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Early-Life Risperidone Administration Alters Maternal-Offspring Interactions and Juvenile Play Fighting

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Abstract

Risperidone is an antipsychotic drug that is approved for use in childhood psychiatric disorders such as autism. One concern regarding the use of this drug in pediatric populations is that it may interfere with social interactions that serve to nurture brain development. This study used rats to assess the impact of risperidone administration on maternal-offspring interactions and juvenile play fighting between cage mates. Mixed-sex litters received daily subcutaneous injections of vehicle or 1.0 or 3.0 mg/kg of risperidone between postnatal days (PNDs) 14–42. Rats were weaned and housed three per cage on PND 21. In observations made between PNDs 14–17, risperidone significantly suppressed several aspects of maternal-offspring interactions at one-hour post-injection. At 23 hours post-injection, pups administered risperidone had lower activity scores and made fewer non-nursing contacts with their moms. In observations of play-fighting behavior made once a week between PNDs 22–42, risperidone profoundly decreased many forms of social interaction at one hour post-injection. At 23 hours post-injection, rats administered risperidone made more non-social contacts with their cage mates, but engaged in less social grooming. Risperidone administration to rats at ages analogous to early childhood through adolescence in humans produces a pattern of abnormal social interactions across the day that could impact how such interactions influence brain development.

Keywords

Antipsychotic; development; dopamine; social behavior; autism; forebrain

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All authors declare no conflict of interest.

The experiments reported here comply with current laws in the United States regarding the care and use of laboratory animals in research. The authors have full control of all primary data and agree to allow the journal to review their data if requested.

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1. Introduction

Over the last decade, antipsychotic drugs (APDs) have been used increasingly to treat a host of psychiatric disorders in children and young adolescents. In 1996, children made up 7% of the total population of APD users – a percentage that doubled by 2005 (Vitiello et al., 2009). The greatest rise in APD prescription rates among different age groups has been for children under the age of 5 (Constantine et al., 2011; Olfson et al., 2012). When used in pediatric populations, over 50% of APD prescriptions target children diagnosed with ADHD, with 20% of prescriptions are directed at children with intellectual disabilities and autism (Olfson et al., 2012). The most widely used APD in children is risperidone, with boys receiving a majority of the prescriptions (Domino & Swartz, 2008).

One primary issue regarding APD use, in adults as well as children, is the propensity for APDs to produce sedation (Cohen et al., 2012). In one study (Lemmon et al., 2011), this side effect was identified as the main reason for APD treatment failure in children. This raises a concern that APD-induced sedation could interfere with a number of daily activities in children with psychiatric and neurological disorders. One aspect of daily experience that may be disrupted by APD medication is social interaction between treated children and their parents, siblings, peers, and educators. Not only is there a concern regarding interactions when drug blood levels peak, but also during their daily trough. It is possible that, at this latter time point, compensatory behavioral reactions to the absence of drug, as is seen in animals (Muller & Seeman, 1978; Tadakoro et al., 2012), could emerge in children. The impact of APDs on social interaction in children is especially important for two reasons. First, alterations in social interaction during development influence brain maturation and later social competence, as reported in many animal studies (see below). Second, deficits in sociability (e.g., autism) are a symptom of most childhood psychiatric and behavioral disorders treated with APDs.

One indirect, yet informative approach to determining how APDs might affect social interaction is to examine their impact on developing, pro-social mammals. This strategy allows for the analysis of the immediate or delayed effects of APDs on social interaction within a controlled environment and without the intervening factors of brain abnormalities, additional medications, and a host of other factors encountered in clinical settings. The rat may serve as one of the better model systems in this regard for three reasons. The effects of APDs on behavior in adult rats are well established (see Bardgett, 2004 for review). The pattern of brain development seen in rats mirrors the trajectory observed in humans (Spear, 2000). Finally, rats demonstrate two well-characterized forms of social behavior during development: maternal-offspring interactions and juvenile play fighting (Cooke & Shukla, 2011; Parent & Meaney, 2008; Pellis, Field, Smith, & Pellis, 1997; Siviý & Panksepp, 2011).

Early in life, rats depend on their dams for nutrients and warmth, and as they approach the end of the pre-weaning stage of development at postnatal day (PND) 21, begin to investigate their environment more and interact more with their siblings. Much research has focused on the maternal-offspring interactions that occur within the first two weeks of life (see Meaney, 2001 for review), especially maternal licking and grooming. However, dams continue to

nurse and carry their pups well into third postnatal week. After weaning, and especially between the onset of puberty and the beginning of adulthood (PNDs 28-45), juvenile rats engage in play-fighting behavior that is marked by agonistic crawl overs and crawl unders, grooming, pouncing, and other social behaviors.

Much research has demonstrated the importance of adequate maternal stimulation during the preweaning period for adult behavior and brain physiology (see Meaney & Szyf, 2005, for review). Likewise, isolation from cage mates and individual variation in play-fighting behavior have been linked to neural and behavioral changes that persist into adulthood (Bell et al., 2010; also see Cooke & Shulka, 2011 for review). If administration of APDs early in life were to disrupt the patterns of maternal-offspring or adolescent social interaction, adult behavioral and brain function may be compromised. To this end, we have recently shown that rats administered risperidone early in life demonstrate locomotor hyperactivity as adults (Bardgett et al., 2013). It has yet to be determined if this change is due to a direct effect of risperidone on the brain or an indirect effect of risperidone on social interaction and other formative experiences during development.

As a means to begin addressing this latter idea, the primary goal of this research was to determine if early-life risperidone administration alters pre- and post-weaning social behaviors in rats. The first purpose of the study was to assess the effects of risperidone on maternal-offspring interactions from PNDs 14-17 at one hour and 23 hours post treatment. These observation times were chosen to capture the peak drug effects on behavior, and to determine if there are any residual or compensatory effects that emerged when blood drug levels should be at their lowest. Previous studies (Olsen et al., 2008; van Beijsterveldt et al., 1994) have shown that the plasma and brain half-lives of risperidone are approximately 1 and 3.5 h respectively. To confirm the time course of elimination from the plasma after injection, plasma risperidone and its active metabolite, 9-OH-risperidone were measured at 1, 5, and 23 hours post-injection. The second purpose of the study was to evaluate the effects of risperidone on play-fighting behaviors observed at 1 hour and 23 h post-administration. Observations were conducted once a week between PNDs 21-42.

2. Materials and methods

2.1 Subjects

A total of 36 Long Evans (18 male and 18 female) rats were used in the behavioral studies. All rats were derived from six litters (and dams) received from Harlan Laboratories (Indianapolis, IN) when the pups were seven days old. Data from one male and female pup per dose group per litter were included in the behavioral analyses. Pups were given subdermal injections of ink in their paws for identification purposes on PND 10. All pups were weaned on PND 21, given a single ear punch, and housed three per cage with members of the same sex. Housing cages were clear polypropylene cages (51 cm long × 26.5 cm wide × 32 cm high) with metal tops containing food and a water bottle that were available to the animals ad libitum. The lighting in the housing room for the rats was kept on a 12 hour on/off schedule with lights on at 06:30. Drug treatments and testing were performed between 08:00 and 14:00. The principles of laboratory animal care and all experimental procedures were carried out according to the Current Guide for the Care and Use of

Laboratory Animals (USPHS) under a protocol approved by the Northern Kentucky University Institutional Animal Care and Use Committee.

2.2 Daily Risperidone Administration

All rats received daily subcutaneous drug injections from PND 14 through PND 42. Rats received injections of 1.0 mg/kg of risperidone, 3.0 mg/kg of risperidone, or the vehicle used for the risperidone solution as a control. When the litters were weaned, rats were assigned to cages such that there was one rat from each treatment group represented within each cage. The National Institute of Mental Health's Chemical Synthesis and Drug Supply program kindly provided the risperidone. The risperidone solution was made by first dissolving it in 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. Daily injections occurred in a room just outside of the housing room immediately after each rat was weighed. The doses were selected on the basis of research demonstrating that developmental administration of these doses leads to behavioral changes during adulthood (Bardgett et al., 2013) and alters neurotransmitter receptor binding (Choi et al., 2009; Choi et al., 2010; Moran-Gates et al., 2007).

2.3 Plasma Risperidone Concentrations

Because this study considered the effects of risperidone on social interactions at 1 and 23 hours post-injection, the plasma concentrations of risperidone and its active metabolite, 9-OH-risperidone were determined at 1, 5, and 23 hours post-injection at PND 14. Thirty-six Long-Evans rats (16 males and 20 females), derived from six litters, were used. Rats received subcutaneous injections of risperidone and were rapidly decapitated at one of the three time points. Trunk blood was collected into ice-cold tubes. Plasma was obtained from centrifugation, and stored at -70°C until assayed. Because of the low volume of plasma from each rat pup, samples were pooled from two pups that were from different litters. Risperidone and 9-hydroxyrisperidone were detected using a modified liquid chromatographic method (LeMoing, Edouard, & Levron, 1993).

2.4 Maternal-Offspring Interactions

Observations of maternal-offspring interactions took place in the animal housing room between PNDs 14-17. Each pup was observed once over this four-day period at 1 hour and 23 hours post drug administration, and always within the same day. Observations were performed for twelve rats were on PND 14, ten rats each on PND 15 and 16, and four rats on PND 17, with approximately equal numbers of rats per risperidone administration group and sex observed each day. Each session involved a 20-minute observation of two pups from different treatment groups with their dam in the home cage. During this session, the other littermates were placed in a separate cage in a different room. The standard cage top was replaced with a clear Plexiglas top to allow for video recording. The two pups were labeled with either a large 'X' or 'O' for identification purposes with a black permanent marker. Each pup was observed for twenty seconds in an alternating manner with a ten second break between observations such that each pup was observed twenty times within the 20-minute session.

The home cage was placed on a table within the housing room and behavior within the cage was recorded with a Canon digital video camcorder centered above the cage. For each 20 second observation, the following behaviors were tallied: 1) dam contacting pup, 2) dam carrying pup, 3) pup contacting dam, 4) pup making a nursing contact, and 5) pup contacting the other pup. The duration of pup nursing and non-nursing activity was rated separately within each 20-second observation period using the following scale: 0 (never), 1 (rarely - activity less than 3 seconds), 2 (sometimes - activity more than 3 seconds), and 3 (always active). Nursing activity was defined as attempts to nurse or actual nursing on the part of the pup, and non-nursing activity was defined as all other forms of ambulatory activity. At the beginning and end of each observation, the proximity of the observed pup from the mother was measured on a scale of 1 (opposite side of cage) to 4 (in contact with dam). Total scores compiled over the 20-minute session for each measure were used for all statistical analyses. The observer was blind to the treatment status of the pup.

2.5 Play-Fighting Observations

The second experiment measured play fighting among three cage mates once a week for three weeks (PND 22-42) for 12 minutes. On each of the three weeks of testing, each animal was observed at 1 and 23 hours post drug administration. The animals were marked with a large 'X' or 'O' with a marker or left unmarked for identification purposes. The recording and observation process was the same as described as above, except each animal was observed for 20 seconds eight times over the 12 minute session. For each 20 second observation, receptive or expressive instances of the following social behaviors exhibited by the target rat were tallied separately: boxing, pounces, pins, grooming, sniffing, crawling over, crawling under, and general contacts (i.e., the target rat comes into contact with another rat). We used the definitions of these various behaviors provided by Poole and Fish (1976), Parent and Meaney (2008) and Varlinskaya and Spear (2008) as guides for distinguishing these different behaviors and for determining which rat initiated boxing, pins, and pounces. During observations, the duration of time spent self-grooming and being active (defined as ambulatory motion) was rated separately on the scale of 0 (never) to 3 (always) defined above. The proximity between the target pup and the nearest other pup was measured on a scale of 1 (opposite side of cage) to 4 (in contact with another pup) at the beginning and end of the observation period. Total scores compiled for each rat across the 12 minutes session on each measure were used in the statistical analysis. The observer was blind to the treatment status of the pup.

2.6 Statistical Analysis

For the maternal-offspring observations, data for each measure were analyzed using a three-way analysis of variance (ANOVA) with drug treatment and gender as between-subjects factors and time (one hour and 23 hour) as a within-subject factor. Since these analyses indicated no treatment x gender interactions for any of the measures, subsequent tests were conducted using a two-way ANOVA with treatment and time as factors. For the play-fighting observations, a three-way ANOVA was also used to analyze the data for each measure with drug treatment and gender as between-subjects factors and week as a within-subject factor. Data for 1 or 23 hours post-administration were analyzed separately. Alpha

levels were set at $p < .05$ for all analysis of variance tests. Post-hoc testing was performed using a Fisher PLSD test with a significance level set at $p < .05$.

3. Results

3.1 Plasma Risperidone Concentrations

Plasma concentrations of risperidone and its active metabolite, 9-OH-risperidone, were determined at 1, 5, and 23 hours after subcutaneous administration of the 3.0 mg/kg dose of risperidone. The risperidone concentrations found at one hour after injection of the 3.0 mg/kg dose (Table 1) were 1–3 times greater than the plasma levels reported in rats after injection of risperidone doses between 0.6 – 1.0 mg/kg (Kapur, VanderSpek, Brownlee, & Nobrega, 2003; Olsen, Brennum, & Kreilgaard, 2008). At five hours, the plasma risperidone concentration was at a level consistent with therapeutic plasma levels found in humans (Kapur, Zipursky, & Remington, 1999). Risperidone concentrations were below the limit of detection at 23 hours post-injection. The plasma concentrations of 9-OH-risperidone followed the same time course as the pattern seen for risperidone. The rapid peak and decline of each compound within hours after administration are consistent with other time-course studies in rats of plasma risperidone concentrations following subcutaneous injection (van Beijsterveldt et al., 1994; Olsen et al., 2008).

3.2 Maternal-Offspring Interactions

The effects of risperidone treatment on maternal-offspring interactions were studied at one and 23 hours post-treatment from PND 14-17. Initial statistical analyses did not reveal any effects of sex or sex x drug administration interactions on any of the measures; also there were no interactions between drug administration and postnatal test day. Therefore the statistical analyses presented here used a mixed-factor, two-way analysis of variance that assessed the main effects of post-injection time (one and 23 hours) and drug administration (vehicle, risperidone 1.0 or 3.0 mg/kg). There were no significant effects of drug administration or interactions between drug administration and post-injection time on the number of contacts made by the mom with the observed pup, or the number of times that the observed pup was carried by the mom, or the observed pup's proximity to the dam. However, there were several significant effects of drug administration or interactions between drug administration and post-injection time on the remaining measures of maternal-offspring interactions.

Pups administered risperidone made fewer contacts with their moms at one and 23 hours post-injection (Drug effect: $F(2, 33) = 4.24, p = .02$) (Figure 1a). At one hour post-injection, both risperidone groups made fewer non-nursing contacts with their mom than the vehicle group ($p = .04$ & $.05$ for the respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle). Although the number of contacts for all groups increased at 23 hours, the vehicle-administered pups continued to make more contacts with the mom than the risperidone-administered pups ($p = .03$ & $.06$ respectively for the 1.0 and 3.0 mg dose groups versus vehicle). Pups administered risperidone also made fewer contacts with the other observed pup at one hour post-injection (Drug x time interaction: $F(2, 33) = 3.49, p = .04; p = .007$

& .01 for the respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle) (Figure 1b). At 23 hours post-injection, there was no significant difference between the drug groups.

As for nursing-related behaviors, there were significant drug x time interactions found for the number of nursing contacts and the amount of time spent nursing (Drug x time interactions: $F(2, 33) = 9.77, p < .001$, & $F(2, 33) = 9.42, p < .001$ for each respective measure). At one but not 23 hours post-injection, pups that were administered either dose of risperidone made significantly fewer nursing contacts ($p = .004$ & $.007$ for the respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle) (Figure 1c) and spent less time nursing ($p = .01$ & $.003$ for the respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle) (Figure 1d). Finally, there was a significant drug x time interaction ($F(2, 33) = 9.52, p < .001$) for the activity measure (Figure 1e). Pups that received either risperidone dose were significantly less active one hour after injection ($p < .001$ for each comparison of 1.0 and 3.0 mg dose groups to vehicle). At 23 hours post-injection, all groups were more active but the 3.0 mg dose group was significantly less active than the vehicle group ($p = .02$) and there was a trend towards less activity in the 1.0 mg group in comparison to vehicle ($p = .09$).

3.3 Play-Fighting Interactions

The effects of risperidone on play-fighting behaviors were observed once a week at one and 23 hours post-injection over the last three weeks of drug administration. Because data from three weeks of play-fighting observations were analyzed as opposed to the single week of data generated in the maternal-offspring observations, separate analyses were conducted on the one hour and 23 hour post-injection, play-fighting data using sex and drug as between-groups factors and week as a repeated-measure.

At one hour post-injection, there were no significant effects of week or week x drug interactions and no significant gender x drug interactions, so the data are presented in Table 1 as a function of drug group. At this time, rats administered vehicle directed the following behaviors towards their cage mates more than rats administered either dose of risperidone: social pouncing (Drug effect: $F(2, 33) = 5.71, p = .007$; $p = .008$ & $.005$ for respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle), social grooming (Drug effect: $F(2, 33) = 6.82, p = .003$; $p = .004$ & $.002$ for respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle), and social sniffing (Drug effect: $F(2, 33) = 6.72, p = .004$; $p = .003$ for each comparison of the risperidone groups to vehicle) (Table 2). Rats administered risperidone were less likely to make general contacts with their cage mates (Drug effect: $F(2, 33) = 35.37, p < .001$; $p < .001$ for each comparison of the risperidone groups to vehicle), but were more likely to receive such contacts from their cage mates that were administered vehicle (Drug effect: $F(2, 33) = 19.79, p < .001$; $p < .001$ for each comparison of the risperidone groups to vehicle) (Table 2). Rats administered risperidone were more likely to be located farther away from the nearest cage mate when observed (Drug effect: $F(2, 33) = 7.37, p = .002$; $p = .02$ & $< .001$ for respective comparisons of the 1.0 and 3.0 mg dose groups to vehicle), and their activity and self-grooming scores were significantly lower at one hour post-injection (Respective drug effect's on activity and self-grooming: $F(2, 33) = 36.08$ & $17.21, p < .001$ for each measure; $p < .001$ for all comparisons of the risperidone

groups to vehicle) (Table 2). Significant drug effects were absent for the following measures: receptive or expressive boxing and pins, receptive pounces, social grooming, and social sniffing, and crawling over or under. These non-significant effects were likely due to the very low incidences of these behaviors.

At 23 hours post-injection, rats administered risperidone engaged in less expressive social grooming than the rats administered vehicle (Drug effect: $F(2, 33) = 10.21, p < .001$) (Figure 2a). Post-hoc tests indicated that these drug effects were significant during the first two weeks of the play-fighting observations ($p = .02$ & $.03$ for respective comparisons between the 1.0 mg and vehicle groups during the first two weeks; $p = .01$ & $.001$ for respective comparisons between the 3.0 mg and vehicle groups during the same two weeks). On the other hand, risperidone-administered rats made more general contacts with their cage mates during the second and third weeks of observation (Drug x time interaction: $F(4, 66) = 4.80, p = .002$) (Figure 2c). During the first week of observations, there was a trend towards less frequent general contacts in the rats administered the high risperidone dose in comparison to the vehicle group ($p = .08$). However during the second and third observation weeks, rats in both risperidone groups made more contacts with cage mates than the vehicle rats ($p = .03$ & $.03$ for respective comparisons between the 1.0 mg and vehicle groups during the last two weeks; $p = .009$ & $< .001$ for respective comparisons between the 3.0 mg and vehicle groups during the same two weeks). Likewise, the rats in the risperidone groups received fewer non-social contacts from their cage mates during the last two weeks of observations (Drug x time interaction: $F(4, 66) = 4.06, p = .005$) (Figure 2d). There was a statistical trend towards rats in the low risperidone dose group ($p = .10$) and high risperidone dose group ($p = .08$) receiving fewer general contacts during the second week of testing. There was a similar trend during the third week for the high risperidone group ($p = .07$). On the other hand, rats administered the high risperidone dose received significantly more general contacts during the first observation week relative to the vehicle group ($p = .01$).

Significant effects of the week of testing were found for the crawl under, proximity to the nearest cage mate, and pup activity measures. Rats crawled under their cage mates less (Time effect: $F(2, 66) = 4.63, p = .01$; $p = .01$ for each comparison of PNDs 29-35 and PNDs 36-42 to PNDs 22-28) (Figure 2b) and were less active as the three weeks of testing progressed (Time effect: $F(2, 66) = 3.19, p = .05$; $p = .02$ and $.07$ for respective comparisons of PNDs 29-35 and PNDs 36-42 to PNDs 21-28) (Figure 2f). Proximity to the nearest cage mate increased over the three weeks (Time effect: $F(2, 66) = 11.87, p < .0001$; $p = .0037$ and $< .0001$ for respective comparisons of PNDs 29-35 and PNDs 36-42 to PNDs 22-28) (Figure 2e). Finally, the time spent self-grooming was greater during PNDs 36-42 in comparison to the level measured during PNDs 29-35 (Time effect: $F(2, 60) = 3.72, p = .03$; $p = .0074$ for comparison between PNDs 29-35 and PNDs 36-42) (Figure 2g). Interestingly, there was an overall drug x sex interaction ($F(2, 30) = 4.39, p = .02$) (Figure 2h) for this measure. Male rats administered vehicle engaged in more self-grooming than did male rats administered the high risperidone dose ($p = .003$) and the females administered vehicle ($p = .008$). In comparison to the vehicle males, there was a trend towards less time spent grooming in the females administered the low ($p = .06$) or high doses ($p = .07$) of risperidone.

4.0 Discussion

This is the first study to assess the effects of risperidone, the most widely prescribed APD in children, on maternal-offspring interactions and juvenile play fighting in rats. Given risperidone's ability to suppress ongoing behavior in adult rats (Bardgett, Humphrey, & Csernansky, 2002), it was not surprising that several indices of social and non-social behavior were reduced in rat pups one hour after risperidone administration. These data are nonetheless important to report since reductions in ongoing behavior during brain development could alter the rate or trajectory of neural maturation or indirectly modify it via reduced maternal or peer interactions. A unique aspect of this study was its consideration of the effects of risperidone administration at 23 hours after drug injection, when risperidone was not detected in the plasma. Given the profound behavioral effects of risperidone seen immediately after administration, this approach was included in order to determine if compensatory changes in behavior occurred in the relative absence of drug. Several aspects of maternal-offspring interactions and play-fighting behavior were significantly altered at 23 hours post-risperidone administration, although not all changes (e.g., risperidone-treated juveniles made more general contacts with conspecifics but engaged in less social grooming) could be characterized as compensatory.

Several studies have assessed the behavior of rat dams and their pups after maternal APD administration (Li, Budin, Fleming, & Kapur, 2005; Li, He, & Heupel, 2011; Pereira et al., 2011). To our knowledge, this is the first report of the effects of APDs on maternal-offspring interactions after direct drug administration to the pups. Administration of older APDs, such as haloperidol and clozapine, suppresses ongoing behavior in preweaning and juvenile rats (Coyle, Napier, & Breese, 1985; Wiley, 2008). Therefore, it was of little surprise that risperidone reduced many indices of pup interactions with the dam at one hour post-administration. Notably, the number of non-nursing-type contacts made by the dam with the pups did not differ between the drug groups. This suggests that dams still attend to drug-treated pups despite their decrease in ongoing behavior.

At 23 hours post-injection, pups administered risperidone made fewer non-nursing contacts with their dams, and were slightly less active than the vehicle control pups. These changes were not a simple consequence of reductions in all behaviors since the rats administered risperidone were not significantly different from the vehicle rats on any other measure taken at the same time. There was a mean increase in the time spent nursing in each of the former groups that could explain some of their decreases in pup-mom, non-nursing contacts and overall pup activity. These results demonstrate that risperidone administration during the preweaning period not only alters behavior and social interactions immediately after administration but also modifies normal patterns of behavior and social interaction long after administration.

There are some caveats to the maternal-offspring observations. First, each pup was tested only once between PNDs 14-17, and the data collected over this time were collapsed. It is possible that this approach missed possible maturational changes that occurred over these four days. This concern is mitigated by the finding that there were no statistical interactions between drug administration x PND for any of the measures. Another issue entails the

timing of the observations during the third postnatal week, since most studies of maternal-offspring interactions tend to focus on the first two weeks of life (Parent & Meaney, 2008; Power & Moore, 1986), with the third week of life marked by less licking and grooming by the dam. However, since few APDs are prescribed very early in life, it was felt that starting risperidone administration at PND 14 and characterizing its behavioral effects thereafter would represent a more clinically relevant approach.

In the observations of play fighting, it was found that risperidone suppressed many aspects of social behavior immediately after injection. Expressive behaviors, such as social pouncing, grooming, and sniffing, and general contacts with cage mates were dramatically reduced by risperidone. As a consequence, vehicle-treated rats received fewer general contacts during the same period. These changes are likely related to the reduction in general activity elicited by risperidone. These effects were observed consistently across the three weeks of testing, and there were no significant interactions between risperidone administration and sex.

While this is the first study to examine the effects of risperidone on play-fighting behavior, earlier studies (Beatty, Costello, & Berry, 1984; Holloway & Thor, 1985) assessed play-fighting behavior after haloperidol administration. These studies found that haloperidol administered up to 30 minutes before testing decreased play-fighting behavior and activity in younger (PNDs 26–32; Beatty et al., 1984) and older (PNDs 36–41; Holloway & Thor, 1985) juvenile rats. The actions of risperidone observed in the present study are consistent with these reports. Commonalities in the affinities of risperidone and haloperidol for D₁, D₂, D₃, D₄, and α_1 receptors in rat brain (Bymaster et al., 1996; Schotte et al., 1996) suggest that antagonism of one or more of these receptors accounts for the similar acute effects of haloperidol and risperidone on juvenile play fighting and preweaning social interactions.

At 23 hours post-injection, rats administered risperidone contacted other pups more but less of this contact was of a social nature. Rats administered risperidone made more general contacts with their cage mates during the second and third weeks of testing and likewise received fewer contacts during those times. But they also engaged in less social grooming of their cage mates, especially between PNDs 22 and 35. Male rats that received the high dose of risperidone even groomed themselves less when compared to vehicle-treated males. It appears, as was discovered with maternal-offspring interactions, that not all elements of social play return to normal during the trough of daily risperidone administration, but are reconstituted in a way that could alter the nurturing influence of such play on behavioral and brain development.

Some studies of juvenile social behavior (e.g., Sivi, Crawford, Akopian, & Walsh, 2011; Taylor, 1980; Varlinskaya & Spear, 2008; see Pellis, Field, Smith, & Pellis, 1997 for review) have incorporated social isolation prior to testing and testing with unfamiliar conspecifics as a means to elicit play fighting among rats (Varlinskaya & Spear, 2008). However, other studies (Beatty et al., 1984; Parent & Meaney, 2008) have examined juvenile social interactions among group-housed, familiar rats. We took this latter approach to minimize any stress associated with exposure to social isolation, novel environments, or unfamiliar peers. This approach likely biased our study towards lower social interaction and

play-fighting scores, but was intended to allow for a more naturalistic account of risperidone's impact on social behaviors among familiar peers. Future research should consider if social isolation and interaction with unfamiliar rats can differentially affect social play in juveniles administered risperidone. It may also be worthwhile, as a comparison, to examine adult social interactions after risperidone administration, although such an approach would need to be mindful of the qualitatively different nature of rat social interactions during adulthood relative to adolescence (see Varlinskaya & Spear, 2008 for a review).

What remains to be discovered is how chronic (or even acute) APD administration during development modifies the function of brain circuits involved in maternal-offspring or sibling interactions. Social play requires several brain regions that also regulate sexual, aggressive, and social behavior (Cooke & Shukla, 2011; Siviý & Panksepp, 2011). Many of these regions use dopamine as a neurotransmitter, which has been associated with play-fighting intensity. For example, Siviý et al. (2011) linked the deficits in play-fighting behavior seen in Fisher 344 rats (as compared to Sprague-Dawley rats) to reductions in dopamine content, turnover, and release in the frontal cortex and striatum. These decreases are of interest, since early-life haloperidol administration also leads to deficient forebrain dopamine content during adulthood (Cuomo et al., 1981; Cuomo et al., 1983; Scalzo & Spear, 1985). While early-life risperidone administration has yet to be linked to changes in neurotransmitter content or release, it does modify dopamine, serotonin, and glutamate receptor binding in the forebrain upon cessation of treatment during adolescence (Choi et al., 2009; Choi et al., 2010; Moran-Gates et al., 2007). However, these studies of early-life APD administration do not address their more immediate effects on neurotransmitter function at earlier pre- and post-pubertal time points, which would be germane to the goal of linking APD-induced alterations in play to specific modifications in brain neurotransmitter function.

We deliberately chose to examine the effects of risperidone in normal rats and not in an animal model of a single childhood psychiatric disorder since APDs are used to treat several different disorders, and are likely used in some cases where pathophysiology is absent. Moreover, a universally accepted animal model of any single pediatric psychiatric disorder (e.g., autism, ADHD, disruptive behavioral disorder) has yet to be established. Nonetheless, it is noteworthy that risperidone is approved for use in children with autism, a disorder that is characterized by impaired social interaction. Some clinical studies (Jesner, Aref-Adib, & Coren, 2007; Scahill et al., 2013), but not all (Kent et al., 2013), have shown that risperidone alleviates social withdrawal in autism. From a translational point of view, our results suggest that the clinical impact of risperidone on developmental social behavior be monitored not only during the peak of active treatment but also throughout an entire dosing period. Our findings also highlight the need to limit APD doses that reduce behaviors required not only for ongoing social relationships but normal brain development. For example, we have recently shown that early-life risperidone administration causes locomotor hyperactivity during adulthood (Bardgett et al., 2013). The data described here suggest that such changes in adult behavior may not only be rooted in the direct effects of early-life APDs on neuronal function, but also linked indirectly to alterations in social behavior produced by APDs during development.

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Highlights

- Risperidone reduces rat pup interactions with dams immediately after administration.
- At 23 hours post-risperidone administration, pups contact dams less.
- Juvenile play fighting is decreased immediately after risperidone administration.
- At 23 hours post-risperidone administration, juveniles engage in less social grooming.

Fig. 1.

Measures of maternal-offspring interactions at 1 and 23 hours post-injection. Data represent means $\pm$ s.e.m. A single asterisk indicates a significant difference between the Vehicle group and the Risp 3.0 group at a single post-injection time point. Double asterisks indicate statistically significant differences between the Vehicle group and both the Risp 1.0 and Risp 3.0 groups at a single post-injection time point. There was a trend towards a difference between Vehicle and Risp 1.0 group at 23 hours in a. and e. as indicated by the *p* values. *n* = 12 for each group.

Fig. 2.

Measures of play-fighting behavior at 23 hours post-injection across three weeks of testing (a.-g.). In h., the data from the three weeks are averaged as a function of sex. Data represent mean total score $\pm$ s.e.m. A single asterisk indicates a significant difference between the Vehicle group and the Risp 3.0 group during a given week, except in h. where an asterisk differentiates Vehicle males from Risp 3.0 males and Vehicle females. Double asterisks indicate statistically significant differences between the Vehicle group and both Risp 1.0 and Risp 3.0 groups during a given week. There were trends towards a difference between Vehicle and Risp 3.0 group at PNDs 22-28 in c., and at PNDs 29-35 and PNDs 36-42 in d., as indicated by the *p* values. In h., there were trends towards lower self-grooming scores in the female rats from the Risp 1.0 and 3.0 groups relative to the males from the Vehicle group. *n* = 12 for each group, except for h. where *n* = 6 per group.

Table 1

Plasma concentrations of risperidone and its metabolite, 9-OH-risperidone

Drug	Time post-injection		
	1 hour	5 hours	23 hours
Risperidone (ng/ml)	550.7 $\pm$ 13.5	14.0 $\pm$ 1.3	Trace < 2.0
9-OH-risperidone (ng/ml)	379.3 $\pm$ 18.0	144.8 $\pm$ 9.1	Trace < 2.0

Data represent mean $\pm$ s.e.m.. Samples were pooled from two rats, and there were 6, 5, and 5 samples for each time point, respectively. At 23 hours post-injection, the concentrations were below 2.0 ng/ml.

Table 2

Risperidone reduces play-fighting behavior at one hour post-administration.

Behavioral measure	Vehicle	Risperidone group	
		1.0 mg/kg	3.0 mg/kg
Social pouncing	0.47 ± 0.19	0.03 ± 0.03	0.00 ± 0.00
Social grooming	0.40 ± 0.14	0.03 ± 0.03	0.00 ± 0.00
Social sniffing	0.53 ± 0.13	0.14 ± 0.06	0.14 ± 0.05
General contacts made	3.39 ± 0.49	0.39 ± 0.15	0.22 ± 0.10
General contacts received	0.75 ± 0.15	3.17 ± 0.38	3.61 ± 0.44
Proximity to nearest pup	30.52 ± 0.31	28.16 ± 0.83	27.04 ± 0.71
Activity	10.69 ± 1.38	1.81 ± 0.49	1.61 ± 0.30
Self-grooming	3.42 ± 0.78	0.17 ± 0.09	0.11 ± 0.06

Data represent total scores from each week averaged across the three weeks ± s.e.m.. See the Materials and Methods (section 2.5) for definitions of each measure. n =12 per group. Scores for the vehicle group were significantly different ($p < .05$) from each risperidone-treated group as indicated by boldface.