Department of Psychological Science

A Comparison of Two Antipsychotic Drugs on Behavioral Development in Rats

Honors in Psychology Project

2017-18

Emily C. Baltes Thompson

Northern Kentucky University

Dr. Mark Bardgett, Faculty Mentor

A Comparison of Two Antipsychotic Drugs on Behavioral Development in Rats

Emily C. Baltes Thompson

Northern Kentucky University

Faculty Mentor: Dr. Mark Bardgett

Honors in Psychology Program

Department of Psychological Science

Northern Kentucky University

Student: *Emily C. Baltes Thompson*

Project Title: A Comparison of Two Antipsychotic Drugs on Behavioral Development in Rats

Signatures below signify that the student has successfully completed the requirements of the Honors in Psychology Project.

Project Mentor: Dr. Mark Bardgett	*Date* 5/3/18
Committee Member: Dr. Cecile Marczinski	*Date* 5/3/18
Committee Member: Dr. Justin Yates	*Date* 5/3/18

Abstract

The rates of atypical antipsychotic drug prescriptions in pediatric populations has risen over the last two decades, but little is known about the long-term consequences of these drugs on brain and behavioral development. In rats, developmental administration of risperidone, the most widely-used antipsychotic drug in children, elicits locomotor hyperactivity that can persist into adulthood. It is not known whether developmental risperidone administration can impact other behaviors later in life, and what the effects of other antipsychotics are on the same behavioral measures. The purpose of this study was to compare the effects of early-life risperidone administration on locomotor activity and reward sensitivity to similarly timed administration of aripiprazole, the second most commonly used antipsychotic drug in young children. Pups (n = 12 per group) received subcutaneous daily injections of 7.0 mg/kg aripiprazole, 21.0 mg/kg aripiprazole, 3.0 mg/kg risperidone, or vehicle between postnatal days 14-28. Acute risperidone administration during development suppressed activity immediately after injection but was associated with elevated levels of spontaneous locomotor activity during adulthood. These effects were not observed in rats administered aripiprazole early in life. Neither drug affected locomotor responses to amphetamine or MK-801 during adulthood, but each drug slightly, yet significantly, increased sucrose preference – a measure of reward sensitivity. These data suggest that the impact of early-life aripiprazole on brain development is less dramatic than the impact of early-life risperidone, but that both drugs modestly increase sensitivity to reward.

A Comparison of Two Antipsychotic Drugs on Behavioral Development in Rats

For decades, antipsychotic drugs have served as a primary tool for treating psychiatric disorders in adults (Bardgett, 2004; Stubbeman, Brown, Yates, & Bardgett, 2017). Recently, the number of children and young adolescents receiving these medications has increased, despite a small number of human clinical studies urging caution and a minimum of evidence for long-term effects on brain and behavioral development (Bardgett et al., 2013; Iñiguez, Cortez, Crawford, & McDougall, 2007; Vitiello et al., 2009). This increase is likely due to the introduction of second-generation, or atypical, antipsychotic drugs which, when compared to their predecessors, generally have fewer acute adverse effects. Moreover, these drugs are used to treat psychiatric conditions in children under circumstances where other forms of psychiatric treatment are not available (Olfson, Blanco, Liu, Moreno, & Laje, 2006; Vitiello et al., 2009).

The most commonly prescribed atypical antipsychotics in children are risperidone and aripiprazole. These drugs been approved for the treatment of adolescents with schizophrenia and bipolar disorder, as well as for children ages 5-17 with behavioral problems associated with autism (Olfson, Blanco, Liu, Wang, & Correll, 2012; Varela et al., 2014; Vitiello et al., 2009). However, most children are provided off-label prescriptions for these medications to treat attention-deficit/hyperactivity disorder (ADHD), pervasive developmental disorder, depression, anxiety, and conduct disorder (De Santis, Lian, Huang, & Deng, 2016; Iñiguez et al., 2007; Olfson et al., 2012). Antipsychotic drugs are thought to exert their therapeutic effects via their antagonistic/partial agonism of the dopamine D_2 and serotonin 5-HT_{1A} and 5-HT_{2A}/5HT_{2C} receptors. These receptors and the neurotransmitters that bind to them are involved in multiple critical neurodevelopmental processes that makes receptor blockade during development particularly concerning (De Santis et. al., 2016).

Risperidone primarily targets 5-HT_{2A} and D_2 receptors, along with D_3, D_4, adrenergic α_1 and α_2 and histamine H_1 receptors (Choi, Moran-Gates, Gardner, & Tarazi, 2010; Stubbeman et al., 2017). Like other atypical antipsychotics, risperidone has greater affinity for 5-HT_{2A} receptors as compared to D_2 receptors (Choi et. al., 2010). Previous research has indicated that long-term treatment with risperidone differentially affects dopamine receptor density in adult and juvenile rats. In juvenile rats, risperidone increases D_1 receptor density in the nucleus accumbens and caudate-putamen, but elevates D_2 receptors in the medial prefrontal cortex and hippocampus and D_4 receptors in the nucleus accumbens, caudate-putamen, and hippocampus in juvenile and adult rats (Moran-Gates, Grady, Park, Baldessarini, & Tarazi, 2007). Long-term risperidone administration also increases hippocampal 5-HT_{1A} receptors in juvenile but not adult rats, although such treatment leads to lower cortical 5-HT_{2A} receptor densities regardless of age. However, a lower dose of risperidone is required for the same effect in younger rats (Choi et. al., 2010).

Aripiprazole has a unique mechanism of action, possessing an affinity for the D_2 receptor that is similar to the traditional antipsychotic drug haloperidol, but lower than other atypical antipsychotics including risperidone (Mamo et al, 2007). It acts as a partial agonist at the receptor as opposed to a full antagonist like all other antipsychotic drugs (Mamo et al., 2007; Natesan, Reckless, Nobrega, Fletcher, & Kapur, 2006; Varela et al., 2014). As a partial agonist, aripiprazole prevents the full activation of the D_2 receptor by dopamine but can actually increase dopamine transmission depending on cell-line and assay condition if dopamine is absent (Natesan et al., 2006). Aripiprazole also serves as an antagonist at 5-HT_2 receptors and as a

partial agonist at 5-HT_{1A} receptors, though it is unique from most other atypical antipsychotics in that it possesses a lower affinity for 5-HT_2 receptors compared to D_2 receptors (Mamo et al., 2007; Natesan et al., 2006; Varela et al., 2014).

Though atypical antipsychotic drugs like risperidone are associated with fewer acute adverse effects, they are not devoid of them (Bernagie, Danckaerts, Wampers, & De Hert, 2016). Specifically, by affecting the dopaminergic pathways involved in motor control, prolactin secretion, cognition, and motivation, these medications can cause extrapyramidal symptoms (tardive dyskinesia, dystonia, akathisia, and parkinsonism), increased prolactin levels, and neurolepsis (Bernagie et al., 2016). Patients less than 20 years of age have been shown to be more vulnerable to these acute adverse effects (Bernagie et al., 2016).

In contrast, aripiprazole possesses a comparatively low risk of inducing extrapyramidal side effects at effective doses (10-30 mg per day) (Mamo et al., 2007; Varela et al., 2014). Patients receiving doses in this range exhibit very high D_2 receptor occupancy (>80%) relative to the occupancy rates (60%-65%) achieved by clinically effective doses of most other antipsychotics (Mamo et al., 2007). It should be noted that people with schizophrenia who are treated with aripiprazole are at an increased risk for extrapyramidal side effects when the drug occupies more than 90% of D_2 receptors, but clinical doses rarely produce this level of receptor occupancy (Mamo et al., 2007; Natesan et al., 2006).

As discussed above, the acute effects of early-life atypical antipsychotic drug administration have been reported in several studies, but research evaluating the long-term effects of early-life antipsychotic drug administration is mostly absent. Therefore, the purpose of this study was to compare the behavioral effects of early-life administration of aripiprazole and risperidone respectively in rats at several points throughout development. To this end, we

employed several known behavioral measures of antipsychotic drug action.

Locomotor activity in rats can be defined as ambulatory horizontal movement. This simple behavior has been used widely to determine the efficacy of antipsychotic drug administration or the long-term impact of such drugs on behavioral and brain function (Bardgett, 2004; Bardgett et al., 2013; Varela et al., 2014). For example, Stevens, Gannon, Griffith, and Bardgett (2016) examined the emergence timeline of persistently elevated locomotor activity resulting from early-life risperidone administration. Their research indicated that adult rats treated with daily risperidone displayed greater activity levels within 24 hours of their first injection, but developing rats did not experience this effect until the start of their fourth week of treatment. One week after cessation of administration, adult rats no longer demonstrated hyperactivity in comparison to controls, but the same could not be said for their juvenile counterparts. Previous research has also indicated that rats receiving early-life risperidone are hyperactive for several months after the cessation of treatment. Bardgett et al. (2013) found that increases in locomotor activity due to risperidone administration within the first two months of life persisted from one-week post-treatment through roughly nine months of age.

Aripiprazole has not been shown to impact locomotor activity specifically, but very little research has been done on the subject (De Santis, Pan, Lian, Huang, & Deng, 2014). The present study addressed this void in the literature as well as specifically examined whether aripiprazole produces the same delayed-onset compensatory hyperactivity in developing rats as risperidone.

Forebrain dopamine systems are not only known as targets of antipsychotic drug action but are perhaps better known as neurochemical targets for drugs of abuse. One concern regarding developmental exposure to antipsychotic drugs is that it may alter the sensitivity of forebrain dopamine systems to drugs of abuse, possibly enhancing the acute rewarding responses elicited

by such drugs or increasing liability to dependence. Amphetamine, a psychostimulant, increases dopamine release, blocks dopamine and norepinephrine reuptake, and inhibits monamine oxidase from metabolizing dopamine in the synapse (Iversen, Iversen, Bloom, & Roth, 2008). Particularly active in the nucleus accumbens and caudate putamen – regions of the brain known to be impacted by antipsychotic drug action – amphetamine stimulates motor activity in a dose-dependent manner (Stubbeman et al., 2017). The sensitivity to this stimulation can be evaluated by measuring locomotor activity, and previous findings indicate that rats administered risperidone early in life are hypersensitive to the locomotor effects of amphetamine application (Stubbeman et al., 2017). Tadokoro et al. (2012) evaluated dopamine release and locomotor activity induced by methamphetamine (an analogue of amphetamine) in adult rats chronically administered aripiprazole and found that aripiprazole did not modify dopamine or locomotor responses to methamphetamine. The present study sought to determine whether animals treated with aripiprazole early in life demonstrate the hypersensitivity to amphetamine observed in rats administered risperidone early in life.

Another neurotransmitter system important in antipsychotic drug action as well as sub-serving neural responses to drugs of abuse is the glutamate system. Glutamate is found in abundance in many of the same dopaminergic regions of the forebrain (e.g., frontal cortex, nucleus accumbens, caudate-putamen), with glutamate and dopamine nerve terminals working in tangent to alter post-synaptic function in many of these regions. Glutamate can bind to three general receptor subtypes: NMDA, AMPA, and metabotropic glutamate receptors. The NMDA receptor is of particular interest here because it possesses high affinity for a different category of abused drugs, namely phencyclidine and ketamine. It is possible that developmental exposure to

antipsychotic drugs indirectly alters NMDA receptor function through direct modifications of dopaminergic systems. To ascertain the effects of risperidone and aripiprazole on NMDA receptor function, we administered dizocilpine (MK-801), an uncompetitive N-methyl-D-aspartate (NMDA) receptor antagonist, and measured locomotor activity responses. MK-801 is associated with increased dopamine output in the nucleus accumbens, striatum, and frontal cortex, as well as impacting 5-HT neurotransmission (Tuplin, Stocco, & Holahan, 2015). Acute administration of MK-801 and other NMDA receptor antagonists in rats results in behaviors modeling symptoms of schizophrenia including hyperactivity, stereotypic behaviors, and cognitive deficits (Bradford, Savage, Jones, & Kalinichev, 2010). The hyperactivity produced by MK-801 in rats can be reduced by acute administration of aripiprazole and risperidone respectively (Bradford et al., 2010; Tulpin et al., 2015). In the current study, we sought to determine if administration of aripiprazole and risperidone during development alters locomotor responses to MK-801 during adulthood.

Much of the work here focused on possible modifications by early-life antipsychotic drug administration of locomotor responses to drugs of abuse. However, it may be of interest to gain a better appreciation of whether reward mechanisms in the brain are also modified by early-life antipsychotic drugs. As a first step towards this goal, we compared sucrose preference between adult rats administered risperidone, aripiprazole, and vehicle early in life. Presumably, greater intake of sucrose in a free-choice preference test would suggest greater sensitivity to rewarding effects of the sucrose solution. For example, Gulley and colleagues (Kang, Wu, Galvez, & Gulley, 2016) found that sucrose preference is reduced in rats during withdrawal from chronic amphetamine treatment, indicating that decreases in sucrose preference can be associated with anhedonia. Sucrose preference was studied here since it was assessed after rats were tested for

locomotor responses to amphetamine and MK-801 and did not involve further exposure to the latter drugs in tests of reward such as drug-induced conditional place preference.

Children with ADHD and disruptive behavior disorders are at an increased risk for substance abuse and addiction, including the abuse of the very psychostimulants used to treat the disorders (Faraone & Wilens, 2007; Harstad, Levy, & Committee on Substance Abuse, 2014; Levy et al., 2014; Wilens et al., 2008). Previous research from our lab (Stubbeman et al., 2017) indicates that those treated with antipsychotics early in life may have an increased sensitivity to such psychostimulants and implies that these individuals may be at an even higher risk for substance abuse. The study performed by Tadokoro et. al. (2012) also provided evidence that aripiprazole is able to reduce the dopamine sensitivity caused by the chronic application of haloperidol, indicating a potential for attenuating the potential for increased risk for substance abuse. It was expected that the effects of aripiprazole on each behavioral measure would be less marked than the effects of risperidone, based on the latter drug's action as a partial D_2 receptor agonist, and the literature reviewed above that generally suggest a less dramatic behavioral phenotype of rats treated with aripiprazole. By pursuing this line of inquiry, we hoped to generate data that has implications for the treatment of pediatric psychiatric disorders as well as to provide basic information regarding effects of early-life antipsychotic drug treatment on brain and behavioral maturation.

Method

Subjects

Forty-eight male (n=24) and female (n=24) Long Evans rats were used. Pregnant mothers were purchased from Envigo Bioproducts (Indianapolis, IN) and arrived in the animal facility on

gestational day 14. On postnatal day (PND) 7, pups were sexed and cross fostered into litters of 8 such that each litter contained four rats of each sex. They were paw tagged for identification purposes. All pups were weaned on PND 21, re-housed into same-sex pairs, and ear clipped for identification within their home cage. Rats had access to food and water, and the housing room lights went 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 procedures and animal care.

Drugs

Rats were randomly assigned to one of four treatment groups (vehicle, RISP 3.0, ARI 7.0, and ARI 21.0 mg/kg of body weight). Only one dose of risperidone was chosen for study since previous work has assessed dose-dependent effects of risperidone and the selected dose has been the most effective one reported in those studies (Bardgett et al, 2013; Stevens et al., 2016; Stubbeman et al., 2017). Rats were weighed and administered their assigned treatment subcutaneously once a day from PND 14 through 28.

Risperidone. RISP was provided by the National Institute of Mental Health's Chemical Synthesis and Drug Supply program. It was dissolved in a small volume of 10% glacial acetic acid, brought to volume with 0.9% saline, and pH adjusted with 1M sodium hydroxide to roughly 6.3. This solution was injected subcutaneously at a volume of 2.0 ml/kg of body weight. The selected dose was based upon previous work in our lab (Bardgett et. al., 2013; Stubbeman et al., 2017).

Aripiprazole. ARI (RTI; Research Triangle Park, SC) was dissolved in a small volume of 45% 2 hydroxypropyl-b-cyclodextrin (HBC) solution to produce a 6.0 mg/ml concentration based on an approach reported by Varela et. al. (2014). These doses were selected from a range reported in the literature (Tadokoro et al., 2012; Tuplin et al., 2015; Varela et al., 2014). This

solution was injected subcutaneously at 3.5 ml/kg of body weight to serve as the 21.0 mg/kg dose. To produce the 7.0 mg/kg dose, the 6.0 mg/ml concentration was diluted with dH_20 to 2.0 mg/ml and administered at a volume of 3.5 m/kg of body weight.

Vehicle. The vehicle solution consisted of 0.9% saline and 10% glacial acetic acid and pH adjusted to ~6.37 with sodium hydroxide. This was injected subcutaneously at a volume of 2.0 ml/kg of body weight.

Amphetamine. Rats from the four treatment groups were equally divided into four groups of 12 rats, and two groups were tested once per day on either PND 67 and 70, or PND 68 and 71. AMPH was administered via subcutaneous injection on the assigned day. The order of AMPH administration was counterbalanced within each treatment group and within males and females, such that half of each treatment/sex group received AMPH on the first day of testing and the other half received AMPH on the second day of testing. D-amphetamine sulfate (Sigma Aldrich; St. Louis, MO) was dissolved in 0.9% saline at a concentration of 1.0 mg/ml and injected at a volume of 1.0 ml/kg of body weight. The doses are based on those reported in recent studies from our lab (Stubbeman et al., 2017).

Dizocilpine (MK-801). Subjects from the four treatment groups were equally divided into four groups of 12 rats, and one group was tested once a week for three weeks starting on PND 74. MK-801 (Sigma) was injected subcutaneously. Two doses of MK-801 (0.05 & 0.1 mg/kg of body weight) and a 0.9% saline control were used with the selected doses based on previously published behavioral studies from our lab (Bardgett, Points, Roflow, Blankenship, & Griffith, 2009). The order of MK-801 administration was counterbalanced within each treatment group, and within males and females, across the three weeks of testing, such that an equal number of rats from each treatment/sex group got each dose during each week of testing.

MK-801 was dissolved in 0.9% saline and injected at 1.0 ml/kg of body weight.

Locomotor activity

Rats were assessed for locomotor activity throughout the study using clear polypropylene cages (51 cm long × 26.5 cm wide × 32 cm high) inserted into Kinder Scientific Smart-Frames (Poway, CA). Locomotor activity was measured by the number of photobeam breaks recorded during each test session. Testing occurred in a room separate from where the home cages were kept and occurred in a darkened environment except where otherwise noted.

Treatment response. All 48 rats were tested on PND 14 for one hour post-injection, and then again 23 hours later for 20 minutes preceding the following day's treatment administration. These tests were repeated on PND 21 and PND 28. This testing allowed for a comparison of the immediate and delayed effects of ARI and RISP on locomotor activity.

Long-term effect of treatment on spontaneous activity during adulthood. On PNDs 60-64, locomotor data were collected again for one hour to compare the long-term effects of early-life ARI and RISP administration on locomotor activity. Additionally, this procedure served to habituate the rats to the testing environment prior to AMPH administration and testing.

Locomotor response to amphetamine. Locomotor activity after amphetamine or saline administration was recorded in two groups of 12 rats on PND 67 and 69 or in the remaining two groups of 12 rats on PND 68 and 70. On each testing day, locomotor activity was recorded for 30 minutes prior to drug administration to generate baseline locomotor activity data, and then again for three hours after receiving an injection of amphetamine or saline. Each group of 12 rats was balanced for antipsychotic drug treatment group and sex, and the order of saline and amphetamine injections was balanced within each group across the two testing days.

Locomotor responses to MK-801. Once a week for three weeks, beginning on PND 74, locomotor activity was tested before and after MK-801 injection. Rats were placed in locomotor chambers for 30 minutes prior to drug administration to establish baseline locomotor activity, and then again for five hours after receiving an injection of the assigned dose. Balancing and counterbalancing of antipsychotic drug treatment, sex, and administration of different doses of MK-801 occurred as described for the study of locomotor responses to amphetamine.

Sucrose Preference

Beginning on PND 95, a sucrose preference test was conducted based on a protocol published by Isingrini et al. (2016). Starting at 8 am, rats were placed in a locomotor chamber with access to food and two bottles containing tap water and 3% sucrose solution. For 11.5 hours, rats were tested with the lights on, then the bottles were weighed and replaced, their positions on top of the cages swapped to ensure that positional bias was controlled for, and tested again for 11.5 hours in the dark (beginning at 8 p.m.). The side placement of the bottle containing sucrose was balanced as a function of antipsychotic drug treatment and sex. Locomotor activity was also recorded across the entire 23-hour test.

Statistical Analyses

The number of photobeam breaks generated during the tests of the immediate, delayed, and long-term effects of ARI and RISP were analyzed separately for each test using a three-way analysis of variance (ANOVA) with treatment and sex as between subjects factors and time across testing (compiled in five-minute bins) as a within subjects factor. The same analysis was applied to the number of photobeam breaks generated after saline injection in the amphetamine and MK-801 studies (separately for each study), but the data were compiled into 30-minute bins across the 3-5 hours of testing. The effects of amphetamine or MK-801 (each dose) on activity

were analyzed by subtracting the saline data at each time point from the activity recorded at the corresponding time point for each drug/dose. These difference scores were analyzed using a three-way ANOVA with treatment and sex as between subjects factors and time across testing (compiled in 30-minute bins) as a within subjects factor. In the sucrose preference test, the dependent measure was % sucrose solution consumed as a function of total fluid intake. This measure was compared using a three-way ANOVA with treatment and sex as between subjects factors and time (light phase vs. dark phase) as a within subjects factor. Locomotor activity across this testing was compared in the same manner, but with the data compiled into two 11.5-hour bins across the 23 hours of testing.

Results

The first part of the study looked at the effects of each antipsychotic drug on locomotor activity for one hour at PND 14, 21, and 28. A Mauchly's test of sphericity was used in this analysis and nearly all others reported below to determine if the assumption of sphericity was violated in any of within-subjects analysis, such as time or day. When the test indicated that sphericity was violated, all degrees of freedom in such cases were adjusted using Greenhouse-Geisser estimates. There was a significant effect of test day on locomotor activity, $F(1.085, 43.388) = 39.639$, $p < .001$ (Figure 1). Rats appeared to be more active on PND 14 compared to PNDs 21 and 28. There was also a significant treatment effect, $F(3, 40) = 13.643$, $p < .001$. Post hoc testing indicated that the rats that received risperidone were significantly less active than all other treatment groups [Fishers Protected Least Significant Difference (PLSD), $p < .001$]. There was no main effect of sex or interactions between sex and the other variables.

The second part of the study looked at the effects of each antipsychotic drug on

locomotor activity for 20 minutes at 23 hours post-injection at PND 15, 22, and 29. There was a significant effect of test day, $F(1.261, 50.428) = 145.049, p < .001$ (Figure 2). Rats appeared to be less active on PNDs 22 and 29 relative to PND 15. There were no main effects of treatment or sex or interactions between these and the other variables; however, the rats that received risperidone 23 hours previously appeared to be slightly more active than the other treatment groups starting on PND 22.

We then evaluated the long-term effects of each antipsychotic drug on locomotor activity for one hour on five consecutive days between PNDs 60-64. There was a significant effect of test day, $F(4, 160) = 30.242, p < .001$, and a main effect of sex, $F(1, 40) = 24.495, p < .001$ (Figure 3). Additionally, there was a statistically significant interaction between test day and sex, $F(4, 160) = 6.121, p < .001$. Females were more active than males, particularly on the last three days of testing (PLSD $p \leq .001$). There was a significant treatment effect as well, $F(3, 40) = 8.607, p < .001$. Post hoc testing indicated that the rats that received risperidone were significantly more active than all other treatment groups (PLSD, $p \leq .002$).

Locomotor responses to amphetamine and saline were evaluated in separate three-hour sessions that occurred between PNDs 67-71. The data were compiled every 30 minutes across the three-hour session. Moreover, the data were calculated and analyzed as difference scores between the locomotor activity recorded at each time point after amphetamine injection minus the activity recorded at the same time point after saline injection. There was a significant main effect of time, $F(3.107, 124.284) = 85.327, p < .001$, and of sex $F(1, 40) = 18.303, p < .001$, but no main effect of treatment (Figure 4). There was also a significant interaction between time and sex, $F(3.107, 124.284) = 6.015, p = .001$. Female rats were more active than males at 60-150 minutes after amphetamine injection (PLSD $p \leq .024$).

An analysis of the saline data revealed significant main effects of time, $F(3.837, 153.491) = 76.059$, $p < .001$, sex, $F(1, 40) = 8.821$, $p = .005$, and treatment, $F(3, 40) = 3.315$, $p = .029$ (Figure 5). Post hoc testing indicated that risperidone-treated animals displayed higher levels of locomotor activity than all other groups (PLSD, $p \leq .010$) except the low dose of aripiprazole. There were no significant interactions between any of the variables.

Locomotor responses to one of two doses of MK-801 (0.05 and 0.1 mg/kg) and saline were recorded for 5 hours in separate sessions carried out between PNDs 74-92. The data for each dose were analyzed separately since we assessed the difference between the locomotor activity recorded at each post-injection time point in response to a single dose of MK-801 minus the activity recorded at the same time points after saline injection. When the response to the lower dose of MK-801 was assessed in this way, significant effects of time, $F(4.582, 183.268) = 47.762$, $p < .001$, and sex, $F(1, 40) = 78.078$, $p < .001$, were observed (Figure 6). There was also a significant interaction between time and sex, $F(4.582, 183.268) = 18.191$, $p < .001$. Females were more active than males at 60-210 minutes after injection of this dose of MK-801 (PLSD $p \leq .002$). There was no main effect of treatment or interactions between treatment and the other variables.

When the difference scores for the higher dose (0.1 mg/kg) of MK-801 were analyzed, significant effects of time, $F(3.261, 130.443) = 84.281$, $p < .001$, and sex, $F(1, 40) = 321.046$, $p < .001$, were revealed (Figure 7). There was also a significant interaction between time and sex, $F(3.261, 130.443) = 38.951$, $p < .001$. Females displayed greater activity levels than males at 60-300 minutes after injection of this higher dose of MK-801 (PLSD $p < .001$). There was no main effect of treatment or interactions between treatment and the other variables.

An analysis of the saline data acquired during the MK-801 experiment indicated

significant main effects of time, $F(3.689, 147.575) = 75.558$, $p < .001$, sex, $F(1, 40) = 12.300$, $p < .001$, and treatment, $F(3, 40) = 4.255$, $p = .011$ (Figure 8). Post hoc testing indicated rats that received risperidone were more active than vehicle animals (PLSD, $p = .001$), but no other significant differences emerged.

During the week of PND 95, a 23-hour sucrose preference test was conducted by recording the amount of liquid consumed after 11.5 hours in a well-lit environment and again after 11.5 hours in the dark. Data are reported as a ratio of 3% sucrose intake to total intake. When the data were analyzed, there was a significant main effect of light condition, $F(1, 39) = 5.206$, $p = .028$, as well as an interaction between light condition and treatment, $F(3, 39) = 3.394$, $p = .027$ (Figure 9). Post hoc testing indicated that rats in the risperidone and low-dose aripiprazole groups drank more than vehicle rats in the light condition (PLSD, $p \leq .047$), but no significant differences were observed between the treatment groups when the lights were off.

During the sucrose preference test, locomotor activity was recorded in two 11.5-hour blocks across the light/dark cycle. There was a significant effect of sex, $F(1, 40) = 21.526$, $p < .001$ (Figure 10). There was no main effect of treatment or time nor interactions between the variables.

Discussion

The purpose of this study was to compare the behavioral effects of early-life administration of aripiprazole and risperidone respectively in rats at several points throughout development. During the two weeks of initial drug treatment, the immediate effects of the atypical antipsychotics were evaluated at PND 14, 21, and 28 for one hour. Early-life risperidone caused a significant drop in locomotor activity compared to all other treatment groups. This is consistent with Stevens et al. (2016), who also reported dramatic suppression of ongoing

locomotor activity in rat pups administered the same dose of risperidone on PNDs 14-42. These data suggest that the neurotransmitter mechanisms responsible for risperidone-induced decreases in locomotor behavior are fully mature as early as PND 14.

In contrast, there was no significant acute effect of aripiprazole treatment on locomotor behavior on PND 14. However, on PND 21, pups given the lower dose of aripiprazole demonstrated a slight decrease in activity, and, on PND 28, both aripiprazole doses elicited a trend-level decrease in activity. These data suggest that there may be a cumulative effect of aripiprazole drug administration over time. The relatively weaker effects of aripiprazole on locomotor suppression may be related to its somewhat weaker affinity for D_1 receptors when compared to risperidone (Farah, 2005). Previous research has shown that antagonism of D_1 receptors can reduce ongoing locomotor activity (Bardgett & Henry, 1999; Bardgett, Depenbrock, Downs, Points, & Green, 2009).

Twenty-three hours post-injection, 20-minute tests on PND 15-29 showed that risperidone-treated animals appeared to be slightly, but not significantly, more active than the other groups starting on PND 22. Stevens et al. (2016) found that rats administered risperidone over the same developmental period demonstrated significantly higher rates of locomotor activity, but only after PND 29, suggesting that had risperidone administration continued in the present study, hyperactivity may have emerged in the risperidone-administered rats. In contrast, the data did not suggest any lingering or compensatory effects of aripiprazole on locomotor activity at the end of the daily dosing period on PND 15-29.

In examining whether exposure to risperidone or aripiprazole early in life had significant effects on the development of brain circuits responsible for regulating activity during adulthood, one-hour locomotor tests were administered starting at two months of age. These tests revealed

that the risperidone rats were significantly more active than their counterparts. This is consistent with previous research indicating that rats administered early-life risperidone are hyperactive for several months post-treatment (Bardgett et al., 2013). The aripiprazole-treated animals also showed a slight, non-significant increase in activity, but it was not reliably seen across the five test days. This lack of effect was also reported by De Santis et al. (2014) who showed that rats administered aripiprazole via their food between PND 21-42 did not differ from controls in terms of levels of locomotor activity. However, our study used a higher daily dose of aripiprazole and tested rats for longer daily sessions and for more days, yet, despite these methodological differences, still found no significant effect of aripiprazole.

The expression of hyperactivity by adult rats administered risperidone early in life continued into the saline trials accompanying amphetamine-sensitivity testing during PNDs 67-71. Risperidone animals were more active than the other groups with the exception of the low-dose (7.0 mg/kg) of aripiprazole. The latter group demonstrated a mean increase in activity relative to the vehicle group mainly at 30 and 120 minutes into the test, but these mean differences were not statistically significant. The hyperactivity observed in the risperidone-treated animals was again evident in the saline trials during the weeks of MK-801 testing, though the significance was limited to an elevation over vehicle-treated rats. In a previous study by Stubbeman et al. (2017), rats administered risperidone early in life did not demonstrate differential responses in activity after saline injection relative to controls. The presence of these differences in the present study suggests that early-life risperidone administration may alter locomotor responses to the stress of the saline injection or the novelty of the testing cage. Stubbeman et al. (2017) may not have observed similar differences because they performed

extensive habituations of the rats to the testing environment prior to injections relative to the procedures used in the present study.

Because of the group differences in response to the saline injections, we reported the amphetamine response data as difference scores between the locomotor activity recorded at each time point after amphetamine injection minus the activity recorded at the same time point after saline injection. In doing so, we did not find an effect of early-life risperidone or aripiprazole on locomotor responses to amphetamine. The lack of an effect of risperidone is in contrast to previous findings from our lab. Stubbeman et al. (2017) indicated that rats that received early-life risperidone were hypersensitive to the locomotor effects of amphetamine. However, Stubbeman et al. (2017) administered risperidone for four weeks, whereas animals in the present study received only two weeks of antipsychotic treatment early in life. Perhaps the observed hypersensitivity to amphetamine only emerges after more chronic treatment of risperidone, or perhaps, even aripiprazole.

Using the same approach of subtracting the activity observed after MK-801 administration from the activity observed after saline injection, there were no significant effects of early-life risperidone or aripiprazole on locomotor responses to either MK-801 dose. At a neurochemical level, these data suggest that the mechanisms underlying the locomotor hyperactivity produced by NMDA receptor blockade are simply unaffected by early-life antipsychotic drug treatment. But at a practical level, it is possible that the application of MK-801 came too late after the initial atypical antipsychotic administration for an observable effect to occur. Moreover, since MK-801 administration followed extensive locomotor testing and exposure to amphetamine in all rats, the effects of MK-801 could have been confounded by such experiential factors. Further research should address this potential concern.

The locomotor activity recorded during the 23-hour sucrose preference test was void of any observable treatment effects, indicating that any impact of early-life antipsychotic administration had disappeared by the week of PND 95. While Bardgett et al. (2013) found that risperidone-induced hyperactivity in rats persisted through roughly nine months of age, most of the hyperactivity associated with early-life risperidone administration in the present study appears to occur only prior to three months of age. The most likely explanation for this difference is that the former study administered risperidone treatment for four weeks, whereas rats in this study only received daily doses for half that time.

In the sucrose preference test, rats in the risperidone and low-dose aripiprazole groups drank more sucrose than vehicle rats in the light condition, but no significant differences emerged when the lights were off. This provides modest, yet significant, evidence that early-life antipsychotic administration may increase sensitivity to reward. Past work has reported that withdrawal from chronic alcohol (Briones & Woods, 2013) or amphetamine (Kang et al., 2016) or exposure to social stress (Rygula et al., 2005) decreases sucrose preference, suggesting that such reductions may reflect anhedonia or a depressive-like state in animals. The increase in sucrose preference seen in the risperidone and low dose aripiprazole rats could reflect either an elevated hedonic state in such rats or a greater craving for reward. That such an effect would be observed after a low dose of aripiprazole but not a high one is not unprecedented; for example, low but not high doses of aripiprazole can accelerate lever pressing extinction rates (Tuplin et al., 2015). The idea that early-life antipsychotic drug administration could lead to greater craving for reward is worrisome in terms of the implications for drug abuse. However, since the effects seen here are rather modest, any interpretation of them will require further research to resolve their robustness and meaning.

It is important to note that while preparing the aripiprazole solutions, the drug did not completely dissolve into a homogenous solution, particularly in regard to the higher dose. This occurred despite the fact that previously published methods for preparing these solutions were followed (Varela et al., 2014). Before administration each day, the drug solutions were heated and stirred vigorously to achieve relative homogeneity. This raises concerns about actual blood and brain levels of aripiprazole achieved after injection, and its ensuing half-life. Blood sample analysis could give evidence of the physiological concentrations that were achieved in the rats and provide insight into the true efficacy of the administration.

Another explanation for the lack of dramatic behavioral effects seen after aripiprazole administration could be related to the selected doses. Effective behavioral doses of 10-30 mg per day (Mamo et al., 2007; Varela et al., 2014) have been reported, and our doses (7 and 21 mg/kg/day) were within that range. It is possible that higher doses are required to significantly modify brain development, but such high doses are unlikely to be used clinically in pediatric populations. Additionally, the single vehicle control group employed here may not be sufficient as it was created using glacial acetic acid (used in creating our risperidone vehicle solution), but the aripiprazole was dissolved in a HBC solution.

Finally, the relative lack of behavioral effects of aripiprazole, when compared to risperidone, may be simply a consequence of pharmacological differences. In general, the effects of aripiprazole on several behaviors and biological processes (e.g., stimulant-induced locomotor activity, antipsychotic-induced gene expression) seem to be muted in rats when compared to other antipsychotic drugs, such as risperidone or haloperidol, at doses that are roughly equivalent in terms of the level of D_2 receptor binding achieved (Natesan et al., 2006; Tadokoro et al., 2012). This limited behavioral effect of aripiprazole may be related to its affinity profile at non-

D_2 receptors – a profile that is unique in comparison to other atypical antipsychotic drugs, such as risperidone (Farah, 2005).

Future research should address the concerns identified above, but also compare the developmental effects of atypical antipsychotics on a greater array of behavioral processes during adulthood. This study focused primarily on locomotor responses and assayed one measure of reward. There is a myriad of other behaviors that could be studied, such as learning and memory, drug-induced conditioning, or decision-making, all of which can be influenced acutely by antipsychotic drug administration during adulthood (Bardgett 2004; Bardgett, Depenbrock et al., 2009). Moreover, as mentioned in the Introduction, studies could be conducted to examine biological processes affected by early-life antipsychotic drug administration such as drug-induced changes in receptor densities (Moran-Gates et al., 2007; Choi et al., 2010).

Overall, our research confirmed our earlier work showing that early-life administration of risperidone leads to elevated rates of locomotor activity. In comparison, the immediate and delayed effects of aripiprazole on locomotor activity were not as pronounced, although the modest effects of aripiprazole as well as risperidone on sucrose preference merit further inquiry. These preclinical data suggest that the impact of early-life aripiprazole on brain development is less dramatic than the impact of early-life risperidone. At a translational level, this work indicates that the long-term effects of aripiprazole use in human pediatric populations should be less marked than the long-term effects of risperidone. Since the FDA has approved the use of both drugs in children, our data may offer some guidance for prescribing practices in this population.

References

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

Bardgett, M. E., Depenbrock, M., Downs, N., Points, M., & Green, L. (2009). Dopamine modulates effort-based decision-making in rats. *Behavioral Neuroscience, 123,* 242-251. doi: 10.1037/a0014625

Bardgett, M. E. & Henry, J. D. (1999). Locomotor activity and accumbens fos expression driven by ventral hippocampal stimulation requires D1 and D2 receptors. *Neuroscience, 94,* 59-70.

Bardgett, M. E., Points, M., Roflow, J., Blankenship, M., & Griffith, M. S. (2009). Effects of the H3 antagonist, thioperamide, on behavioral alterations induced by systemic MK-801 administration in rats. *Psychopharmacology, 205*, 589-597. doi: 10.1007/s00213-009-1566-8

Bardgett, M., 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

Bernagie, C., Danckaerts, M., Wampers, M., & De Hert, M. (2016). Aripiprazole and acute extrapyramidal symptoms in children and adolescents: A meta-analysis. *CNS Drugs, 30*, 807-818. doi:10.1007/s40263-016-0367-y

Bradford, A. M., Savage, K. M., Jones, D. C., & Kalinichev, M. (2010). Validation and pharmacological characterization of MK-801-induced locomotor hyperactivity in

BALB/C mice as an assay for detection of novel antipsychotics. *Psychopharmacology, 212*, 155-170. doi:10.1007/s00213-010-1938-0

Briones, T. L., & Woods, J. (2013). Chronic binge-like alcohol consumption in adolescence causes depression-like symptoms possibly mediated by the effects of BDNF on neurogenesis. *Neuroscience 254*, 324-334. doi: 10.1016/j.neuroscience.2013.09.031

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

De Santis, M., Pan, B., Lian, J., Huang, X., & Deng, C. (2014). Different effects of bifeprunox, aripiprazole, and haloperidol on body weight gain, food and water intake, and locomotor activity in rats. *Pharmacology, Biochemistry And Behavior, 124,* 167-173. doi:10.1016/j.pbb.2014.06.004

De Santis, M., Lian, J., Huang, X., & Deng, C. (2016). Early antipsychotic treatment in juvenile rats elicits long-term alterations to the dopamine neurotransmitter system. *International Journal of Molecular Sciences, 17*, e1944. doi: 10.3390/ijms17111944

Farah, A. (2005). Atypicality of atypical antipsychotics. *Primary Care Companion Journal of Clinical Psychiatry, 7*, 268-274.

Faraone, S.V., & Wilens, T.E. (2007). Effect of stimulant medications for attention-deficit/hyperactivity disorder on later substance use and the potential for stimulant misuse, abuse, and diversion. *Journal of Clinical Psychiatry, 68*, 15-22.

Harstad, E., Levy, S., & Committee on Substance Abuse (2014). Attention-deficit/hyperactivity disorder and substance abuse. *Pediatrics, 134*, e293-e301. doi: 10.1542/peds.2014-0992

Iñiguez, S. D., Cortez, A. M., Crawford, C. A., & McDougall, S. A. (2007). Effects of aripiprazole and terguride on dopamine synthesis in the dorsal striatum and medial prefrontal cortex of preweanling rats. *Journal of Neural Transmission, 115*, 97-106. doi: 10.1007/s00702-007-0820-7

Isingrini, E., Perret, L., Rainer, Q., Sagueby, S., Moquin, L., Gratton, A., & Giros, B. (2016). Selective genetic disruption of dopaminergic, serotonergic and noradrenergic neurotransmission: insights into motor, emotional and addictive behaviour. *Journal of Psychiatry and Neuroscience, 41*, 169-181.

Iversen, L. L., Iversen, S. D., Bloom, F. E., & Roth, R. H. (2008). *Introduction to neuropsychopharmacology*. Oxford: Oxford University Press.

Kang, S., Wu, M. M., Galvez, R., & Gulley, J. M. (2016). Timing of amphetamine exposure in relation to puberty onset determines its effects on anhedonia, exploratory behavior, and dopamine D(1) receptor expression in young adulthood. *Neuroscience, 339,* 72-84. doi: 10.1016/j.neuroscience.2016.09.044

Levy, S., Katusic, S. K., Colligan, R. C., Weaver, A. L., Killian, J. M., Voigt, R. G., & Barbaresi, W. J. (2014). Childhood ADHD and risk for substance dependence in adulthood: A longitudinal, population-based study. *PLoS One, 9, e105640*. doi: 10.1371/journal.pone.0105640

Mamo, D., Graff, A., Mizrahi, R., Shammi, C. M., Romeyer, F., & Kapur, S. (2007). Differential effects of aripiprazole on D(2), 5-HT(2), and 5-HT(1A) receptor occupancy in patients with schizophrenia: A triple tracer PET study. *The American Journal of Psychiatry, 164*, 1411-1417. doi: 10.1176/appo.ajp.2007.06091479

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

Natesan, S., Reckless, G. E., Nobrega, J. N., Fletcher, P. J., & Kapur, S. (2006). Dissociation between in vivo occupancy and functional antagonism of dopamine D-2 receptors: Comparing aripiprazole to other antipsychotics in animal models. *Neuropsychopharmacology, 31*, 1854-1863. doi: 10.1038/sj.npp.1300983

Olfson, M., Blanco, C., Liu, L., Moreno, C., & Laje, G. (2006). National trends in the outpatient treatment of children and adolescents with antipsychotic drugs. *Archives of General Psychiatry, 63*, 679-685. doi: 10.1001/archpsyc.63.6.679

Olfson, M., Blanco, C., Liu, S., Wang, S., & Correll, C. U. (2012). National trends in the office-based treatment of children, adolescents, and adults with antipsychotics. *JAMA Psychiatry, 69*, 1247-1256. doi: 10.1001/archgenpsychiatry.2012.647

Rygula, R., Abumaria, N., Flügge, G., Fuchs, E., Rüther, E., & Havemann-Reinecke, U. (2005). Anhedonia and motivational deficits in rats: Impact of chronic social stress. *Behavioural Brain Research, 162,* 127-134. doi: 10.1016/j.bbr.2005.03.009

Stevens, R. M., Gannon, M. A., Griffith, M. S., & Bardgett, M. E. (2016). Delayed yet persistent effects of daily risperidone on activity in developing rats. *Behavioral Pharmacology, 27*, 460-469. doi: 10.1097/FBP.0000000000000230

Stubbeman, B. L., Brown, C. J., Yates, J. R., & Bardgett, M. E. (2017). Early-life risperidone enhances locomotor responses to amphetamine during adulthood. *European Journal Of Pharmacology, 812,* 256-263. doi: 10.1016/j.ejphar.2017.07.025

Tadokoro, S., Okamura, N., Sekine, Y., Kanahara, N., Hashimoto, K., & Iyo, M. (2012). Chronic treatment with aripiprazole prevents development of dopamine supersensitivity and potentially supersensitivity psychosis. *Schizophrenia Bulletin, 38*, 1012-1020. doi:10.1093/schbul/sbr006

Tuplin, E. W., Stocco, M. R., & Holahan, M. R. (2015). Attenuation of MK-801-induced behavioral perseveration by typical and atypical antipsychotic pretreatment in rats. *Behavioral Neuroscience, 129*, 399-411. doi:10.1037/bne0000066

Varela, F. A., Der-Ghazarian, T., Lee, R. J., Charntikov, S., Crawford, C. A., & McDougall, S. A. (2014). Repeated aripiprazole treatment causes dopamine D2 receptor up-regulation and dopamine supersensitivity in young rats. *Journal of Psychopharmacology, 28*, 376-386. doi:10.1177/0269881113504016

Vitiello, B., Correll, C., van Zwieten-Boot, B., Zuddas, A., Parellada, M., & Arango, C. (2009). Antipsychotics in children and adolescents: Increasing use, evidence for efficacy and safety concerns. *European Neuropsychopharmacology, 19*, 629-635. doi:10.1016/j.euroneuro.2009.04.008

Wilens, T.E., Adler, L.A., Adams, J., Sgambati, S., Rotrosen, J., Sawtelle, R., Utzinger, L., & Fusillo, S. (2008). Misuse and diversion of stimulants prescribed for ADHD: A systematic review of the literature. *Journal of the American Academy of Child Adolescent Psychiatry 47*, 21-31. doi: 10.1097/chi.0b013e31815a56f1

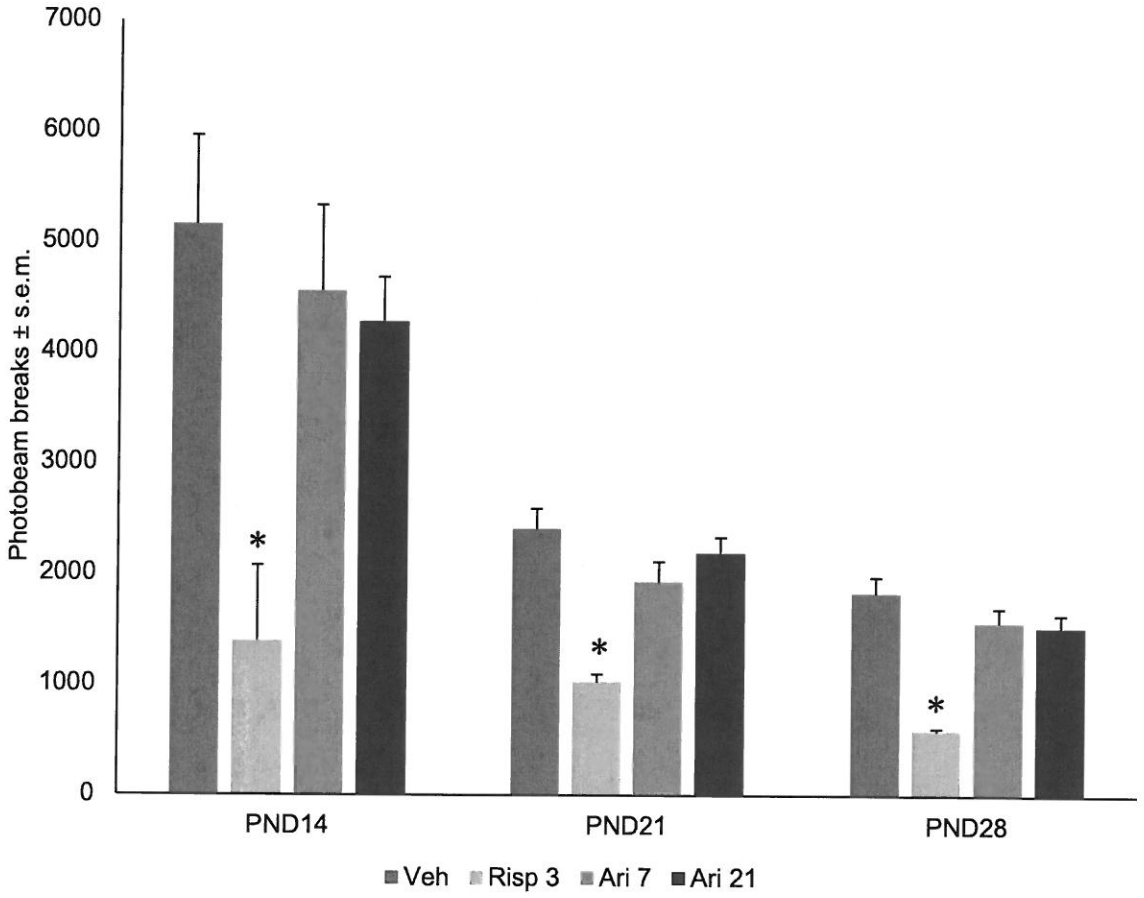

Figure 1. Locomotor activity over one hour on PND 14, 21, and 28 following drug administration. Data represent mean number of photobeam breaks + s.e.m.

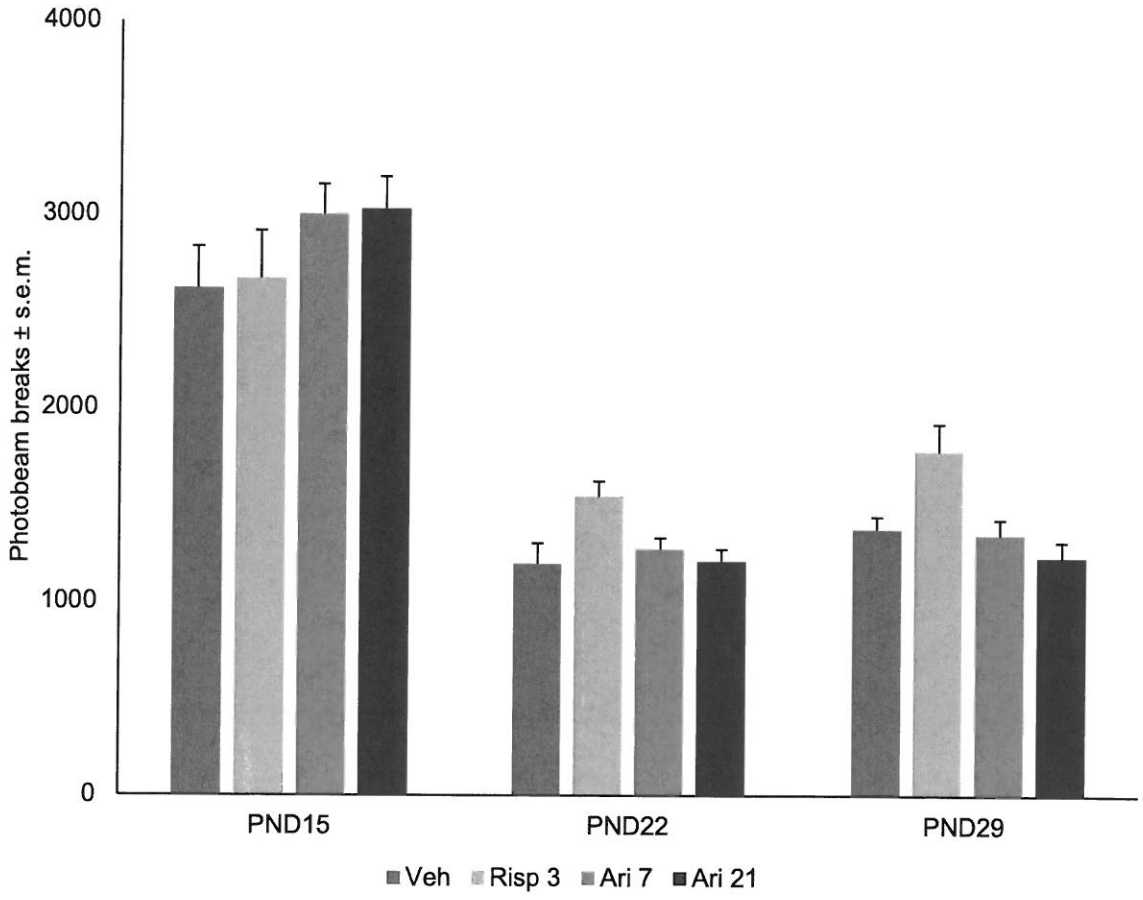

Figure 2. Locomotor activity over 20 minutes 23 hours post-injection on PND 15, 22, & 29. Data represent mean number of photobeam breaks + s.e.m.

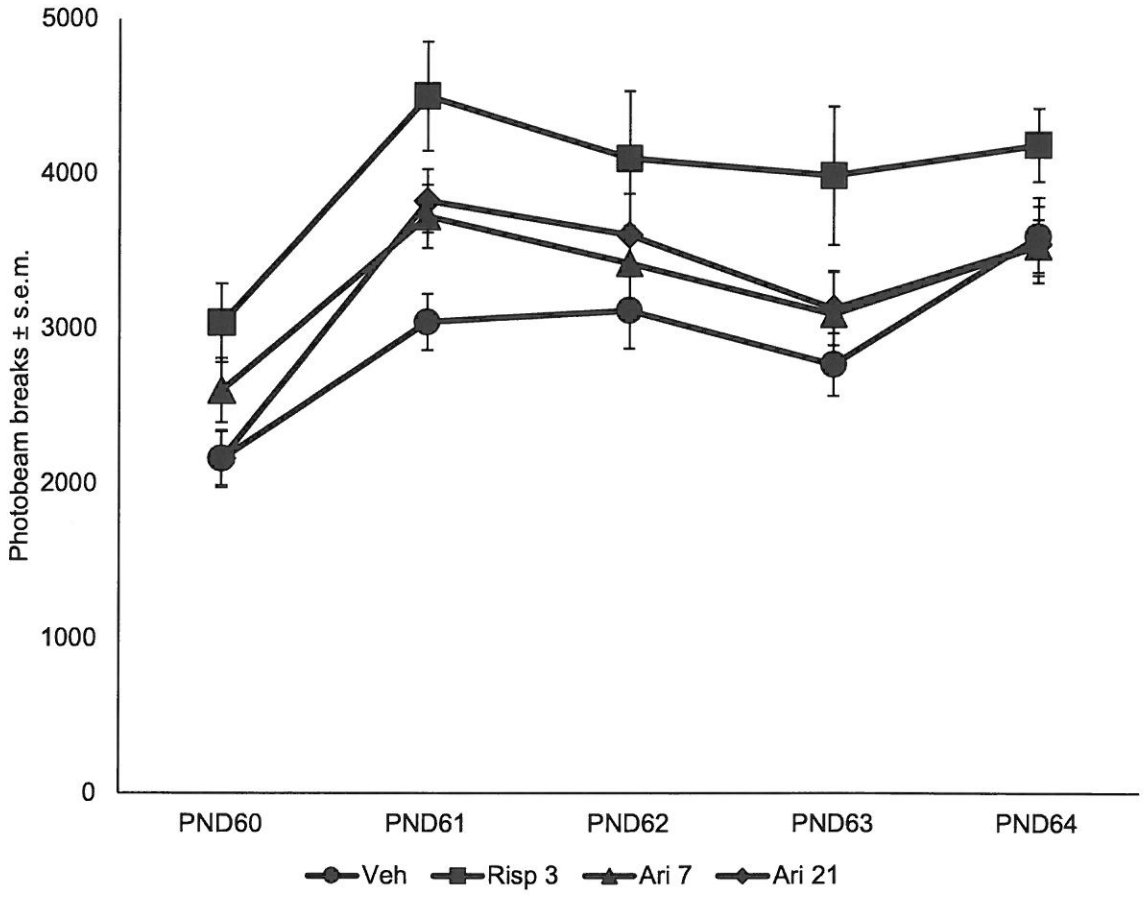

Figure 3. Locomotor activity over one hour on PND 60-64. Data represent mean number of photobeam breaks ± s.e.m.

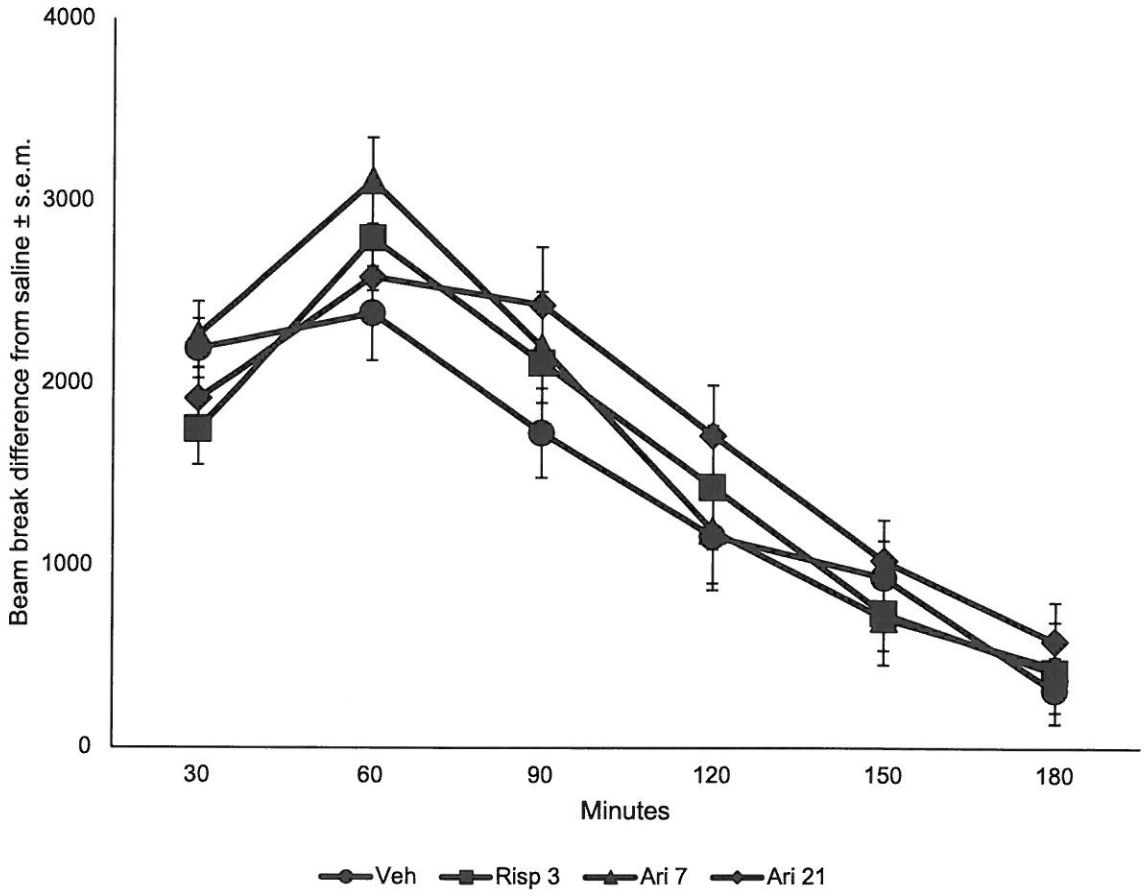

Figure 4. Locomotor response to amphetamine Data represent mean difference in the number of photobeam breaks observed after amphetamine injection minus the number of photobeam breaks observed after saline injection ± s.e.m.

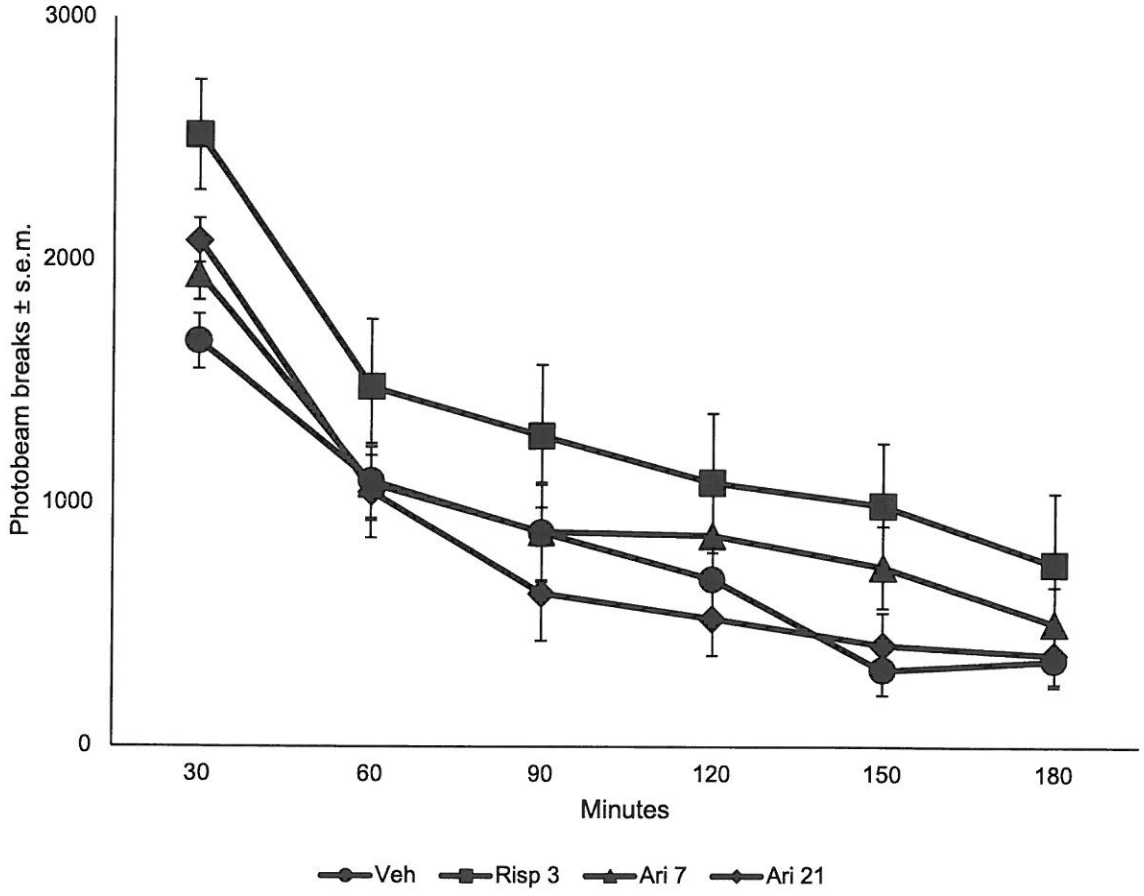

Figure 5. Locomotor activity response to saline during the week of PND 67. Data represent mean number of photobeam breaks ± s.e.m.

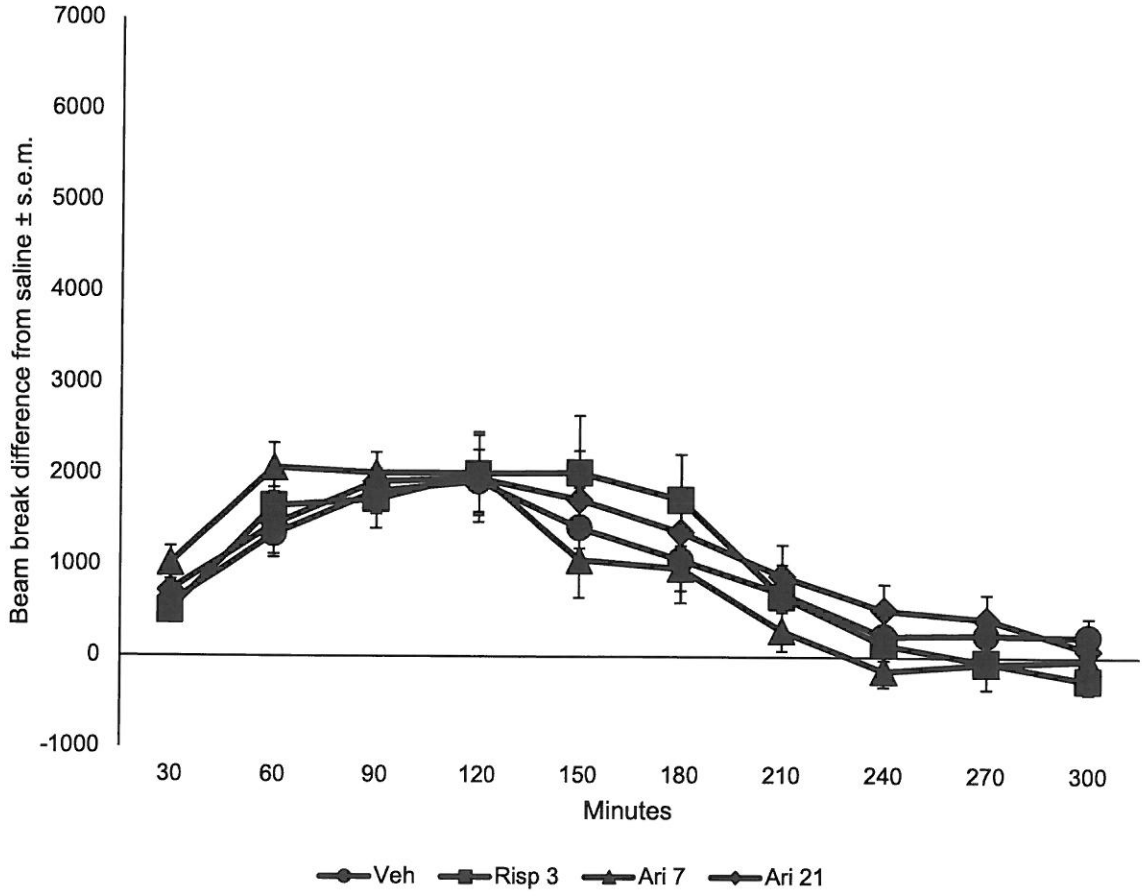

Figure 6. Locomotor response to 0.05 mg/kg of MK-801. Data represent mean difference in the number of photobeam breaks observed after MK-801 injection minus the number of photobeam breaks observed after saline injection ± s.e.m.

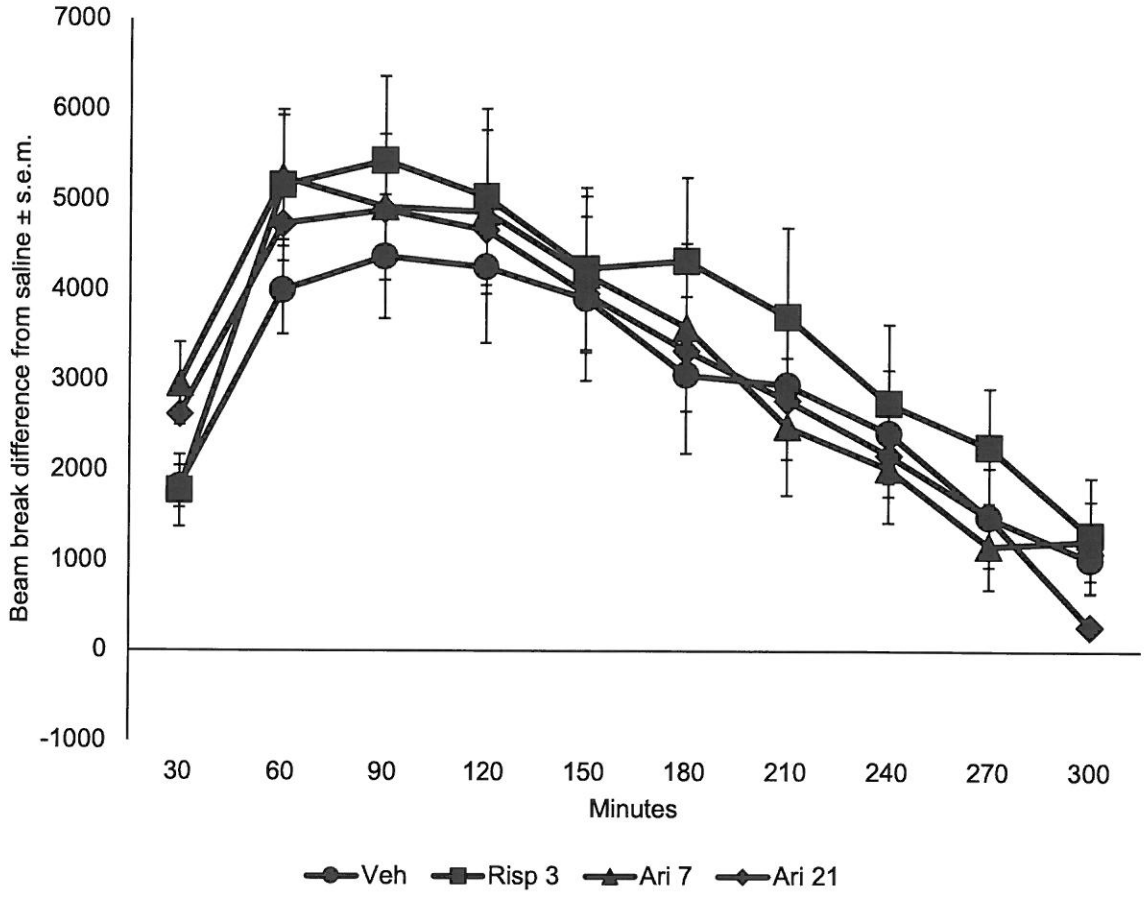

Figure 7. Locomotor response to MK-801 (0.1 mg/kg) reported as difference from saline recorded between PNDs 74-92. Data represent mean difference in the number of photobeam breaks observed after MK-801 injection minus the number of photobeam breaks observed after saline injection ± s.e.m.

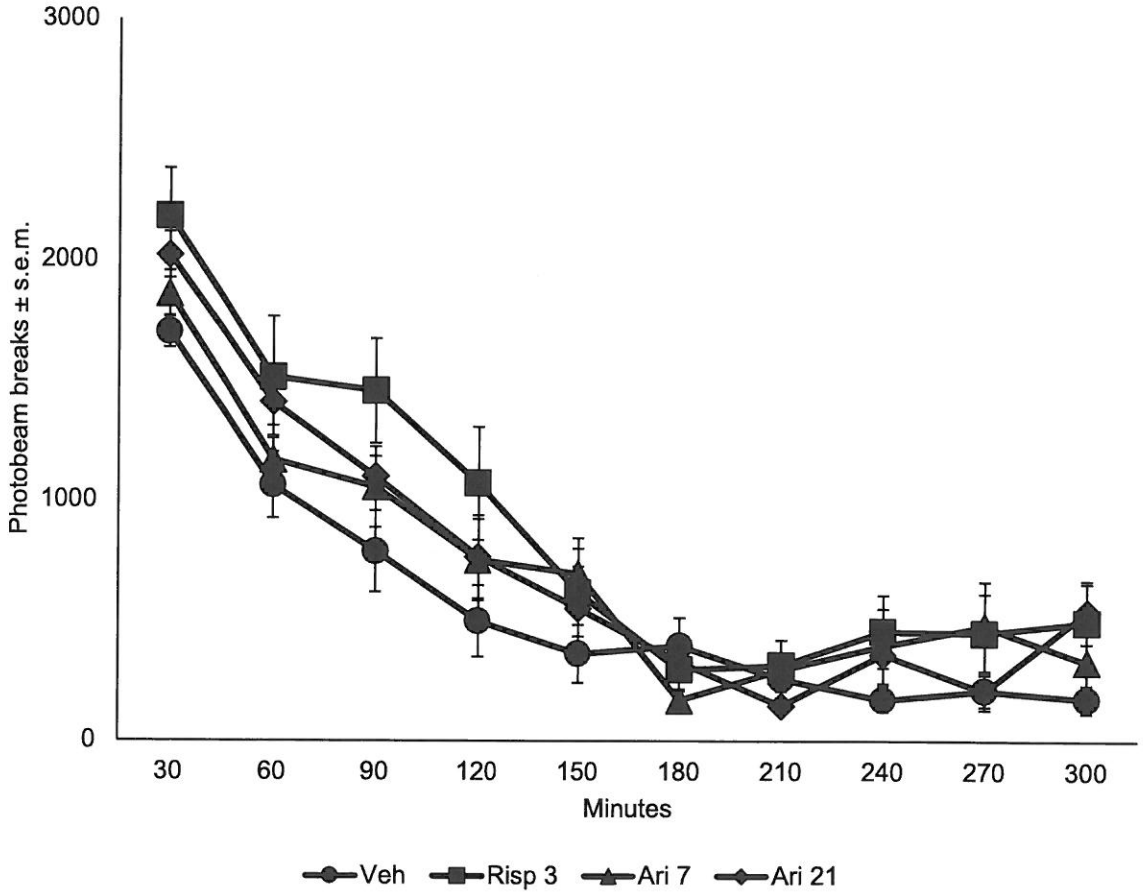

Figure 8. Locomotor activity response to saline recorded between PNDs 74-92. Data represent mean number of photobeam breaks ± s.e.m.

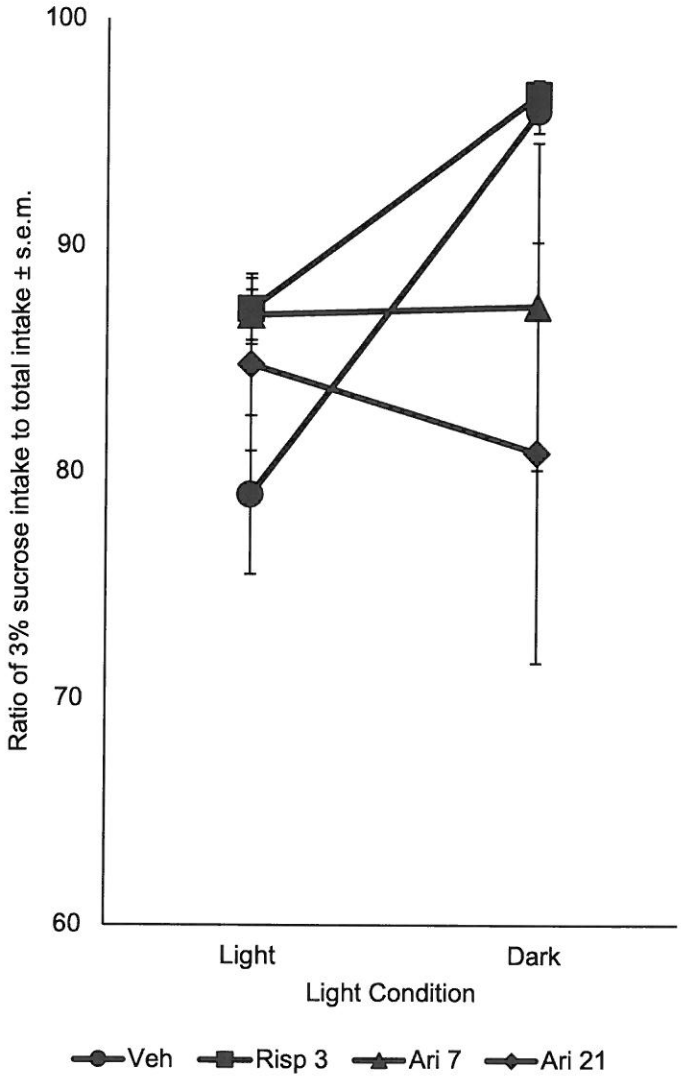

Figure 9. Percent sucrose intake recorded over 23 hours across two 11.5-hour blocks during the week of PND 95. Data represent mean ratio of 3% sucrose intake to overall fluid (3% sucrose solution + water alone solution) intake ± s.e.m.

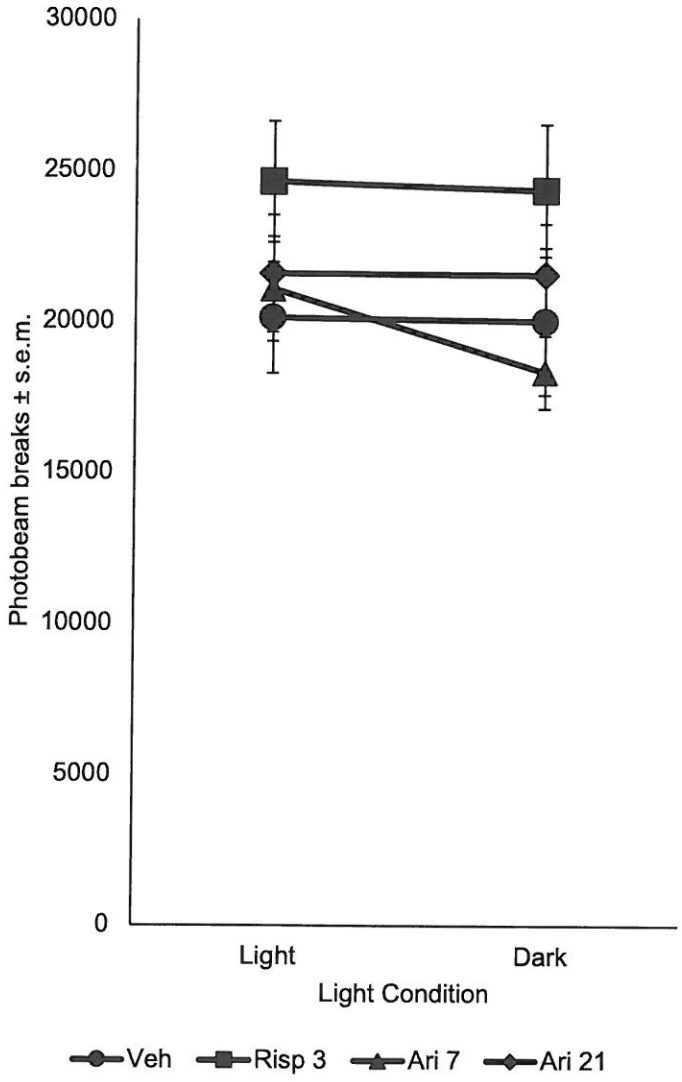

Figure 10. Locomotor activity recorded over 23 hours across two 11.5-hour blocks during the sucrose preference test (week of PND 95). Data represent mean number of photobeam breaks ± s.e.m.