Stimulant and Antipsychotic Drug Co-Administration: Long-Term Effects on Behavioral Development.

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Abstract

The use of antipsychotic (AP) drugs for childhood psychiatric disorders has increased dramatically over the last 20 years. These drugs are prescribed mainly for disorders such as attention-deficit hyperactivity disorder (ADHD). Most children who are prescribed APs also receive treatment with psychostimulant drugs. However, little research has been done addressing the long-term effects of early-life co-administration of APs and stimulant medication. This study examined the long-term behavioral effects of co-administration of risperidone (RISP), the most widely prescribed AP in children and dextroamphetamine (DAMP), a commonly prescribed psychostimulant, during development in male Long-Evans rats. Changes in gross locomotor activity were assessed. It was found that RISP alone decreased activity after administration, and DAMP alone increased activity. The combination of RISP and DAMP did not cause any significant changes in locomotor activity relative to controls. Early-life RISP administration did not increase activity later in life, or have an effect on locomotor responses to a DAMP challenge during adulthood. Early-life DAMP administration led to increased activity during early adulthood, but it did not affect locomotor responses to DAMP challenge. These studies raise concerns about the immediate effects of APs and psychostimulants on children and about the long-term effect of stimulant use during childhood on adult behavior.

Stimulant and Antipsychotic Drug Co-Administration: Long-Term Effects on Behavioral Development.

Overview

The use of AP drugs for childhood psychiatric disorders has increased dramatically over the last 20 years. These drugs are prescribed mainly for disorders such as ADHD, for which they have not been approved. Little research has been done to determine long-term AP exposure during development and the effect on behavior and brain function later in life. Since most children who are prescribed APs also receive concomitant treatment with psychostimulant drugs, research on the long-term effects of early-life AP and stimulant co-administration would be relevant and beneficial.

Clinical uses of APs and mechanism of action

Disorders of psychosis include schizophrenia and schizoaffective disorder, and the most common treatment for these disorders is AP or neuroleptic medication. There are two classes of APs: typical/first generation and atypical/second generation. Typical APs were developed first, and their method of action is characterized by dopamine D_2 receptor antagonism. They mainly alleviate the positive symptoms of schizophrenia such as paranoia, delusions, and agitation. Typical APs are also known to produce extrapyramidal symptoms such as tardive dyskinesia or dystonia. These are irreversible motor disorders that involve involuntary movement especially of the face, hands, and tongue (Lieberman et al., 2005). Atypical or second-generation APs possess a method of action characterized by D_2 receptor affinity in addition to greater affinity for other neurotransmitter receptors such as serotonin and norepinephrine. Second generation AP's mitigate not only the positive symptoms of schizophrenia, but they can also relieve the negative symptoms such as catatonia and extreme apathy. Second generation APs are thought to produce

fewer extrapyramidal symptoms than first generation AP's although high doses have still been shown to produce these unwanted motor side effects (Lieberman et al., 2005). APs often cause metabolic side effects that younger people are more often affected by, and include increases in insulin sensitivity that can lead to diabetes, and hormonal changes that can cause weight gain and sexual dysfunction (Olfson et al., 2012).

RISP is an atypical AP that is FDA-approved for the treatment of schizophrenia in adults and adolescents aged 13-17 years, bipolar I disorder in adults and children and adolescents aged 10-17 years, and irritability related to autism in children and adolescents aged 5-16 years (U.S. Department of Health and Human Services, 1993). It acts mainly as an antagonist for dopamine D_2 and the serotonin 5-HT_{2A} receptors. This antagonism blocks access of dopamine and serotonin to post-synaptic neurons in the mesolimbic and nigrostriatal pathways in the brain, which include the nucleus accumbens and frontal cortex, and the caudate-putamen, respectively (Bardgett, 2004). This pharmacological action may counter the hyperactivity of dopamine neurons that is thought to take place in schizophrenia (Liddle, Lane, & Ngan, 2000). RISP's actions in the mesolimbic pathway have been linked to its ability to alleviate psychosis, whereas its actions in the nigrostriatal pathway have been associated with the ability of high RISP doses to elicit motor side effects (Bardgett, 2004).

Increased use of APs in children

In the past 20 years, APs have become one of the most commonly used medications in the United States and Canada, and their use and prescription are increasing especially in youth (Murphy et al., 2013; Olfson, Blanco, Liu, Wang, & Correll, 2012). A study by Olfson and colleagues (2012) found that the number of clinician visits that involved AP prescriptions rose approximately four-fold between the mid-1990's to the mid-2000's (Olfson et al., 2012). The

rate at which APs are prescribed to children has, in some cases, surpassed the use of APs in adults. For example, a significantly larger portion of psychiatric visits involving APs occurred for children (67.7%) and adolescents (71.6%) than for adults (50.3%)(Olfson et al., 2012). According to a large survey collected on 1,419 Canadian children, 31% were prescribed RISP for ADHD - a disorder for which RISP use has not been approved by the FDA - followed by 21% who received it for psychosis (Murphy et al., 2013). Not only is the amount of off-label use alarming, but most AP prescriptions to youth are written by general practitioners and not by psychiatric specialists (Murphy et al., 2013).

Although APs have immediate physical side effects in children, little is known about the long-term effects of AP use during youth on behavioral and neural development. A recent study by Bardgett and colleagues (2013) showed that when RISP is administered to young rats, they show hyperactivity later in life. They are also more sensitive to the locomotor-activating effects of the stimulant drug, DAMP, when the latter drug is administered acutely during adulthood (Stubbeman, Brown, & Bardgett, 2014). One possible mechanism for this behavioral outcome is an increase in forebrain dopamine receptors, since early-life treatment with RISP up-regulates these receptors (Moran-Gates et al., 2007). However, early-life AP administration also reduces forebrain dopamine synthesis later in life (Cuomo et al., 1981; Velley et al., 1975; Vinnish et al., 2013). Thus, early-life AP administration could lead to a hyper-dopaminergic state (e.g., more dopamine receptors) coupled to a hypo-dopaminergic state (e.g., less dopamine) that would not necessarily offset each other at a behavioral or neural level.

AP and stimulant co-administration in children

Co-prescription of APs and stimulants is common in youth. A study done in Canada from 2000-2007 involving 1270 subjects reported that approximately 20% of AP users aged 16-25

were also prescribed a stimulant (Murphy et al., 2013). A study in the United States reported even higher percentages with 54.1% of subjects aged 0-13 and 30.3% of subjects aged 14-20 being prescribed AP's and stimulants at the same time (Olfson et al., 2012). Like the situation with the use of APs alone in children, there is no information about the long-term consequences of combined AP-stimulant exposure at an early age.

Psychostimulants and their effects on brain development

In the last decade, stimulant prescriptions in young people have increased. A 12-year study examining the trends of stimulant use in children and adolescents in the United States found that stimulant use increased by an estimated 3.4% (Zuvekas & Vitiello, 2012). The same study also reported that approximately 3.5% of children in the U.S receive stimulant medication. Taking the growth rate and population size into account (U.S. Census Bureau, 2013), an estimated 2.6 million children in the U.S. were prescribed stimulants in 2014 (Zuvekas & Vitiello, 2012).

Stimulants are drugs that increase arousal, and are intended to improve physical or mental processes. Dextroamphetamine (DAMP) or Dexedrine is a central nervous system stimulant that is FDA approved for the treatment of ADHD and narcolepsy in people over the age of six (United States Department of Health and Human Services, 2007). Although the exact mechanism of DAMP's alleviation of ADHD is unknown, it is known that DAMP and other amphetamines elicit the release of monoamines such as dopamine and norepinephrine from the neurons (Anderson & Navalta, 2004). DAMP preferentially stimulates nigrostriatal and mesolimbic circuits, and activation of these circuits by DAMP has been implicated in its euphoric and cognitive effects (Willson, Wilman, Bell, Asghar, & Silverstone, 2004).

Effects of stimulant use on neural and behavioral development

Stimulant use during development may have permanent, tangible effects on brain structure and function. Stimulant use during early development in rats reduces dopamine transmission and extracellular dopamine levels in the brain later in life (Anderson, 2005). Rats administered amphetamine early in life self-administer significantly higher amounts of cocaine as adults (Brandon et al., 2001). This suggests that these animals possess lower dopamine levels as they may be self-administering drugs in order to boost their dopamine levels. It has also been hypothesized that amphetamine affects neurodevelopment specifically by increasing dendritic branching of dopamine neurons in the prefrontal cortex (Diaz Heijtz et al., 2003). This could be viewed as problematic since it may represent a disruption of the natural, age-normative "pruning" of dopaminergic synapses or receptors (Berman, Kuczenski, McCracken, & London, 2008; Diaz Heijtz et al., 2003).

Behavioral outcomes after co-administration of APs and stimulants during development

At first glance, RISP and DAMP seem to work against each other at a synaptic level. Risperdone antagonizes D_2 receptors on postsynaptic neurons, which serves to block dopamine signals. It also antagonizes presynaptic D_2 autoreceptors, which leads to increased dopamine synthesis and release (Cuomo et al., 1981; Velley et al., 1975). In comparison, DAMP increases dopamine synthesis and release (Anderson & Navalta, 2004).

However, as reviewed above, studies assessing the long-term effects of AP or stimulant administration during development suggest that each drug produces a similar outcome, namely decreased dopamine neurotransmission. It is possible that the excessive release of dopamine caused by a combination of an AP, such as RISP, and a psychostimulant, such as DAMP, would exhaust presynaptic dopamine stores (Cuomo et al., 1981; Velley et al., 1975; Anderson, 2005)

more than either drug alone. Since the brain is developing during such co-administration, the affected neurons may *permanently* their capacity to synthesize and release dopamine, as opposed to the *temporary* compensatory reaction that could occur in adulthood after AP and DAMP co-administration.

This hypothesized decrease in dopamine synthesis and release may create an ADHD-like behavioral state (Blum, Chen, & Oscar-Berman, 2008) in the rat that is more exaggerated than the hyperactive state seen in adult rats treated with RISP alone during development (Bardgett et al., 2013). Moreover, the AP and DAMP combination during development may also enhance sensitivity to psychostimulants such as DAMP or cocaine during adulthood, since early-life exposure to either of the former drugs alone appears to increase the behavioral effects of DAMP during adulthood (Stubbeman et al., 2014) or the likelihood to self-administer cocaine (Brandon et al., 2001).

Purpose

This study examined the long-term behavioral effects of RISP and DAMP co-administration during development. The main dependent variable was locomotor activity based on previous work that has shown this behavior is altered after early-life RISP administration (Bardgett et al., 2013). The expected deficiency in the dopaminergic system of adult rats administered both drugs early in life was hypothesized to increase locomotor activity and locomotor sensitivity to DAMP more than administration of either drug alone or vehicle during development.

Method

Animals and housing

Thirty-six Long-Evans male rats were used. Pregnant mothers were purchased from Harlan Bioproducts (Indianapolis, IN) and arrived in the animal facility on gestational day 14. On postnatal day (PND) 8, pups were identified by sex, litters culled to four males, and paw-tagged for identification purposes. Subjects were weaned on PND 21 and ear clipped for identification. Upon weaning, subjects were housed two per cage with continuous access to food and water, except where noted. The lighting schedule consisted of lights on at 6:30 a.m. and off at 6:30 p.m. The Northern Kentucky University Institutional Animal Care and Use Committee approved all of the proposed procedures and animal care.

Drug administration

Subjects were randomly assigned to one of four treatment groups (n = 9 per group): 1) RISP and DAMP, 2) RISP, 3) DAMP, and 4) vehicle (Table 1). Once assigned, rats were weighed and administered two injections daily on PNDs 14 through 28.

Table 1: Manipulated Drug Treatment Groups

1. RISP and DAMP	2. RISP and Saline
3. Vehicle and DAMP	4. Vehicle and Saline

Rats were weighed and administered subcutaneous injections of either RISP or vehicle daily on PNDs 14 through 28. RISP was dissolved in a small volume of 10% glacial acetic acid, brought to volume with saline, and adjusted for a pH ~6.2 with sodium hydroxide. The 3.0 mg/kg/day dosage was based on the dose used in previous studies of developmental RISP administration in rats (Bardgett et al., 2013). The vehicle consisted of 10% glacial acetic acid, brought to volume with saline, and adjusted for a pH ~6.2 with sodium hydroxide. Injections

were administered subcutaneously at a volume of 2.0 mL/kg of body weight. RISP was provided by the National Institute of Mental Health's Chemical Synthesis and Drug Supply program.

Immediately after receiving an injection of RISP or vehicle, each rat also received a subcutaneous injection of DAMP or a 0.9% saline vehicle daily from PNDs 14-28. DAMP (D-amphetamine, Sigma) was dissolved in 0.9% sterile saline. The dosage (1.0 mg/kg) was based on the work of Sherill, Stanis, and Gulley (2013) that demonstrated adverse behavioral outcomes for rats administered doses of DAMP between 1 and 3 mg/kg during adolescence. Injections were administered subcutaneously at a volume of 2.0 mL/kg of body weight.

Locomotor activity

Locomotor activity was measured using a clear polypropylene cage inserted into a Kinder Scientific Smart-Frame (Kinder Scientific, Poway, CA). Locomotor activity was measured by the quantity of photobeam breaks generated by each rat during each 5-minute time bin. Testing occurred during the rats' light phase in a darkened room separate from the animal housing room.

On PNDs 14, 21, and 28, rats were tested for 30 minutes prior to drug administration and then for 90 minutes after drug administration. This testing allowed for determination of the initial effects of the drugs on activity after the first injection on PND 14 and for the monitoring of changes in the locomotor response to each drug combination over time, as well as potential changes in basal locomotor activity during the pre-injection period.

Locomotor activity was also evaluated later in development in a procedure similar to the study done by Bardgett et al. (2013). Beginning at PND 52, each rat was tested for an hour a day for four consecutive days. Once a week for three days thereafter, rats were tested twice a week for two hours. During the first weekly test, the rats were placed in the testing chamber for 30 minutes, removed, and received a subcutaneous injection of saline, and then were returned to the

chamber for 90 minutes. During the second weekly test, each rat received a subcutaneous injection of either saline, or 1.0 or 3.0 mg/kg of DAMP. The order in which each rat received the three different doses over the three weeks was balanced within the groups.

Analyses

For the locomotor testing performed between PNDs 14-28, the number of photobeam breaks generated by each individual rat over two hours of daily testing was analyzed using an analysis of variance (ANOVA). The data for the 30-minute pre-injection and 90-minute post-injection periods were analyzed separately. For each data set, the total number of photobeam breaks generated each day was compared as a function of RISP administration, DAMP administration, and PND (repeated measure) with a three-way ANOVA. The locomotor data generated across the four consecutive days of testing starting at PND 52 were analyzed in a similar manner. The locomotor data generated across the three weeks of testing with the different DAMP challenge doses were compared between the early-life drug groups over the 90-minute test as a function of early-life RISP administration, early-life DAMP administration, and DAMP challenge dose with a three-way ANOVA. The baseline data generated prior to each of the DAMP challenge injections were analyzed as a function of both early-life drug groups and PND (repeated measure) with a three-way ANOVA. Finally, the locomotor data generated after the DAMP challenge during adulthood were also analyzed for the influence of PND by comparing the effects of DAMP challenge dose as a function of PND (repeated measure) with a two-way ANOVA.

The alpha level for all statistical tests was $p < 0.05$. Post-hoc testing was performed using a paired or independent samples t-test, or a Fishers Least Significant Difference (LSD) test.

Results

Locomotor activity during drug administration

The first sessions of locomotor testing were used to determine the initial effects of the drugs on activity, to monitor changes in regards to each drug combination over time, and to observe potential changes in basal activity at ~23 hours after drug injections.

The locomotor data gathered over 30 minutes prior to injection for each treatment group were combined into a single data point at PNDs 14, 21, and 28 and analyzed with a repeated-measures, three-way ANOVA. There was no main effect of RISP or DAMP or interactions between these variables with age (Figure 1). There was a significant age effect on locomotor activity, $F(2, 31) = 22.92, p < .001$, with total activity increasing over the three test days. A two-tailed, paired samples t-test was used to evaluate the differences in activity over the three PNDs tested. Rats were more active on PND 21 than on PND 14, $t(35) = -5.77, p < .001$, and were more active on PND 28 when compared to PND 21, $t(35) = -6.45, p < .001$.

The locomotor data gathered over 90 minutes after drug injection for each treatment group were combined into a single data point separately for each of the three PNDs and analyzed with a repeated-measures, three-way ANOVA. There was a significant effect of age, $F(2, 31) = 22.10, p < .001$ (Figure 2). There were also significant effects of RISP, $F(1, 32) = 237.36, p < .001$, and DAMP, $F(1, 32) = 161.88, p < .001$, on locomotor activity, as well as significant interactions between age and RISP, $F(2, 31) = 15.06, p < .001$, age and DAMP, $F(2, 31) = 10.49, p < .001$, and age, RISP, and DAMP, $F(2, 31) = 11.82, p < .001$. On PNDs 21 and 28, rats receiving RISP alone exhibited significantly lower levels of activity than the vehicle-saline control group ($p = .009$). An independent samples t-test revealed no significant differences in the effects of RISP across age. For the DAMP and age interaction, an independent samples t-test

indicated that the ability of DAMP to elevate locomotor activity increased across the three testing days. Post-hoc comparisons using Fishers LSD test revealed that rats receiving DAMP alone exhibited significantly more locomotor activity than the other three treatment groups on each test day ($p \leq .001$). Paired samples t-tests indicated that the activity produced by DAMP alone was higher on PNDs 21 ($p < .001$) and 28 ($p = .003$) than PND 14.

Locomotor activity in young adult rats after early-life RISP/DAMP administration

Locomotor activity was assessed for one hour a day on PNDs 52-55 to determine the long-term effects of early-life exposure to each drug combination. The locomotor data for each treatment group were combined into a single data point for each of the four days and analyzed with a repeated-measures, three-way ANOVA. A significant effect was found for age, $F(3, 29) = 3.94$, $p = .018$ (Figure 3). A two-tailed, paired samples t-test was used to evaluate the differences between each of the four PNDs tested. Rats were more active on PND 52 than on PND 53, $t(34) = 2.51$, $p = .017$, or PND 54, $t(34) = 2.76$, $p = .009$, and were more active on PND 55 than on PND 53, $t(34) = -2.24$, $p = .032$, or PND 54, $t(34) = -2.65$, $p = .012$. There was also a non-significant trend for the interaction between age and DAMP, $F(3, 29) = 2.84$, $p = .055$. An independent samples t-test was performed to further interrogate this trend and it was found that on PND 54, rats who received DAMP during development were more active than rats that did not, $t(33) = 2.33$, $p = .026$.

From PNDs 59-81, additional locomotor testing was done in order to determine if early-life RISP or DAMP administration altered locomotor responses to DAMP challenge during adulthood. On PNDs 59, 66, and 78, the rats were given saline injections and tested in order to reduce the development of an association between injections alone with the subjective effects of

DAMP. Locomotor testing on PNDs 62, 69, and 81was performed in order to determine each group's response to DAMP.

The locomotor data gathered over 30 minutes prior to injection on PNDs 62, 69, and 81 were combined into a single data point separately for each of the three test days and analyzed with a repeated-measures, three-way ANOVA. There was a significant effect for age, $F(2, 22) = 5.24$, $p = .014$ (Figure 4). A two-tailed, paired samples t-test was used to evaluate the differences between each of the three PNDs tested. Rats were more active on PND 62 than on PND 69, $t(34) = 3.20$, $p = .003$, or PND 81, $t(34) = 3.12$, $p = .004$.

The locomotor data gathered over the 90 minutes after injection for each DAMP challenge treatment group were combined into a single data point separately for each DAMP dose and analyzed with a repeated-measures, three-way ANOVA. There was a significant effect of DAMP dose on activity levels, $F(2, 23) = 15.66$, $p < .001$(Figure 5). Post-hoc comparisons using Fishers LSD test revealed that when rats were administered the 1.0 mg/kg ($p < .001$) or 3.0 mg/kg DAMP doses ($p < .001$), they showed significantly more activity than when they were administered saline. There were no significant main effects of early-life RISP or DAMP treatment, or interactions with DAMP challenge dose.

We also determined if locomotor responses to DAMP challenge varied as a function of test day, using a repeated-measures, two-way ANOVA. There was a significant interaction between test day and DAMP dose, $F(4, 44) = 17.55$, $p < .001$(Figure 6). Post-hoc comparisons using Fishers LSD test to evaluate the age by DAMP interaction indicated that on PND 62, all DAMP dose groups were significantly different ($p < .001$) from one another, with rats administered DAMP 3.0 mg/kg showing the highest locomotor activity, followed by rats administered DAMP 1.0 mg/kg, and saline. On PND 69, rats receiving either DAMP 1.0 mg/kg

or DAMP 1.0 mg/kg showed higher locomotor activity than rats given saline (both comparisons to saline, $p < .001$). On PND 81, all dose groups were significantly different ($p < .001$) from one another, with rats administered DAMP 1.0 mg/kg showing the highest locomotor activity, followed by rats administered DAMP 3.0 mg/kg, and saline.

Discussion

This is one of the first studies that examined the behavioral effects of combined AP and stimulant administration on young, developing rats. With the high prevalence of this co-administration in the pediatric population (Olfson et al., 2012), this important topic clearly merits investigation. RISP and DAMP administration had immediate effects on behavior, with DAMP increasing and RISP decreasing activity. DAMP administration early in development also led to more activity later in life, but in contrast to previous reports (Bardgett et al., 2013), RISP did not. The combination of the two drugs did not significantly alter behavior immediately after administration during development, but such exposure was associated with greater activity later in life.

Short-term behavioral effects

RISP significantly decreased ongoing activity in rats immediately after administration, consistent with previous work from our lab (Stevens, Gannon, Griffith, & Bardgett, submitted). The suppressive effect of RISP was significant on PND 21 and 28 and not on PND 14, although there was a mean difference suggestive of lower activity in the RISP group on the latter day. The greater differences seen on PNDs 21 and 28 might be attributable to the control group (comparison) demonstrating more activity on these test days. The ability of RISP to decrease ongoing activity early in postnatal life indicates that the receptor targets of RISP that mediate its suppressive effects on behavior are fully functional at this developmental stage.

DAMP dramatically increased locomotor activity on PNDs 14, 21, and 28. The effect became greater with repeated administration, indicating either a developmental increase in the sensitivity of dopamine/norepinephrine transporters or possible sensitization effect independent of age. This latter effect is widely known to occur in adult rats after repeated amphetamine administration (Adriani, Chiarotti, & Laviola, 1998). It has also been shown that slightly older rats (PND 33-43) demonstrated greater amphetamine sensitization as measured by locomotor activity, but not stereotypy, than adult rats (PND 61-71) (Adriani et al., 1998). At the least, our results indicate that dopamine/norepinephrine transporters are more sensitive to DAMP during the 3rd and 4th weeks of postnatal life in rats relative to earlier in development.

Rats co-administered DAMP and RISP showed levels of locomotor activity that were similar to the levels displayed by the control group. This may indicate that the pharmacological effects of these two medications essentially cancel each other out. This would be feasible if it is assumed that RISP blocks the dopamine D_2 receptor, which would negate the effect of additional dopamine being released by DAMP. It would be interesting in future research to assess the interactions of different doses of RISP and DAMP in order to determine if high doses of one drug combined with low doses of another continue to negate their individual effects or somehow synergize their effects on dopamine release or D_2 blockade.

In the 30-minute pre-injection data, we did not see any compensatory changes in activity in rats administered RISP, DAMP, or combination of the two 23.6 hours beforehand on PNDs 21 or 28. Previous work by our lab demonstrated that rats administered RISP were hyperactive 23 hours after their previous RISP injection (Stevens et al., submitted). However, the study did not observe compensatory activity until the developing rats had been chronically administered RISP for four consecutive weeks, whereas the rats in the present study were only administered RISP

for two weeks. Adult rats administered DAMP have been shown to be hypoactive 20 hours after injection, either due to withdrawal or as a compensatory reaction (White & White, 2006). Since we did not observe such a reaction at 23 hours post-injection on PND 21 or 28, it is possible that withdrawal or compensatory changes do not occur after developmental exposure or that it occurred earlier than when we tested the rats post-injection.

Long-term behavioral effects

Rats who were administered DAMP, regardless of co-injection, during PNDs 14-28 showed significantly higher activity on PND 54. Since this was the third consecutive day of testing, DAMP administration during development may disrupt an adult rat's ability to habituate. Another study (Carlezon, Mague, & Andersen, 2003) demonstrated this same effect in adult rats administered methylphenidate (Ritalin) from PNDs 20-35. The researchers hypothesized that this effect was mediated either by an attention deficit that didn't allow the animals to properly recognize the familiar testing environment, or by sustained elevation in anxiety, which undermined the development of a normal relaxation response to a familiar environment. Since this effect was observed even in rats co-administered RISP, it may suggest that early-life DAMP exposure permanently modifies cognitive function via a non-D_2 or non-dopaminergic mechanism. However, as is clear from the data, the combination of RISP and DAMP did not significantly heighten this effect.

This study did not find the hypothesized increase in locomotor activity resulting from early-life RISP administration. One explanation for this lack of replication is that the rats were given RISP for two weeks during development, and other studies administered RISP for four weeks (Bardgett et al., 2013; Stevens et al., submitted; Stubbeman et al., 2014). It is possible that the effects occur during the third or fourth week of administration. This also could have been due

to an increased handling of the animals during PNDs 14-28. This study included repeated testing during this time, and previous studies did not (Bardgett et al., 2013). This may have over-acclimated the animals to the testing environment. The rats were also given two injections during development, which also could have served as another form of handling not performed in previous studies (Stubbeman et al., 2014; Bardgett et al., 2013). Finally, it is possible that since each RISP-administered pup was housed with a very active DAMP-administered pup, the latter pup provided stimulation to former one via additional contacts that countered the lethargy produced by RISP.

Response to amphetamine later in life

Increased locomotor activity was observed in all rats administered the 1.0 and 3.0 mg/kg DAMP doses during adulthood. The 3.0 mg/kg DAMP dose produced the most activity on PND 62 and 69, but the 1.0 mg/kg dose produced more activity on PND 81. This latter effect was unexpected; however, it is possible that on PND 81 the higher DAMP dose increased stereotypy and fine motor movement at the expense of locomotor activity, which occurs with high doses of AMPH (White & White, 2006). Since all rats had been exposed to the low DAMP dose by PND 81, they may have experienced a sensitized response to the high dose of DAMP on that day, which would have likely caused more stereotypy, which was not assessed, than locomotor activity. Future work will need to include measures of stereotypy to test this possibility.

Early-life RISP, DAMP, or RISP+DAMP administration was not associated with changes in the response to DAMP in adulthood. It had been previously shown that rats given RISP during development exhibit an increase in DAMP sensitivity during adulthood (Stubbeman et al., 2014). In the latter study, rats were habituated to the testing chambers for 24 hours on two occasions prior to DAMP challenge and were left in the chambers for 27 hours after DAMP injection

during adulthood. The RISP rats were also administered RISP for four weeks (PND 14-42) as opposed to two weeks. The methodological differences may explain why early-life RISP administration in the present study did not elicit an enhanced response to DAMP challenge during adulthood.

Future goals

Most children who receive an AP and stimulant combination are diagnosed with ADHD (Olfson et al., 2012). While the present study considered the effects of this combination in "normal" rats, future work in this area may benefit from considering the developmental effects of these drugs in a rat ADHD model. There are several face-valid, behavioral rodent models that demonstrate ADHD-like symptoms. One of the most commonly used models is the spontaneously hypertensive rat, which displays high levels of inattention, impulsivity, and hyperactivity, consistent with the diagnostic criterion for ADHD (Sagvolden, 2009).

This study did not look at biological changes in the brains of the animals, which could have yielded valuable information. Both RISP (Cuomo et al., 1981; Moran-Gates et al., 2007; Velley et al., 1975; Vinnish et al., 2013) and DAMP (Diaz Heijtz et al., 2003) affect the dopaminergic system in the brain, and there may be critical changes produced by early-life administration of these drugs. For example, early-life administration of APs alone decreases dopamine turnover (Cuomo et al., 1981) and increases dopamine receptors (Moran-Gates et al., 2007), while early-life DAMP reduces synaptic pruning in the prefrontal cortex (Diaz-Heijtz et al., 2003). It would be interesting to determine if these single drug effects would be offset by the presence of the other, as appears to be the case at the behavioral level early in development, or whether they synergize. It would also be worthwhile to consider the effects of the drug combination or each drug individually on non-dopaminergic function since some of the

behavioral effects suggest that early-life administration of these drugs may be affecting such systems.

Clinical implications

The results of this study may have implications for the treatment of children with psychotherapeutic drugs. First, RISP significantly decreased activity on PNDs 21 and 28. If the results can be generalized to children, then RISP suppression of behavior would likely be detrimental in pediatric populations. Decreased behavior is strongly correlated with increased body weight, lowered metabolic function, higher rates of anxiety and depression, and lowered academic performance (Strong et al., 2005). This effect could be amplified by the metabolic side effects of RISP, which younger people are more susceptible to, and include increases in insulin sensitivity that can lead to diabetes, and weight gain caused by RISP-induced hormonal changes (Olfson et al., 2012).

Second, DAMP increased activity during administration in the rat model. If this effect were observed in humans, then children may experience hyperactivity during the school and early career years. This hyperactivity could have negative results behaviorally and socially. It has been shown that strong arousal can impair performance during mentally challenging tasks (Diamond, Campbell, Park, Halonen, & Zoladz, 2007). It has also been shown that children with hyperactivity can be disruptive to certain social situations (i.e. educational settings, religious traditions, etc.), and unfortunately bear the brunt of disciplinary action (Whalen, Henker, & Hinshaw, 1985). The effects of both RISP and DAMP in the study illustrate an inverted-u model of performance and activity (Diamond, et al., 2007) (Figure 7). However, it should be noted that stimulants generally work to decrease activity in children diagnosed with ADHD (Seeman, &

Madras, 1998). As such, the application of our results may be limited to those children with a mild form of the disorder or who have been misdiagnosed.

Finally, the rats receiving both RISP and DAMP exhibited activity levels very similar to the control group. This suggests that co-administration of these medications may produce a "happy medium" in terms of its effect on symptoms. The contradictory biological effects of these two medications appear to essentially cancel each other out in terms of their combined effect on behavior. However, there should not mitigate interest in continuing research on the long-term effects of psychotherapeutic drugs that are prescribed to pediatric populations. It is always risky to increase the amount of drugs given to a patient because of adverse effects, and an emphasis on lowering existing doses should be taken into account. There is also the potential of long-term changes, as was indicated in the present study by the enhanced activity seen in young adult rats administered DAMP early in life. There is a great deal to be learned in this area of developmental psychopharmacology, and, until more definitive information is generated, greater caution should be taken with the future minds of this world.

References

Adriani, W., Chiarotti, F., & Laviola, G. (1998). Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice. *Behavioral neuroscience, 112,* 1152. doi:10.1037/0735-7044.112.5.1152

Anderson, S.L. (2005). Stimulants and the developing brain. *Trends in Pharmacological Development, 26,* 237-243. doi:10.1016/j.tips.2005.03.009

Andersen, S.L., & Navalta, C.P. (2004). Altering the course of neurodevelopment: A framework for understanding the enduring effects of psychotropic drugs. *International Journal of Developmental Neuroscience, 22,* 423–440. doi:10.1016/j.ijdevneu.2004.06.002

Arnt, J. (1995). Differential effects of classical and newer antipsychotics on the hypermotility induced by two dose levels of D-amphetamine. *European Journal of Pharmacology, 283,* 55-62. doi:10.1016/0014-2999(95)00292-S

Baddeley, A. (1992). Working memory: The interface between memory and cognition. *Science, 255,* 556-559. doi:10.1126/science.1736359

Bardgett, M. E. (2004). Behavioral models of atypical antipsychotic drug action. In J. Csernansky & J. Laurello (Eds.), *Atypical antipsychotics: From bench to bedside* (pp 61-63). New York, NY: Marcel Dekker.

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. *Experimental and Clinical Psychopharmacology, 21,* 259-267. doi:10.1037/a0031972

Berman, S. M., Kuczenski, R., McCracken, J. T., & London, E. D. (2008). Potential adverse effects of amphetamine treatment on brain and behavior: A review. *Molecular Psychiatry, 14,* 123-142. doi:10.1038/mp.2008.90

Birnbaum, M. L., Saito, E., Gerhard, T., Winterstein, A., Olfson, M., Kane, J. M., & Correll, C. (2013). Pharmacoepidemiology of antipsychotic use in youth with ADHD: Trends and clinical applications. *Current Psychiatry Report, 15,* 537-543. doi:10.1007/s11920-013-0382-3

Blum, K., Chen, A. L., & Oscar-Berman, M. (2008). Attention-deficit-hyperactivity disorder and reward deficiency syndrome. *Neuropsychiatric Disease and Treatment, 4,* 893-918. doi:http://dx.doi.org/10.2147/NDT.S2627

Brandon, C.L. et al. (2001) Enhanced reactivity and vulnerability to cocaine following methylphenidate treatment in adolescent rats. *Neuropsychopharmacology, 25,* 651–666. doi:10.1016/S0893-133X(01)00281-0

Carlezon, W. A., Mague, S. D., & Andersen, S. L. (2003). Enduring behavioral effects of early exposure to methylphenidate in rats. *Biological psychiatry, 54,* 1330-1337. doi:http://dx.doi.org/10.1016/j.biopsych.2003.08.020

Choi, Y. K., Gardner, M.P., & Tarazi, F. I. (2009) Effects of risperidone on glutamate receptor subtypes in developing rat brain. *European Neuropsychopharmacology, 19,* 77-84. doi: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. *European Neuropsychopharmacology, 20,* 187-194. doi:10.1016/j.euroneuro.2009.09.002

Cools, R., & D'Esposito, M. (2011). Inverted-U–Shaped Dopamine actions on human working memory and cognitive control. *Biological Psychiatry, 69,* 113-125. doi:10.1016/j.biopsych.2011.03.028

Correll, C.U., Lencz, T., Malhotra, A.K. (2011). Antipsychotic drugs and obesity. *Trends in Molecular Medicine, 2,* 97-107. doi:10.1016/j.molmed.2010.10.010

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. doi:10.1007/BF00432686

Diamond, D. M., Campbell, A. M., Park, C. R., Halonen, J., & Zoladz, P. R. (2007). The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. *Neural Plasticity, 2007,* 1-33. doi:10.1155/2007/60803

Diaz Heijtz, R., Kolb, B., & Forssberg, H. (2003). Can a therapeutic dose of amphetamine during pre-adolescence modify the pattern of synaptic organization in the brain? *European Journal of Neuroscience, 18,* 3394–3399.

Guzman, F. (2013, August 27). *Mechanism of action of risperidone.* Retrieved from http://psychopharmacologyinstitute.com/

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. *Journal of Pharmacology & Experimental Therapeutics, 305,* 625-631. doi:10.1124/jpet.102.046987

Laviola, G., Pascucci, T., & Pieretti, S. (2001). Striatal dopamine sensitization to D-amphetamine in periadolescent but not in adult rats. *Pharmacology Biochemistry and Behavior, 68,* 115-124. doi:10.1016/S0091-3057(00)00430-5

Lieberman, J. A., Stroup, T. S., McEvoy, J. P., Swartz, M. S., Rosenheck, R. A., Perkins, D. O., ... & Hsiao, J. K. (2005). Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. *New England Journal of Medicine, 353,* 1209-1223. doi:10.1056/NEJMoa051688

Liddle, P.F., Lane, C.J., & Ngan E.T.C. (2000). Immediate effects of risperidone on cortico-striato-thalamic loops and the hippocampus. *British Journal of Psychiatry, 177,* 402-407. doi:10.1192/bjp.177.5.402

Milstein, J. A., Elnabawi, A., Vinish, M., Swanson, T., Enos, J. K., Bailey, A. M., ... & Frost, D. O. (2013). Olanzapine treatment of adolescent rats causes enduring specific memory impairments and alters cortical development and function. *PloS One, 8,* 1-17. doi:10.1371/journal.pone.0057308

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,* 1-13. doi:10.1186/1471-244X-13-198

Moran-Gates, T., Grady, C., Shik Park, Y., Baldessarini, R. J., & Tarazi, F. I. (2007). Effects of risperidone on dopamine receptor subtypes in developing rat brain. *European Neuropsychopharmacology, 17,* 448-455. doi:10.1016/j.euroneuro.2006.10.004

Olfson, M., Blanco, C., Liu, S., Wang, S., & Correll, C. (2012). National trends in the office-based treatment of children, adolescents, and adults with antipsychotics. *Archives of General Psychiatry, 69,* 1247-1256. doi:10.001/archgenpsychiatry.2012.647

Robinson, P. W., Newby, T. J., & Ganzell, S. L. (1981). A token system for a class of underachieving hyperactive children. Journal of Applied Behavior Analysis, *14*, 307-315. doi:10.1901/jaba.1981.14-307

Sagvolden, T., Johansen, E. B., Wøien, G., Walaas, S. I., Storm-Mathisen, J., Bergersen, L. H., ... & Faraone, S. V. (2009). The spontaneously hypertensive rat model of ADHD–the importance of selecting the appropriate reference strain. *Neuropharmacology*, *57,* 619-626. doi:10.1016/j.neuropharm.2009.08.004

Seeman, P., & Madras, B. K. (1998). Anti-hyperactivity medication: methylphenidate and amphetamine. *Molecular psychiatry*, *3,* 386-396. doi:10.1038/sj.mp.4000421

Sherrill, L. K., Stanis, J. J., & Gulley, J. M. (2013). Age-dependent effects of repeated amphetamine exposure on working memory in rats. *Behavioural Brain Research, 242,* 84-94. doi:10.1016/j.bbr.2012.12.044

Stevens, R. M., Gannon, M., & Bardgett, M. E. (2013, May). *The NMDA antagonist, MK-801, impairs delayed non-matching-to-sample memory.* Paper presented at the annual Meeting of the Midwestern Psychological Association, Chicago, IL.

Stevens, R. M., Gannon, M., Griffith, M. S., & Bardgett, M. E. (submitted). The immediate and delayed locomotor effects of risperidone in developing and adult rats. *Behavioural Pharmacology.*

Strong, W. B., Malina, R. M., Blimkie, C. J., Daniels, S. R., Dishman, R. K., Gutin, B., ... & Trudeau, F. (2005). Evidence based physical activity for school-age youth. *The Journal of Pediatrics, 146,* 732-737. doi:http://dx.doi.org/10.1016/j.jpeds.2005.01.055

Stubbeman, B. L., Brown, C. J., & Bardgett, M. E. (2014, November). *Early-life risperidone administration enhances locomotor responses to amphetamine during adulthood.* Paper presented at the annual Meeting of the Society for Neuroscience, Washington, D.C.

U.S. Department of Health and Human Services. (2007). Dexedrine FDA Label. *The United States Food and Drug Administration.* Retrieved from www.fda.gov.

U.S. Department of Health and Human Services. (1993). Risperidal FDA Label. *The United States Food and Drug Administration.* Retrieved from www.fda.gov.

U.S. Census Bureau. (2013). Estimates of the population of the United States by single years of age, color, and sex: 1900 to 1959. *Current Population Reports*. Retrieved from www.childstats.gov.

Velley, L., Blanc, G., Tassin, J. P., Thierry, A. M., & Glowinski, J. (1975). Inhibition of striatal dopamine synthesis in rats injected chronically with neuroleptics in their early life. *Naunyn Schmiedeberg's Archives of Pharmacology, 288,* 97-102. doi:10.1007/BF00501817

Whalen, C. K., Henker, B., & Hinshaw, S. P. (1985). Cognitive-behavioral therapies for hyperactive children: Premises, problems, and prospects. *Journal of Abnormal Child Psychology, 13,* 391-409. doi:10.1007/BF00912724

White, W., & White, I. M. (2006). An activity indicator o acute withdrawal depends on amphetamine dose in rats. *Physiology & Behavior, 87,* 368-376. doi:10.1016/j.physbeh.2005.10.009

Willson, M. C., Wilman, A. H., Bell, E. C., Asghar, S. J., & Silverstone, P. H. (2004). Dextroamphetamine causes a change in regional brain activity in vivo during cognitive tasks: a functional magnetic resonance imaging study of blood oxygen level-dependent response. *Biological Psychiatry, 56*, 284-291.

Zuvekas, S. H., & Vitiello, B. (2012). Stimulant medication use in children: A 12-year perspective. *American Journal of Psychiatry, 169,* 160-166.

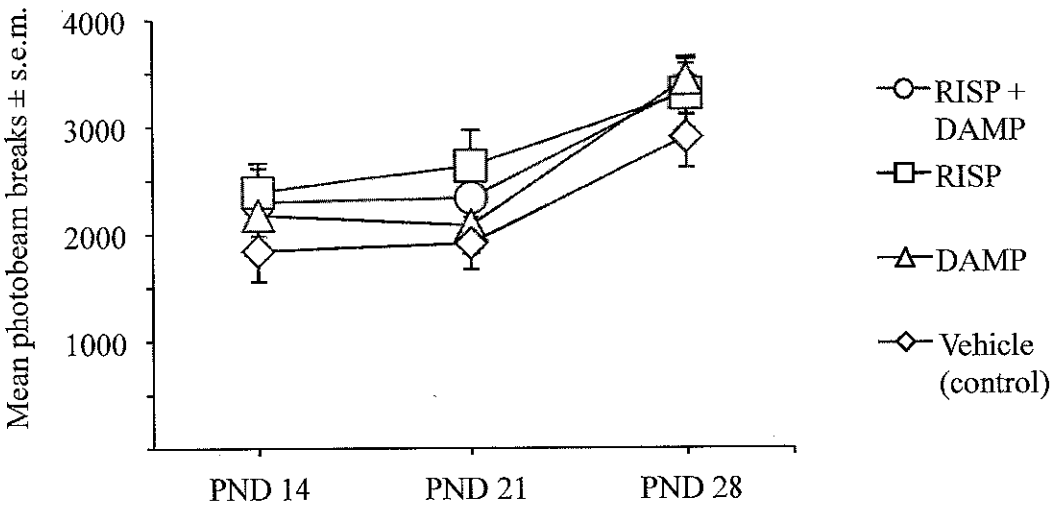

Figure 1. Comparison of total locomotor activity over 30 minutes prior to drug injection on PNDs 14, 21, and 28. No significant differences were found between the drug administration groups. Data represent means ± s.e.m.

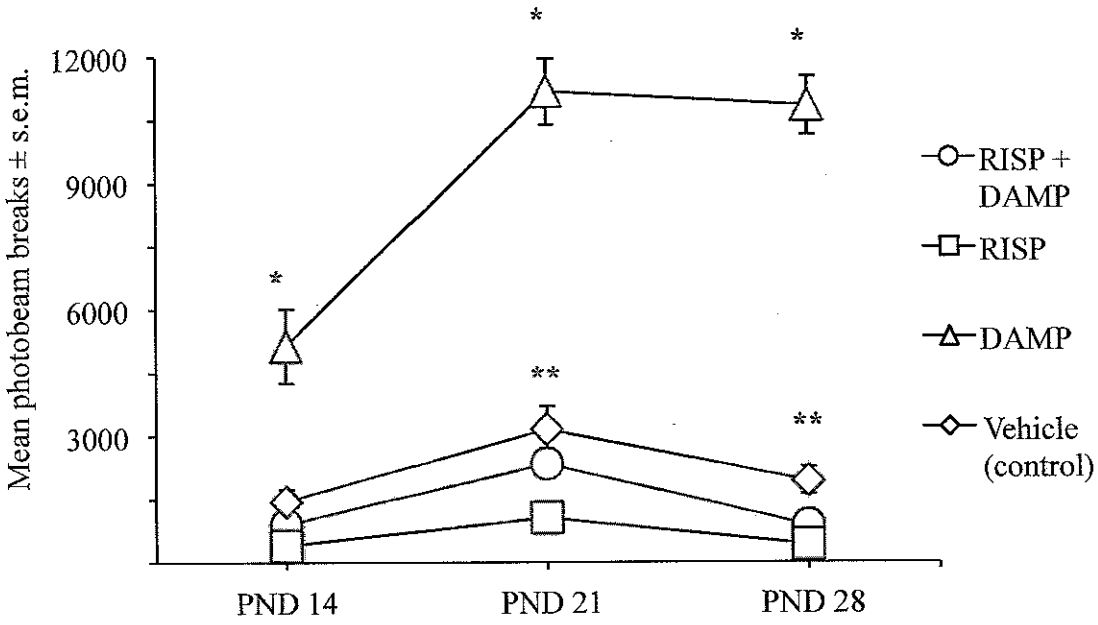

Figure 2. Comparison of total locomotor activity over 90 minutes after drug injection on PNDs 14, 21, and 28. Rats receiving DAMP alone showed significantly more activity than the other three treatment groups on all test days, as indicated by the single asterisk. The effect of DAMP was greater on PNDs 21 and 28 in comparison to PND 14. Rats receiving RISP alone showed significantly lower activity than rats receiving vehicle alone on PNDs 21 and 28, as indicated by the double asterisks. Data represent means ± s.e.m.

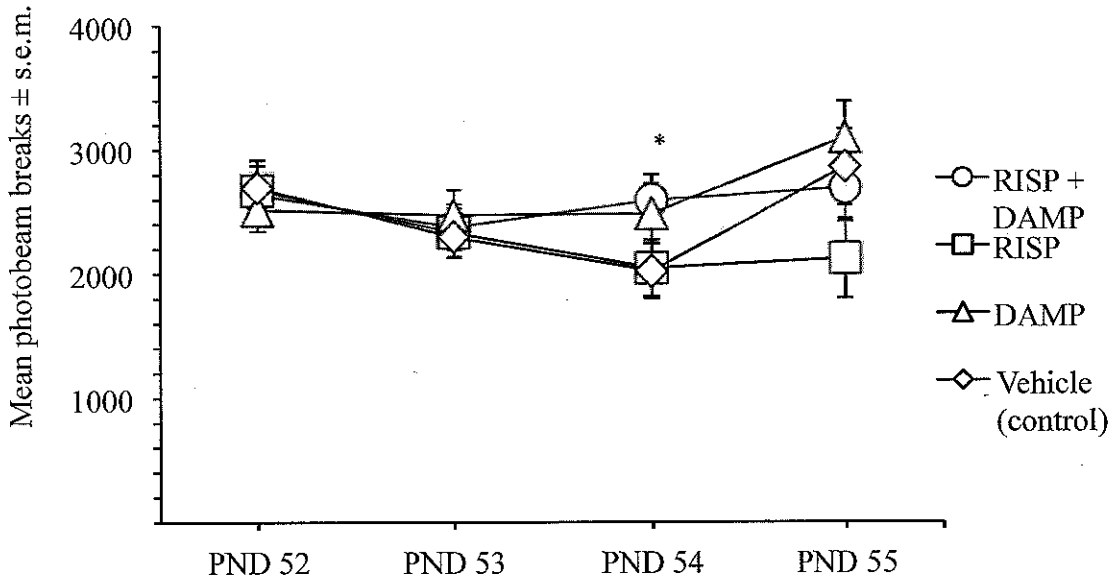

Figure 3. Comparison of total locomotor activity over 60 minutes on PNDs 52-55. On PND 54, rats that received DAMP early in life showed significantly more activity than rats that did not receive DAMP during development as indicated by an asterisk. Data represent means ± s.e.m.

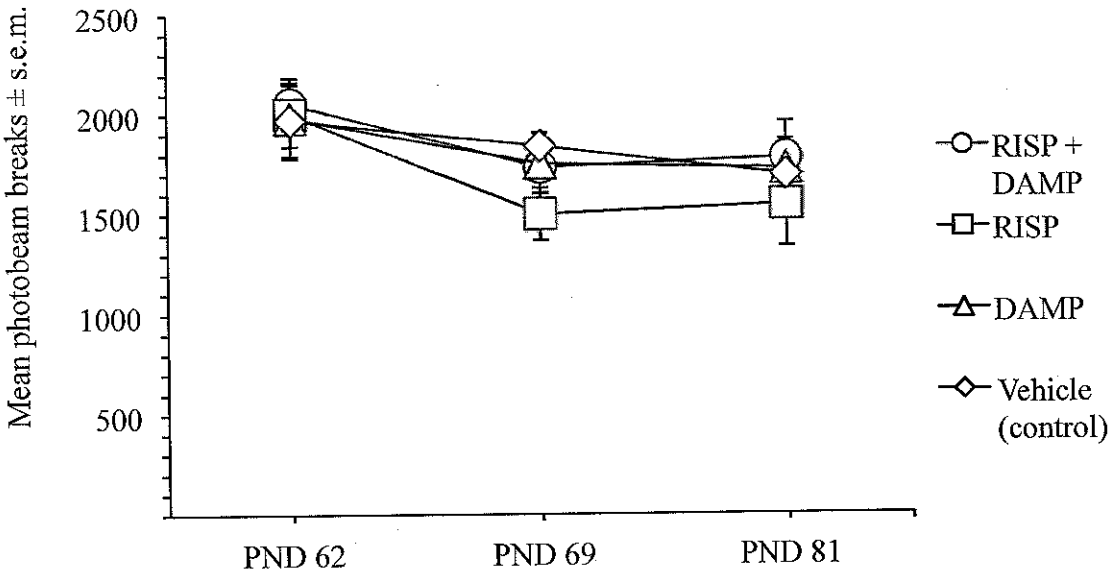

Figure 4. Comparison of total locomotor activity over 30 minutes prior to drug injection on PND 62, 69, and 81. No significant differences were found between the early-life drug administration groups. Data represent means ± s.e.m.

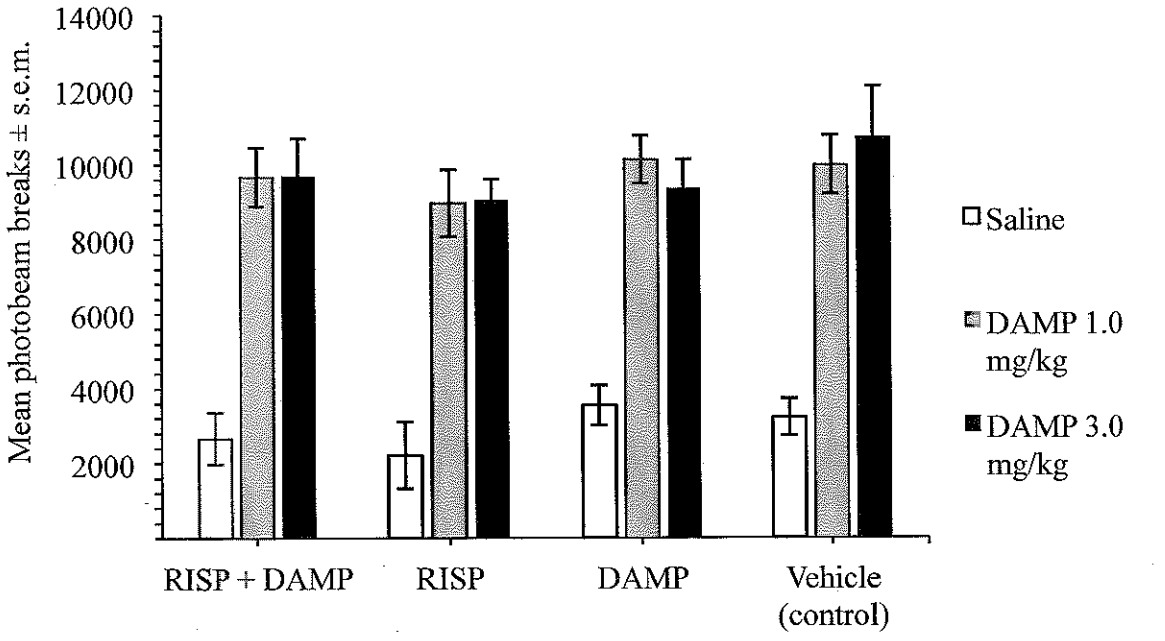

Figure 5. Comparison of total locomotor activity over 90 minutes after DAMP injection on PNDs 62, 69, and 81 as a function of drug treatment group. Both DAMP doses significantly increased activity when compared to saline. No significant differences were found between the early-life drug administration groups. Data represent means ± s.e.m.

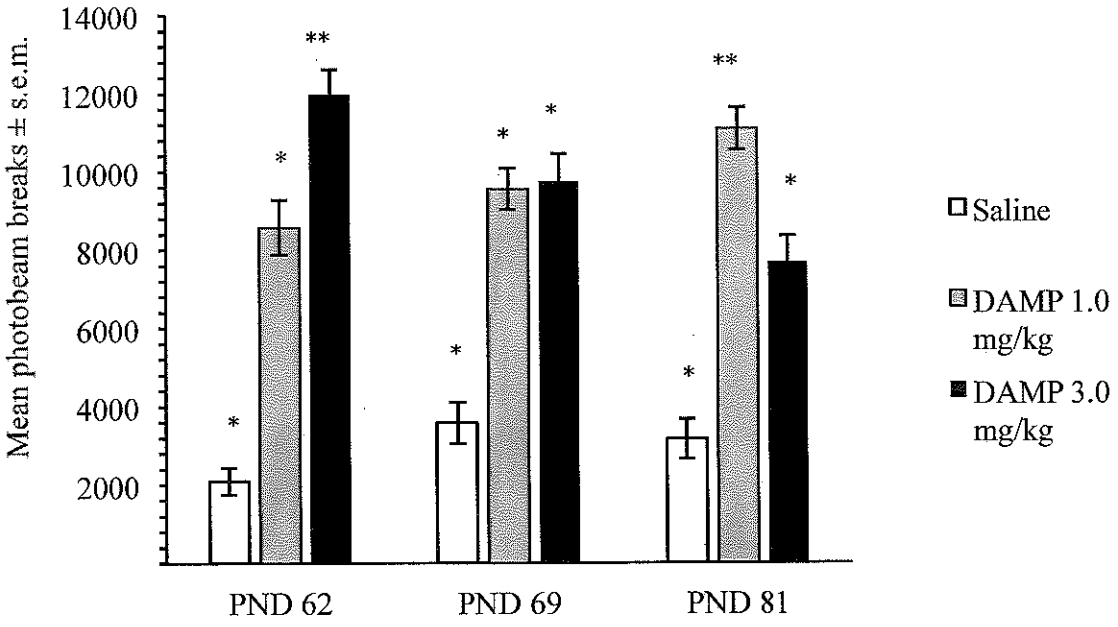

Figure 6. Comparison of total locomotor activity over 90 minutes after DAMP injection as a function of test day (PNDs 62, 69, and 81) and DAMP dose. At PND 62, the DAMP 3.0 mg/kg group was significantly more active than the saline and DAMP 1.0 mg/kg groups, as indicated by the double asterisks, and the DAMP 1.0 mg/kg group was significantly more active than the saline group as indicated by the single asterisk. At PND 69, the rats administered either dose of DAMP were active than the saline group as indicated by single asterisks. At PND 81, the DAMP 1.0 mg/kg group was more active than the DAMP 3.0 mg/kg group, as indicated by the double asterisks, and the latter group was more active than the saline group, as indicated by the single asterisk. Data represent means ± s.e.m.

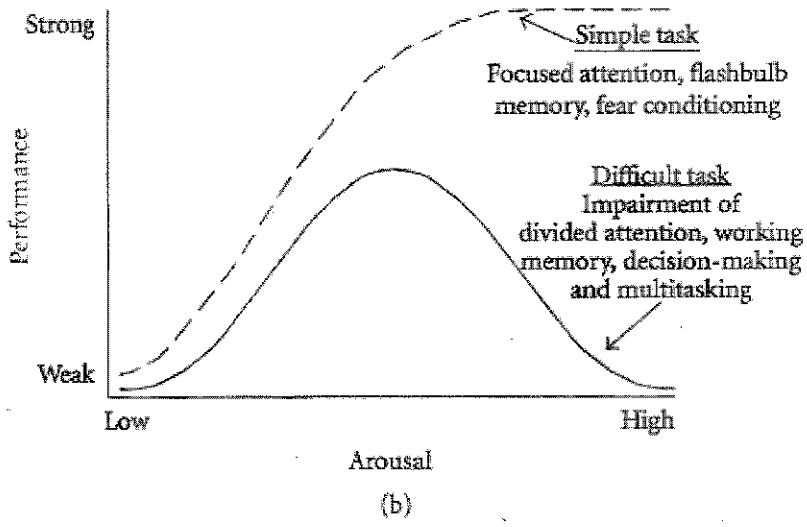

Figure 7. Yerkes-Dodson inverted-u model of arousal and performance (Diamond et al., 2007). Low arousal impairs overall performance, while high arousal enhances performance on simple tasks, but impairs performance on more challenging cognitive tasks.