daily injections, and emerges in rats administered a lower dose of risperidone after one week of drug administration.

One explanation for this compensatory response could be an increase in forebrain D₂ receptors that has been seen after repeated risperidone administration (Moran-Gates et al., 2007). How soon this occurs after repeated risperidone administration is not yet known, but D₂ receptor binding capacity can be elevated within 48 h of continuous haloperidol administration (Samaha et al., 2008), suggesting that this process may be fairly rapid. Such an increase in dopamine receptor availability during a daily trough in plasma risperidone levels may enable synaptic dopamine to have a greater post-synaptic effect and elicit higher levels of locomotor activity. However, it should be noted that chronic APD administration in adulthood also increases the sensitivity of D₂ autoreceptors (Nowycky & Roth, 1977), which, depending on the synaptic dopamine levels, could serve to limit or exacerbate the compensatory hyperactivity observed after risperidone administration.

What is most noteworthy about the compensatory change in locomotor activity is that it did not emerge in the young rats until the third week of administration. The activity level of young rats that received daily risperidone did not differ from controls after 1, 7, or 14 days of injections (i.e., on PNDs 15, 22, and 29) when tested 23.6 hours after the last injection. This is consistent with a recent study (Varela et al., 2014) that found that haloperidol treatment between PNDs 10–20 did not modify locomotor responses to saline on PNDs 21, 24, or 28. As discussed above, elevations in D₂ receptor density may be required for compensatory hyperactivity. If true, then the delayed emergence of this phenomenon in younger rats may indicate that APD-induced D₂ receptor up-regulation does not occur as rapidly early in postnatal development. However, Varela and colleagues (2014) recently reported that daily administration of haloperidol or aripiprazole between PND 10–20 elevates striatal D₂ receptor binding by PND 20 – a finding that does not align with this hypothesis. On the other hand, given that D₂ receptor expression normally peaks in the striatum at PND 28 and in the medial prefrontal cortex at PND 60 (Tarazi & Baldessarini,

2000), it is possible that, despite the ability of APDs to elevate D₂ receptors early in life (Varela et al., 2014), a full complement of D₂ receptors needs to be present or that dopamine receptor responsiveness to dopamine needs to be fully functional (Andersen, 2002) before APD-induced modifications can elicit the same behavioral changes in younger rats that they produce in adults.

The present study confirms that high levels of locomotor activity persist beyond the end of early-life risperidone administration. There was also a significant interaction between drug administration and sex: females administered risperidone early in life were more active than females administered vehicle across the entire testing period, whereas this effect, while statistically significant, was not quite as marked or consistent in the males administered risperidone early in life. The greater level of activity observed in the female rats at PND 49 that were administered risperidone during development fits with sex differences reported by others. For example, Wiley and Evans (2008, 2009) found that female rats between PNDs 30–39 appear to be more sensitive to the cataleptic effects of haloperidol and clozapine, and are more likely to demonstrate locomotor suppression after haloperidol administration. Since risperidone targets D₂ receptors, among other receptor sites, it is worth noting that striatal D₂ receptor densities are lower in adolescent female rats (Andersen et al., 1997), and that dopamine depletion, a state not completely unlike the one effected by APD administration, increases forebrain D₁ receptor immunoreactivity in young female but not male rats (Freund et al., 2014). Finally, electrically-evoked dopamine release is greater after cocaine and haloperidol administration in female rats relative to males (Walker et al., 2006). Given these data, the enhanced locomotor activity seen in females after the cessation of risperidone administration may be linked to developmental sex differences in dopamine receptor expression and dopamine's actions within forebrain synapses.

Spontaneous locomotor activity can be elevated in adult rodents 3–10 days after the cessation of chronic daily injections with haloperidol, thioridazine, or clozapine administration (see Muller & Seeman, 1979 for review). More recent studies of adult rats have demonstrated that locomotor responses to dopamine agonists are elevated after the cessation of chronic haloperidol or olanzapine treatment administered via minipump, but not through daily injections (Samaha et al., 2007; 2008; Bedard et al., 2011; 2013). Like this latter finding, adult rats administered daily risperidone injections for four weeks did not exhibit increased levels of locomotor activity one week after the last risperidone injection. More importantly, the absence of increased locomotor activity after risperidone administration in adulthood was in contrast to the persistent elevation in activity observed after early-life risperidone administration. Apparently, the biochemical changes that support the compensatory activity observed in rats maintained on daily risperidone injections, while quicker to appear within the course of treatment, are more transient when administration occurs in adulthood as opposed to early postnatal development.

We hypothesize that the diminution of dopamine receptor signaling during development caused by chronic risperidone administration produces a tonic state of adult forebrain dopamine function that begets a persistently greater level of locomotor activity. In the case of adult APD administration, the forebrain dopamine system has already been organized. So while adult APD administration rapidly alters dopamine function, these alterations are not

permanent, and forebrain dopamine function returns to its normal state within a swifter time frame than seen after early-life APD administration. Given the direct antagonism by risperidone of D₂ and 5HT_{2A} receptors, changes in these receptors induced by chronic risperidone (Moran-Gates et al., 2007; Choi et al., 2010) are the most likely contributors to the persistent behavioral changes seen in the risperidone-administered developing rats. However, one interesting difference between young versus adult rats is the ability of chronic risperidone administration to increase striatal D₁ receptor binding in the former group but not the latter group (Moran-Gates et al., 2007). Since agonists for D₁ receptors increase locomotor activity in rats (Diaz-Heijtz & Castellanos, 2006), changes in D₁ receptor expression could also explain the lasting elevation of locomotor activity found after daily risperidone administration during development. While risperidone has poor affinity for D₁ receptors, interactions between D₁ and D₂ receptors are known to occur at cellular, systems, and behavioral levels, such that blockade of the latter receptor could potential modify activity or expression of the former (LaHoste et al, 2000; Park et al., 2013). Moreover, blockade of 5HT_{2A} receptors can also change locomotor responses to D₁ receptor agonists (Bishop et al., 2003; 2005). Thus, long-term blockade of D₂ or 5HT_{2A} receptors could change the number or sensitivity of D₁ receptors in a manner that yields greater levels of locomotor activity. In addition, long-term receptor antagonism could also redirect downstream signaling pathways targeted by these receptors in a manner that permanently modifies protein expression (e.g., neurotrophins) linked to synaptic plasticity or structure (e.g., Thacker et al., 2006).

One factor to consider in interpreting the developmental data generated in the present study is the span of risperidone administration across many distinct phases of development: weaning, post-weaning, puberty, and adolescence (Spear, 2000; Anderson, 2002; Izenwasser, 2005; Marco et al., 2011). With many drugs, including APDs (Wiley, 2008), there are shifts in neurochemical and behavioral sensitivity that may be lost when drug administration encompasses multiple developmental time points. Continued research on the developmental effects of risperidone and other APDs will need to examine their impact during specific epochs of maturation and perhaps identify critical periods of sensitivity. Nonetheless, since many children receive APDs across different phases of development (early preschool through junior high and beyond; see Kalverdijk et al., 2008 and Olfson et al., 2012), preclinical studies will need to balance administration approaches that consider both common treatment practices and the possibility of age or phase-dependent effects.

The findings reported here add to basic and translational knowledge about the long-term effects of early-life APD treatment. The data indicate that while compensatory increases in locomotor activity induced by risperidone administration take longer to emerge in developing animals, they are more permanent than the effects induced by risperidone administration in adult animals. Given that risperidone is the most widely used APD in children (Domino & Swartz, 2008; Olfson et al., 2012), if the changes reported here are analogous to its effect in humans, then the timing, duration, and dose of risperidone used in children (e.g., Gleason et al., 2007) should be monitored closely in order to avoid permanent alterations in normal forebrain development.

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Figure 1.

Locomotor activity recorded for 40 minutes immediately after risperidone injection in a.) developing and b.) adult rats. Data represent mean activity $\pm$ s.e.m. on each postnatal day (PND) in mixed-sex groups ($n=12$ and 10 respectively in developing and adult cohorts). Asterisks indicate a significant difference between vehicle and 3.0 mg/kg risperidone dose on PND 74 . Double asterisks indicate significant differences between vehicle and both risperidone dose groups on each other listed PND. ^ indicates a significant difference between activity for a given dose group on a specific PND in comparison to activity observed in that group on all other PNDs.

Figure 2.

Locomotor activity recorded for 20 minutes at 23.6 h after risperidone injection in a.) developing and b.) adult rats. Data represent mean activity $\pm$ s.e.m. on each postnatal day (PND) in mixed-sex groups (n=12 and 10 respectively in developing and adult cohorts). Asterisks indicate a significant difference between vehicle and 3.0 mg/kg risperidone dose on PND 75. Double asterisks indicate significant differences between vehicle and both risperidone dose groups on each other listed PND.

Figure 3.

Locomotor activity recorded for 60 minutes seven days after last risperidone injection in PND 49 males (a.) and females (b.) and PND 109 males (c.) and females (d.). Data represent mean activity $\pm$ s.e.m (n = six & five per sex per dose at PND 49 and 109 respectively). A single asterisk in indicates a significant difference between rats administered risperidone 3.0 mg/kg in comparison to vehicle, and double asterisks indicate a significant difference between rats administered either risperidone dose and vehicle.

Figure 4.

Body weights recorded in a.) developing and b.) adult female (open symbols) and male (closed symbols) rats across the course of risperidone administration. Data represent mean weight (in grams) $\pm$ s.e.m. ($n = 6$ and 5 per sex in each group in a. and b.). In a., single asterisks indicate a significant difference between the combined vehicle and risperidone 3.0 mg/kg groups on PNDs 28 and 35, and the double asterisk indicates significant differences between the combined vehicle and each combined risperidone dose group on PND 21. In b., the asterisks indicate that male rats administered 3.0 mg/kg of risperidone weighed significantly less than male rats in other two groups on each PND except PND 74.