The effects of chronic exposure to risperidone during adolescence on behavioral rigidity and impulsive choice in adulthood

by

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Abstract

Risperidone is a second-generation antipsychotic that is commonly prescribed in children in adolescence. Its mechanism of action targets both serotonin and dopamine, both of which mediate different types of behavior, such as perseveration and impulsive choice. To test whether there was a long-term effect of chronic risperidone exposure on these behaviors, adolescent and adult mice were exposed to 2.5 mg/kg/day risperidone over a 28-day period, then tested on both a spatial discrimination reversal procedure and a delay discounting procedure 30 days after cessation of the drug. During the reversal procedure, adolescent exposed animals showed more behavioral rigidity. In the delay discounting procedure, both adolescent and adult exposed mice showed lower higher rates of discounting.

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Atypical, or second generation, antipsychotics (SGAs) is a class of drug that are prescribed for treatment of schizophrenia and for other reasons. They are prescribed at high rates both nationally and internationally, with a larger number of prescriptions being for adolescents (Edelsohn et al 2017; Chavez et al 2021; Lee, Vidal, and Findling 2018; Persico et al 2021; Rafaniello et al 2020). Risperidone, a SGA acts as a dopamine (DA) and a serotonin (5-HT) antagonist, with its primary therapeutic benefits deriving from the higher affinity risperidone has for 5-HT receptors compared to DA receptors (Schotte et al 1996). Risperidone has been approved for the management of signs and symptoms of schizophrenia, bipolar disorder, and aggressive behaviors in children or adolescents with autism spectrum disorder or other developmental disabilities (Harrison, Cluxton-Keller, and Gross 2012; Rafaniello et al 2020).

As with all drugs, SGAs have side effects. The use of SGAs increases the likelihood of obesity compared to those who do not use SGAs (Guber, Cortes, and Duan 2022). There are a few explanations that have been offered for why SGAs increase the likelihood of weight gain and obesity. First, it is suggested that SGAs cause an upregulation of neuropeptide Y (NPY), which plays a role in the regulation of food intake (Lian, De Santis, He, and Deng 2015; Wang et al. 2020). It has also been suggested that they cause an upregulation of DA receptors, which leads to a higher likelihood of diet induced obesity when a high fat diet is introduced (Labouesse et al 2018). Upregulation refers to a process in which the number of receptors in a synapse increases due to diminished neurotransmitter binding at post-synaptic receptors. This sometimes occurs after long term exposure to a drug or toxicant that affects a specific neurotransmitter system and is more commonly seen with antagonists. The inverse of upregulation is downregulation, in

which the amount of post-synaptic receptors decreases as a result of oversaturation of neurotransmitters binding to post-synaptic receptors. Downregulation is usually the result of neurotransmitter agonists. Upregulation of a given neurotransmitter receptor leads to a higher sensitivity to that neurotransmitter, while downregulation leads to a lower sensitivity to that neurotransmitter (Rajagopal and Shenoy 2018; Shapira, Gafni, and Sarne 2003; Hadcock and Malbon 1988). In the case of SGAs, there is evidence upregulation occurs across multiple neurotransmitter systems, including 5HT and DA (Maiti et al 2021; Moran-Gates et al 2006; Chouinard et al 2017). In addition, up- and downregulation caused by agonists and antagonists, there is also evidence of these alterations taking place naturally during adolescence as well, making it a period even more sensitive to up- or down-regulation occurring form outside sources (Morgan, May, & Finch 1987; Reynolds & Flores 2021; Rothmond, Weickert, & Webster 2012).

Another prominent side effect of SGAs is the presence of extrapyramidal signs symptoms, or problems with movement and motor control. These are largely split into two categories: Parkinsonian symptoms and tardive dyskinesia. Parkinsonian symptoms manifest in a way that resembles Parkinson's Disease, and are characterized by slow movement, shuffling gait, and resting tremors. These symptoms appear as a result of dopamine D_2 receptors being blocked (Solmi, Pigato, Kane, and Correll 2018). Tardive dyskinesia is characterized by involuntary and uncontrolled movements, with one of the most prominent features to be expressed being writhing of the face (Divac, Prostran, Jakovcevski, and Cerovac 2014). However, the risk is smaller with SGAs compared to the first-generation antipsychotics (FGA) because the SGAs have higher binding coefficients for 5-HT than for D_2 receptors. It can become a concern at higher doses and extended durations of treatment. Parkinsonian symptoms usually manifest as a side effect during the drug use, while tardive dyskinesia is a delayed response to long term usage of antipsychotics.

Adolescence is a sensitive period of neural development. It is the final critical period of neural development before adulthood, marked by increased cell proliferation and selective pruning of DA synapses within the prefrontal cortex and striatum (Andersen et al 2000; Teicher et al 1995) as well as for the development of 5-HT neural pathways, which continue to innervate the frontal lobe during this time period (Lidov et al 1982). It is also a period that is marked by an increase in risky, thrill-seeking behaviors, manifesting in was such as recreational drug use, gambling behaviors, and risky sexual behavior (Adriani and Laviola 2004; Squeglia and Cservenka 2017; Tani, Ponti, and Ghinassi 2020; Guo et al 2002). Impulsive and risk-taking behaviors are altered by exposure to neurotoxicants and drugs during the adolescent period, supporting the idea that alterations to both DA and 5-HT neurotransmission during adolescence can affect these types of behavior, among other long-term changes, such as deficits in learning or memory (Pope et al 2016; Andersen 2003; Salmanzadeh 2020).

There are signs that rodents go through similar adolescent development as humans, as marked by shared biological markers of brain development in both species, including large decrease in both synaptic and receptor densities and the timing of synaptic changing being similar (occurring in subcortical regions before cortical regions), to name a few similarities (Andersen 2003). The exact markers for this developmental period are debated, with some suggesting that the boundaries are postnatal days (PND) 21-60 (Andersen 2003), PND 28-42 (Spear 2000), or PND 25-65 (Vetter-O'Hagen and Spear 2012). In addition, the boundaries that surround adolescence are suggested to be different between rodent species, with the onset of adolescence and puberty being different between rats (suggested between PND 28-70) and mice (suggested between PND 23-52) (Schneider 2013).

5-HT and DA are two neurotransmitters that play important roles in modulating behavior. DA is heavily involved in both motor coordination and reinforcement and impulsive choice. These are mediated by different dopaminergic pathways within the brain, the nigrostriatal pathway, projecting from the substantia nigra up to the striatum; the mesolimbic pathway, projecting from the ventral tegmental area (VTA) to the nucleus accumbens; and the mesocortical pathway, projecting from the ventral tegmental area (VTA) through the prefrontal cortex (PFC) and orbitofrontal cortex (OFC), areas known to mediate impulsive behaviors (Anderson et al, 2000; Teicher et al, 1995).

5-HT, in addition to being involved with behaviors such as motor coordination and reward processing also mediates behaviors involving memory, anxiety, aggression, appetite, as well as other organ systems such as circadian rhythm and regulation of other organ systems. 5-HT is also more widespread than DA, projecting from the Raphé nucleus to the midbrain, hindbrain, frontal cortex, cerebellum, and spinal cord, to name a few examples (Berger, Gray, and Roth 2009: Reith et al 1997).

Two types of behavior that might be influenced due to changes in dopamine or serotonin transmission are perseverative behavior and impulsive choice. Perseverative behavior refers to how resistant to change behavior is after the reinforcement contingencies governing that behavior have changed. Two different modes of perseverative behavior are intradimensional versus extradimensional shifts. An intradimensional shift refers to when the dimension of the stimulus of a response remains the same, but the contingency for reinforcement changes. For example, a mouse is trained to press a lever on the left side of a and ignore the lever on the right side. After training to press the left lever, the contingency for what provides reinforcement changes; the mouse now needs to press the right lever. The dimension of the stimulus stays the

same (i.e. spatial location) but the contingency changes (i.e. instead of pressing the left lever, reinforcement is shifted to the right lever). In an extradimensional shift, similarly to an intradimensional shift, the contingency for reinforcement changes after a certain behavior is learned. In contrast, however, the dimension of the stimulus also changes. For example, take the mouse from the previous example that was taught to follow the spatial location to press a lever in order to receive reinforcement. If the stimulus dimensions changed (e.g. when a light flashes, pressing the left lever provides reinforcement, while if the light is off pressing the right lever provides reinforcement), the dimension of the stimuli has changed, causing an extradimensional shift. Two more clinically relevant examples of the extradimensional shift include the visual discrimination reversal task that is used in animals (Paletz et al 2007) or the Cambridge Neuropsychological Test Automated Batteries (CANTAB) with humans (Fray, Robbins, and Sahakian 1996).

In animals, one way that we assess perseveration is a procedure called spatial discrimination reversal (SDR). In SDR, a choice is given between two options, usually a lever press, with responses on option resulting in reinforcement while responses on the other option have no programmed consequence. Once responding on the correct lever becomes stable, a reversal is implemented where the originally reinforcing lever undergoes extinction, while the previously unreinforced lever begins providing reinforcement. Perseveration is then measured by how long it takes the subject to change their responses under the reversal. Because the mode of response (pressing a lever) remains the same, and just the rule governing reinforcement (specific lever pressed) changes, SDR is an example of an intradimensional shift.

SDR has also been used in various studies to show how substances that affect neurotransmission or alterations in neurotransmitter pathways can affect perseverative behaviors.

In one study, rats were trained in a spatial discrimination procedure which was then followed by reversal learning tasks. Before each session, subjects were administered with different doses of the 5-HT_{2A} antagonist M100907. They found that, at higher doses, subjects made more incorrect responses after the reversal than controls or at lower doses (Boulougouris, Glennon and Robbins, 2008). In a different set of experiments, marmosets were taught sets of discriminations, then underwent surgery to selectively deplete levels of either 5-HT or DA within the OFC. After depletion and once subjects were tested on reversals, the group that underwent 5-HT depletion had significantly more errors than the control or DA depletion groups. The errors this group had were perseverative errors, not learning errors, suggesting an effect of lower 5-HT on perseverative responding (Clarke et al. 2004; Clarke et al. 2007). In a probabilistic reversal task in humans, participants were deprived of tryptophan (a precursor to serotonin) and placed in an MRI while completing a reversal learning task. During this task, they found that participants who underwent the acute tryptophan depletion (ATD) had a stronger BOLD response within their dorsomedial PFC, and this was also associated with reversal errors made during the task, linking serotonin activity in the dorsomedial PFC with reversal learning (Evers et al, 2005).

In addition to being heavily affected by 5-HT neurotransmission, perseverative behaviors are also modulated by DA neurotransmission. Researchers took groups of patients with mild or severe Parkinson's disease, a disease marked by a diminished number of DA cell bodies within the substantia nigra who were either medicated or unmedicated were examined. A visual discrimination task involved clicking a screen that had two colors on it, and trying to choose the correct one within the pairs, and at a certain criterion the correct choice within the pair would reverse. When compared to controls, all groups with Parkinson's disease showed significant impairment, failing the reversal more often. In one study, mice were bred to be born without a

specific gene, Drd2. This gene is responsible for making proteins that are used to make up the D2 auto-receptors in the midbrain. In a similar procedure as described with SDR above, mice underwent a spatial reversal procedure. They found that the subjects that lacked Drd2 required a higher number of trials to reach accuracy after a reversal occurred when compared to control mice (Linden et al 2018). Other studies have looked at the effect that drugs or other environmental toxicants that affect DA neurotransmission have on perseverative behavior. In one study that looked at how mice performed on the SDR task after chronic exposure to cocaine, adolescent mice, beginning on PND 30, received an injection of cocaine every day over the course of fourteen days, then were trained in the SDR procedure, beginning at PND 70. They found that while there was no difference between groups on the initial learning of the discrimination, there was a significant difference between the exposure and control groups after the first reversal. Specifically, the exposure group had more errors and took more time to reach target accuracy after the first reversal (Pope et al 2016). Cocaine is a drug that inhibits reuptake of monoamines DA, 5-HT, and norepinephrine (NE), though it may have a higher affinity for DA reuptake inhibition (Cunningham and Callahan 1991). In another study, mice were exposed to either methylmercury (MeHg), *d*-amphetamine, or a combination of the two. In this study, after exposure to either or both of these substances, mice were first tested on a SDR procedure, followed by testing on a visual discrimination reversal (VDR) procedure. In the VDR procedure, most of the process remains similar to SDR, except rather than following which specific side lever provides reinforcement, the subject must follow which light above the lever to determine the reinforcing lever. The transition from SDR to VDR is an example of an extradimensional shift. They found that any combination of MeHg and/or *d*-amphetamine had a significant increase in number of trials to reach accuracy criterion compared to the control group after the

second reversal, and the MeHg group took significantly more trials during the VDR compared to the control group as well (Boomhower and Newland 2017).

Another type of behavior that is affected by changes in 5-HT and DA neurotransmission is impulsive choice. Impulsive choice is an inability to delay gratification; the putting off of a larger reward that will come later in favor of a worse option that occurs more immediately (Evenden 1999). While impulsive choice can be measured with a variety of procedures, one of the most common among both humans and non-human animals is the discounting procedure (Vanderveldt, Oliveira, and Green 2016). Discounting refers to how much the value of a reinforcer diminishes in relation to some other changing variable. This variable is usually either a delay to the reinforcement (delay discounting) or the likelihood that a reinforcer will occur (probability discounting). Delay discounting measures the value of a reinforcer as its relative value diminishes the longer it takes for reinforcement to arrive. Impulsivity, in this case, would refer to choosing to take a smaller reward sooner as the value of the larger reward has diminished due to the time it would take to receive. Discounting procedures are used to measure levels of impulsivity, and is used in various populations such as drug users or those with neurodevelopmental disorders (Reynolds 2006; Bickel et al. 2012; Brown et al. 2018; Mies et al 2019).

In humans, some studies have been done to study how 5-HT can affect impulsivity via delay discounting. One study viewed how variations in dietary tryptophan can affect levels of discounting in people. In this study, participants in groups were given either a normal, high, or zero tryptophan. This is meant to either overload or deplete the amount of serotonin by affecting the amount of precursor amino acid in the body. Participants were then given a delay discounting task in which they were show colored tiles, and given the choice to earn five yen immediately, or

twenty yen after a longer delay. They found that participants who drank the depletion drink (no tryptophan) chose more small rewards compared to the overloaded and control groups (Schweighofer et al 2008). In another study, rats were given varying doses (±)-1-(2,5-dimethoxy4-iodophenyl)-2-aminopropane (DOI) or 8-OH-DPAT. These substances act as 5-HT_2 agonist and 5-HT_{1A} agonists, respectively. During sessions, subjects were given a choice between two different solutions; a simple glucose solution and a glucose solution that also contained saccharin, with the saccharin solution being the more desirable reinforcer. Within sessions, the delay to receive the saccharin solution adjusted based on choices made during the session; a response that delivered saccharin increased the delay by one second while a response that delivered glucose decreased the delay by one second. The delays within the session were then taken and a mean adjusted delay was found (MAD). This study found that there was a dose dependent effect for both DOI and 8-OH-DPAT on the MAD; in both cases, the MAD decreased with dosing, which means there was a higher number of choices for the sooner, and less preferred glucose reward rather than the saccharin reward as subjects were dosed (Blasio et al. 2012).

Delay discounting has also been shown to be affected by DA neurotransmission. In a study looking at MeHg exposure and delay discounting, adolescent mice were given MeHg in their drinking water over a thirty-nine-day period, then were tested as adults on a delay discounting procedure later in adulthood where animals were given a choice between choosing a single reinforcer presentation immediately, or four reinforcer presentations after a varied delay. They found that at the highest dosing of MeHg, there was more discounting that occurred (Boomhower & Newland 2016). Another animal study looked at DA agonist *d*-amphetamine and three dopamine antagonists in an adjusting delay procedure. After acute exposure to these drugs,

they found that the indifference point (the delay at which the subject is equally likely to pick either the smaller or the larger reinforcer) increased with amphetamine, but decreased with two of the three DA antagonists (Wade, de Wit, and Richards 2000). In contrast, a delay discounting procedure using chronic cocaine exposure, another DA agonist, revealed a steeper delay discounting curve which is indicative of more discounting versus the control animals (i.e., the subjects were more likely to choose the smaller reward over the larger reward at longer delays) (Pope et al 2016). This contradiction can potentially be explained by the different nature of the dosing; while one experiment looked at acute effects of DA agonism, the other looked at effects that chronic DA agonist could have, changing the neurotransmitter system. Another possibility is that the drug effects depended on the baseline level of discounting. Pope, Hutsell, and Newland (2020) showed that acute effects of *d* amphetamine are baseline-dependent, with low discounting levels being increased and high levels being decreased.

While research has been done on how both 5-HT and DA can affect different types of impulsive behaviors, not as much research has been done looking specifically at risperidone, which acts on both dopamine and serotonin neurotransmitter systems. More so, how it affects these systems when taken over a long period of time during a critical period of neural development, and what effects it can have even after cessation of the drug.

The present study was designed to test two main hypotheses. First was whether daily exposure to risperidone would result in irreversible changes in behavior controlled by the neurotransmitter systems affected by risperidone. Second was to determine whether adolescence is a vulnerable period of development that is sensitive to drugs that have effects on 5-HT and dopamine neurotransmitter systems. To do this, two groups (risperidone- exposed and controls) underwent exposure either during the adolescent stage of life or during the adult stage of life, for

a total of four (exposed X life stage) groups. To determine whether exposure levels were high enough to cause direct effect of their own, subjects underwent tests of fine motor coordination during exposure. To determine long-term, irreversible effects, behavioral testing was conducted to assess differences perseverative behavior and impulsivity.

Methods

Subjects

Forty-eight, weanling (21 day old) C57BL/6n mice were purchased from a commercial vendor. They were purchased in two sets of 24 litters, with 2 littermates per litter. The littermates were separated into two different dosing groups. Mice were pair-housed in a temperature- and humidity-controlled AAALAC accredited animal facility (lights on at 6am). A total of 24 of these mice underwent dosing during adolescence. The remaining 24 underwent dosing during early adulthood.

Dosing

Animals in the adolescent group received risperidone in drinking water from postnatal day (PND) 22-60. Animals in the adult group received risperidone in drinking water from PND 110-148. Solutions of risperidone (12.5mg/L) were generated by dissolving risperidone in glacial acetic acid, mixing with tap water, and pH correcting to 5 using NaOH. This concentration was estimated to allow for a target dose of 2.5 mg/kg/day risperidone. This target dose was chosen by a combination of both dose conversions based on body surface area to account for difference in metabolism between humans and mice (Nair and Jacob 2016) as well as matching doses used in other studies involving risperidone (Terry et al. 2007). For the control group, an equivalent amount of acetic acid, without any risperidone added, was added to their drinking water (pH =

5). Water bottles containing either acetic acid alone (control) or acetic acid + risperidone (exposure) were placed in animal cages overnight from approximately 4pm to 1pm.

Behavioral Procedures

Rotarod

Rotatod sessions were conducted using a 5-station Med-Associates© Rotarod for mice (product #ENV-575M). In a session the speed of the rotating rod increased from 3 to 30 rpm over 5 minutes at a constant acceleration of .09 revolutions per second. An infrared beam below the rod detected when an animal fell. Subjects underwent single trial sessions three times per week during their exposure period. Each trial ended either after six minutes elapses, or when the subject fell from the rod, whichever occurred first. The time it took the animal to fall off the rotating beam was recorded as a measure of their fine motor coordination.

Delay Discounting and SDR

Apparatus

Twelve standard operant chambers (ENV007, Med Associates, St. Albans, VT) modified for mice were used for data collection. On the back wall was a single standard mouse lever, and on the front wall were two retractable mouse levers on the right and left sides. An alcove where a dipper system delivered 0.01 cc of a 3:1 solution sweetened condensed milk with water, henceforth called milk, was located between the two front levers. Two Sonalert® tone generators (High tone: 4500 Hz, low tone: 2700 Hz) were located at the top left and right sides of the front wall of the chambers. Boxes were enclosed within sound attenuating chambers. All experimental contingencies were controlled by a computer in an adjacent room at a resolution of 0.01-seconds. Mice were assigned to a particular box and split into two squads, ensuring to counterbalance across groups, that were run at the same time each day, Monday through Friday.

Autoshaping and Training

Beginning on PND 90 for adolescence and PND 178 for adults, underwent the autoshaping procedure described in Reed et al (2006). Briefly, mice were placed into operant boxes overnight, 16hrs. During the sessions, the left or right lever was extended, counterbalanced across subjects, and the light above the inserted lever was lit either for 30 s or until the lever was pressed. Both events resulted in the lever retracting, the light turning off, and a presentation of milk followed by a 5-minute intertrial interval (ITI). Upon 10 lever responses, the session shifted into a fixed ratio (FR) 1 schedule, where a single lever press resulted in a single reinforcer presentation. This session ended after either 100 non-reinforced lever presses or 40 reinforced lever presses. During the following overnight session, if the animal achieved 40 reinforced responses the previous night, the previously unused lever was inserted into the chamber and 100 non-reinforced presses or 40 reinforced presses on this lever resulted in session termination. Following completion of this portion (40 reinforced presses), the rear lever was trained in the same way as the left and right levers. Any subjects that did not acquire lever pressing underwent hand-shaping via a method of successive approximations.

After lever-press training, subjects underwent chain training. During this portion of training, trials began with a pulsing tone and illumination of the house light. A response on the back lever initiated a trial. This response extended one of the two front levers for 15 s, and a response on this lever resulted in a presentation of milk, followed by a 10 s ITI. This chain ensured that the mouse was engaged and, by pressing the back lever, prevented the mouse from positioning itself in front of the active lever. There were 60 trials within a session, and mice continued this training until they completed 50 successful chains within a session across 3 sessions.

SDR Procedure

Following chain training, animals were trained to maintain highly accurate responding on one lever before the reinforcing lever switched using a SDR procedure. Sessions in SDR consisted of 60 trials with each trial separated by a 20 s ITI. In the original discrimination (OD), a back-lever response initiated the trial, causing the two front levers to extend. A response on the lever that is deemed "correct" (left or right, counterbalanced across all animals) resulted in access to milk, followed by an ITI. A response on the "incorrect" lever resulted in no reinforcement and returned to the ITI. A lack of response either in the trial initiation or after the back lever press was considered an omission, resulting in trial termination and ITI. After three consecutive sessions of 51 or more correct responses (85% accuracy), the first reversal (R1) was implemented where the previously reinforcing lever no longer produced reinforcement and the previously non-reinforcing lever produced reinforcement. This switch was un-cued. Animals will have to complete three of these reversals (R1, R2, and R3).

Delay Discounting

Following the SDR procedure, mice were in training for the delay discounting procedure as described in Pope et al (2015). Mice were introduced to a concurrent-chained schedule of reinforcement that contained 6-components and 12 initial-terminal link cycles within each component. The initial link response required an FR1 response. At the beginning of each trial, either the left or right lever (pseudo-randomly determined) was selected as the terminal link. A response on the terminal-link lever initiated a fixed time (FT) interval, during which the light above the terminal lever began flashing, followed by reinforcement presentation of either 1 presentation of milk or 4 presentations of milk. Responses on the lever that was not selected as the terminal link were recorded, but have no programmed responses.

The animals underwent magnitude sensitivity, or isodelay, assessment after they finished preliminary chain training. The smaller reinforcer was always associated with a FT 1.25 s and delivered a single presentation of milk. The larger reinforcer option also remained on an FT 1.25 schedule and resulted in four presentations of milk. This was done in order to determine how often the animal chose the larger reinforcer over the smaller one when both delays were kept constant, allowing us to determine how sensitive the animals were to varying magnitudes of reinforcement. Once responding reached stability, the small and large levers were switched in order to prevent the development of lever side bias.

Following this magnitude sensitivity assessment, subjects began the delay discounting procedure (See Figure 1). It followed the same procedure as the isodelay assessment, except the FT for the larger reward was replaced with six, logarithmically spaced delays (1.25, 2.81, 6.31, 14.12, 31.62, and 70.79 s). At the beginning of each component, the six delays were randomly selected as six of the terminal links through the 12 cycles. The other six terminal links consisted of the smaller reinforcer option. As in the training, an initial link response gave access to the front two levers, one of which was selected as the terminal link. A response on the terminal link initiated the FT delay, during which time the lever remained extended, followed by presentation of reinforcement. Responses on the non-terminal link were recorded but produced no scheduled consequence.

Data Analysis

Data were analysed using R (v. 4.1.3). Subjects were nested in litter to account for effects by litter.

Rotarod data were assessed using a linear mixed effect model using exposure and session as fixed effects and subject as random effect. Results were considered significant if $p < 0.05$.

For the SDR procedure, data were analyzed by measuring the latency to change behavior after reversal. The primary measure was how many sessions it takes subjects to switch the lever they press after a reversal occurs. This was done using a logistic equation, as follows:

$$f(x) = \frac{L}{1 + e^{-k(x-x_0)}}$$

Where L represents the maximum achieved accuracy on the lever after the reversal, k is the growth rate of the curve (the inverse of the slope), and x_0 is the midpoint of the S-shaped curve (or the number of trials it takes the animal to reach half of the transition).

For delay discounting, the data were analyzed using a modified version of the generalized matching law, as follows:

$$\log\frac{B_l}{B_s} = s_m \log\frac{M_l}{M_s} - s_d \log\frac{D_l}{D_s}$$

Where *B* represents responses, *M* represents magnitude of reinforcement, *D* represents delay, subscript l and s represent larger and smaller reinforcement, respectively, and s_m and s_d represent sensitivity to magnitude and delay, respectively (Pope et al 2016). The primary measures obtained during the experiment were the sensitivity to magnitude, which measures how well subjects are able to differentiate between a smaller reinforcer and a larger reinforcer, and sensitivity to delay, which is how well the subjects are able to differentiate between delays.

Results

Body mass and water consumption

Figure 2 shows the body mass, water consumption, and dose intake for the adolescent and adult subjects. Adolescent subjects consistently increased their body masses during the exposure period (Fig. 2A). Body mass was not significantly altered by exposure based on conventional standards of significance ($t(861) = -1.8$,

p = 0.069), nor was maximum body mass significantly altered ($t(861) = 1.76$, $p = 0.061$). Water consumption (Fig. 2B) was significantly altered by exposure ($t(834) = 3.94$, $p < 0.001$), with water consumption being lower in the exposure group. The estimated daily dose for adolescent animals was about 2.5 mg/kg/day risperidone based on their water consumption and body masses (Fig. 2C).

Adults were kept under caloric restriction during the exposure period, and both body mass and water consumption remained stable throughout this time (Fig. 2D and 2E). Despite this, adults in the exposure group had a slightly lower body mass ($t(849) = 3.9$, $p < 0.001$) and slightly higher water consumption ($t(799) = 12.76$, $p < 0.001$). The estimated daily dose for adults in the exposure group was around 2mg/kg/day risperidone based on their water consumption and body masses (Fig. 2F).

Rotarod

Throughout the exposure period, motor coordination was measured via rotarod. Performance began to plateau after 4 sessions, and there was no significant effect of exposure on motor coordination (all p's >0.05).

SDR

There was no significant difference between groups in acquisition of the original discrimination (not shown). Figure 3 shows the fitted function describing reversal 1 (Fig. 3A), reversal 2 (Fig. 3B) and reversal 3 (Fig. 3C) for the animals exposed during adolescence. There was no significant difference for transitioning to a new lever during either reversal 1 or reversal 3 for the risperidone exposed

animals. During reversal 2, risperidone did significantly affect the growth parameter, scale (t(1, 10532 = 2.229, p = 0.026). We see a shallower slope, meaning a slow rate of transition between levers. There was no effect on the magnitude of the transition (t)20532 = 0.118, p = 0.906) or the point at which it was half-way completed (t(10532 = 0.21, p = 0.834).

Figure 4 shows reversal 1 (Fig. 4A), reversal 2 (Fig. 4B) and reversal 3 (Fig. 4C) for the animals exposed to risperidone during adulthood. While similar to adolescents in having no significant difference during reversals 1 and 3, adults differed from adolescents during reversal 2. There was no significant difference in adults for transitioning to a new lever during this reversal between the exposed and control groups in scale, magnitude, or half-point to completion (t(10054) = 1.378, p = 0.168; t(10054) = 0.882, p = 0.378; t(10054) = 0.137, p = 0.891, respectively).

Delay Discounting

Figure 5 shows the effects of adolescent and adult risperidone exposure on delay discounting. The top two panels show the decrease in the ratio of responses as a function of delay, indicating the preference for the larger, later reward. This presentation is the form most used in delay discounting and is helpful for comparing the differences between groups. The bottom two panels show the same information, fitted as the log of the response ratio as a function of log of the delay ratio. This presentation matches the concatenated choice model used for data analysis. In both the adolescent and adult groups, there was a decrease in the response ratio for the risperidone group compared to control, indicating a decrease in the magnitude sensitivity of the exposed subjects. In adolescent-exposed subjects, there was a

significant interaction between exposure and delay ($F(1, 118) = 4.937$, $p = 0.028$), but not a significant main effect of group based on conventional standards of significance ($F(1, 22) = 3.785$, $p = 0.065$). A similar result was seen with the adult-exposed subjects, where there was also a significant interaction between exposure and delay ($F(1, 113) = 7.493$, $p = 0.007$), but no main effect of group ($F(1, 21) = 1.709$, $p = 0.205$). In both cases, the exposed mice discounted at a higher rate than the unexposed mice.

Discussion

The present study was designed to determine if there were any effects of chronic risperidone exposure on body mass, motor function, or long-term behavioral changes in executive function, specifically behavioral flexibility and impulsive behavior. Further, it was designed to test if adolescence is particularly vulnerable to this chronic exposure; therefore, both adolescent and adult subjects were exposed for the same amounts of time to compare the effects. Subjects were given a daily dosage that was low enough that it did not affect growth or produce motor deficits during exposure. We observed differences in behavior in both perseverative behaviors during the SDR procedure, as well as changes in sensitivity to reinforcement magnitude during the delay discounting procedure. These changes were observed over one month after cessation of the drug occurred.

In addition to testing whether chronic exposure to risperidone had lasting effects on impulsive behaviors, we also wanted to test whether these changes were specific to exposure during the adolescent period, or if it is general to exposure during other periods of development as well. To test this, adolescent and adult subjects were exposed to risperidone for the same amount of time and underwent the same battery of experiments. For the SDR experiment, there

was a significant but subtle change in perseverative behavior during the second reversal for adolescent exposed subjects, but there was no such change in the adults. Conversely, during the delay discounting procedure, there was a similar change in behavior from the exposure group that occurred in both the adolescent and adult subjects. This raises the possibility that perhaps some behavioral processes, and some neural pathways are sensitive to the alterations caused by adolescent exposure to risperidone, while other behaviors are just subject to these neurochemical changes, regardless of age. However, even though there was an effect for the adolescents and not the adults, it was very subtle, and any conclusions about the difference between adolescent and adult development should not be made at this time.

Based on water consumption and body mass of the subjects, the adolescent animals received an estimated daily dose of risperidone equal to 2.5 mg/kg/day, which adults received a little less, around 2 mg/kg/day. These dosages can be converted to a human equivalent dosage (HED) by considering differences in body surface area, and, for mice, multiplying the dosage by a factor of 0.081 (Nair and Jacobs, 2016). These numbers assume a mouse weighing about 2g, and a human weighing about 60kg. Through this calculation, we receive an estimated HED of 0.202 mg/kg/day for adolescents and 0.162 mg/kg/day for adults. The target dosage for risperidone in adults is 2-8mg/day (0.03-0.13 mg/kg/day for a 60kg adult). The dosages used in this study were higher than clinical doses for adults but were not high enough to cause any motor deficits seen at high doses of antipsychotic use.

SDR is designed to test for perseverative behavior, or how resistant to change a behavior is. Previously, behaviors exhibited during SDR have been shown to be sensitive to changes such as methylmercury exposure at different stages of development (Boomhower & Newland, 2017; Reed, Paletz, & Newland, 2006) and cocaine exposure (Pope et al, 2016). A common

observation between these studies is that the problems with perseveration tend to occur during the first or second reversal, not during the original discrimination. This finding was replicated in the present study, showing more behavioral rigidity during the second reversal with the adolescent exposed subjects.

Delay discounting is an experiment that is designed to test impulsive choice behavior, or how likely a subject is to pick a smaller reward in the moment in favor of having a larger reward after a delay. In the current study, we observed significant changes in the choice of subjects that were exposed to risperidone, choosing more often for the smaller reward, even when the delay to both rewards is the same. These changes were also replicated in both age groups, showing that there was a long-term effect of exposure independent of what age the risperidone was given. This finding was further replicated in a similar experiment in our lab, using the SGA olanzapine rather than risperidone (manuscript in preparation).

Behaviors that are tested by both SDR and delay discounting are modulated by DA and 5HT neurotransmission (Boulougouris, Glennon and Robbins, 2008; Clarke et al. 2004; Pope et al 2016; Blasio et al. 2012; Boomhower & Newland 2016; Wade, de Wit, and Richards 2000). While the brains of the subjects were left intact and not studied further, the alterations to these behaviors because of exposure to a drug that affects these neurotransmitter systems provides evidence that the areas of the brain that control these behaviors are susceptible to long-lasting changes caused by that exposure of the drug. More specifically, the areas such as the striatum and the PFC, which are involved in reinforcement processes, are affected both by 5-HT and DA, which are the two neurotransmitter systems targeted by risperidone. And while we are unsure which neurotransmitter system is specifically involved in the present, there is evidence that there has been some long-term changes to pertinent areas as a result of chronic exposure.

As with any study, there are a few limitations with this study. One potential limitation is the method of exposure. When doing exposure via consumption, there is less control in the exact dosage and particular subject will receive; it is all based upon how much the animal naturally consumes. This can lead to a modest reduction in the amount of fluid consumed by subjects who are undergoing exposure, potentially due to changes in taste. The method of exposure, however, is also a strength in the study. As opposed injections which allow more control over dosage, using consumption more closely mirrors how risperidone is taken by people outside of a laboratory setting, providing results that are might be more relevant in a broader context. Further, the post-experiment calculations to determine the daily dosing the animals received, followed by converting that to a HED reveal that, while higher than the target dose, was high enough to result in behavioral alterations but not motor deficits.

Mice were exposed chronically to the SGA risperidone during both adolescence and during adulthood. The drug had a significant effect on perseverative behavior when exposed during adolescence but not adulthood, and it had a significant effect on impulsive choice when exposed during both adolescence and adulthood.

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Figures

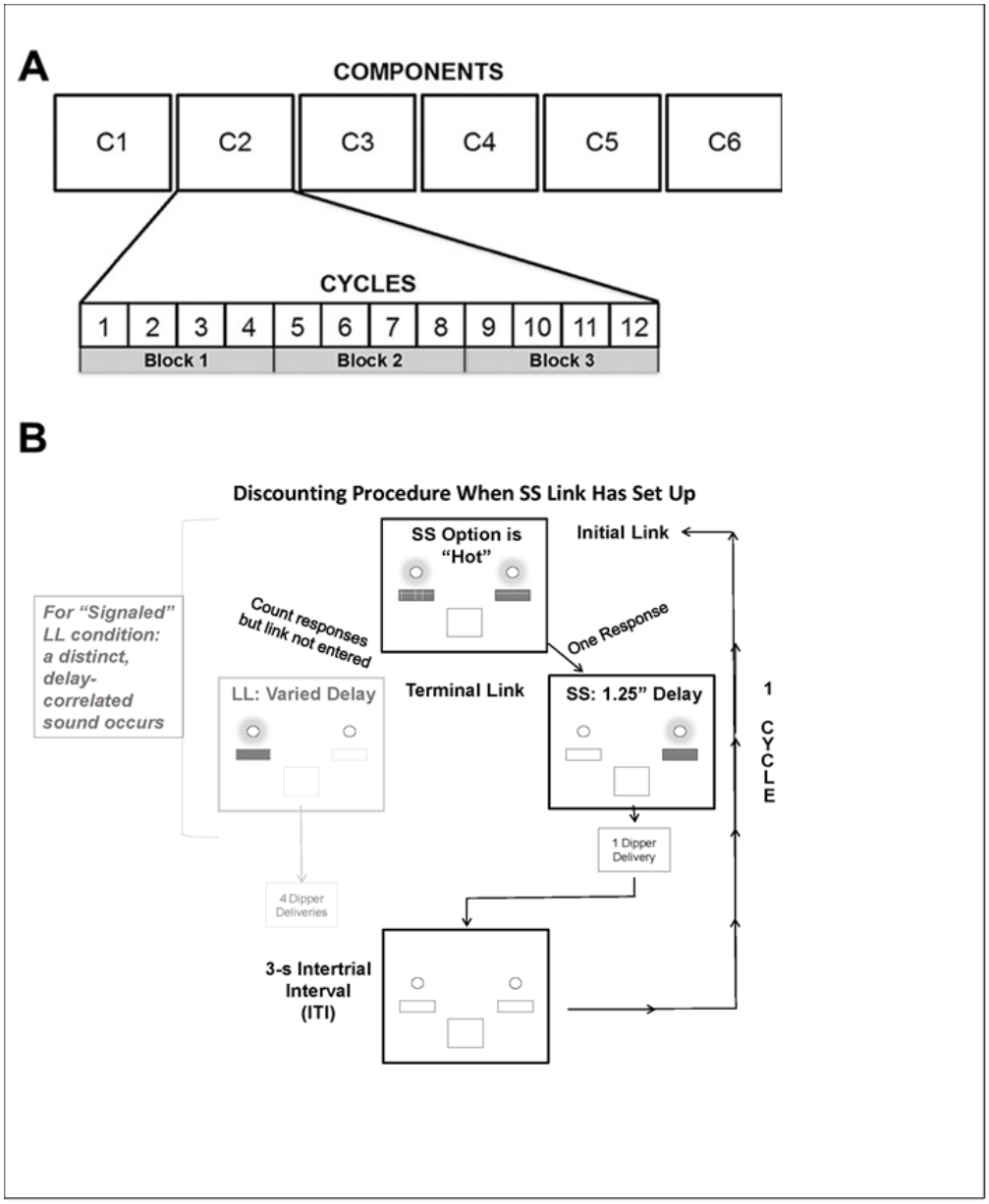

Figure 1. The rapid-acquisition procedure is illustrated in Panel A. Each session comprised six components (C1-C6), each with a different ratio between the delay to the LL reinforcer (1.25 to 70.79 s) and SS one (1.25 s). The ratios were selected randomly (without replacement) at the beginning of the component by choosing a different LL delay. Each component comprised 12 IL-TL cycles, conceptualized as three blocks of four cycles each for data analysis. Panel B shows the concurrent chain schedule. One

lever was designated as “hot” at the beginning of a cycle. A single initial-link (choice phase) response to that lever provided access one of the two terminal-link (delay phase) delays. Responses to the other lever were recorded and used for data analysis but were otherwise without effect. The SS and LL terminal links provided one and four deliveries, respectively, of sweetened condensed milk. During “Signaled” trials, a unique auditory was correlated with the LL durations. During “Unsignaled” trials, the same auditory stimulus occurred regardless of LL duration. A 3-s inter-component interval followed reinforcer deliveries and then a new cycle began with a new initial link. (Additional details in text. Adapted from Pope, et al, 2015, with permission)

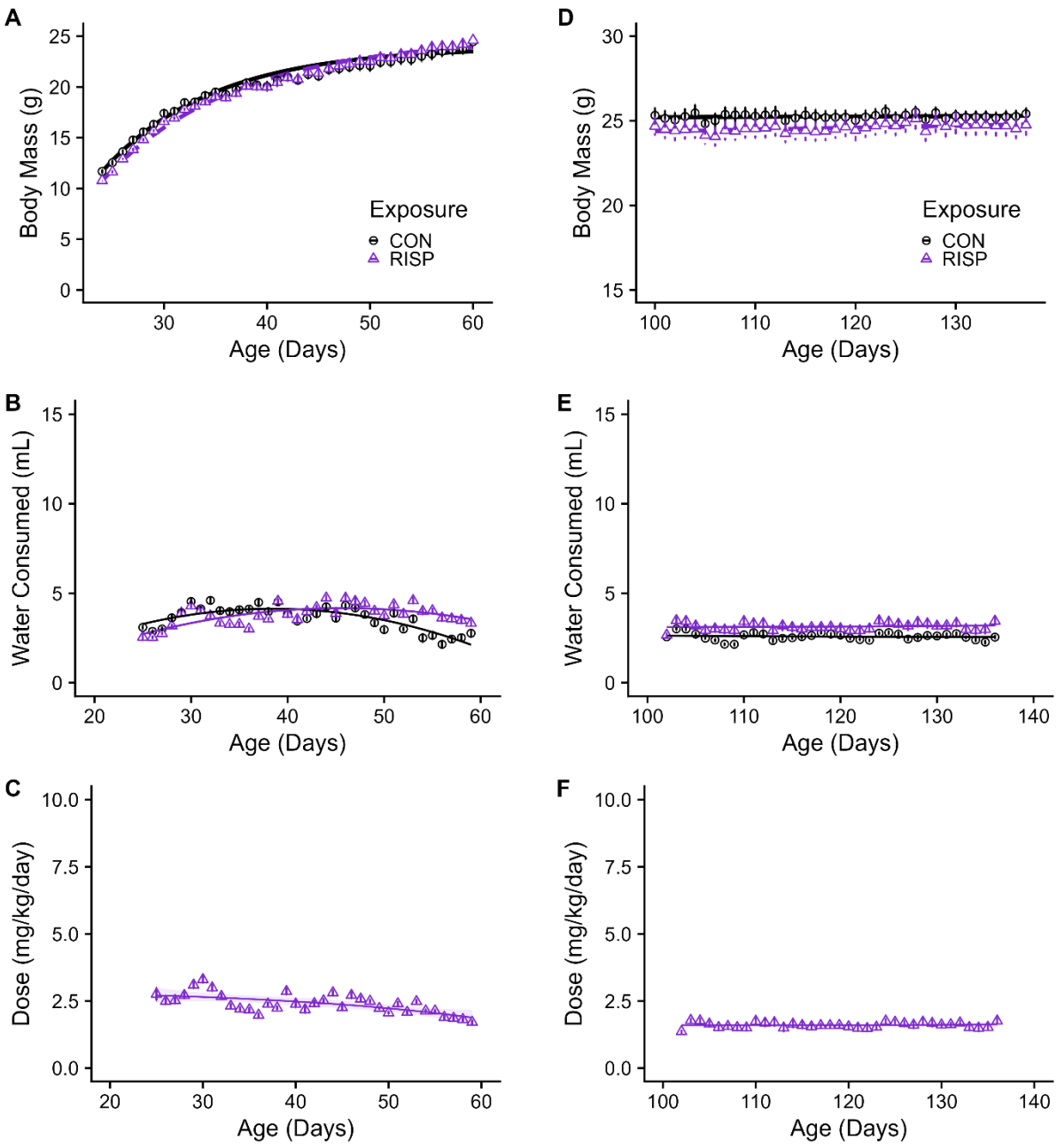

Figure 2. Results of adolescent (left) and adult (right) body mass (top), water consumption (middle) and daily estimated dosage (bottom).

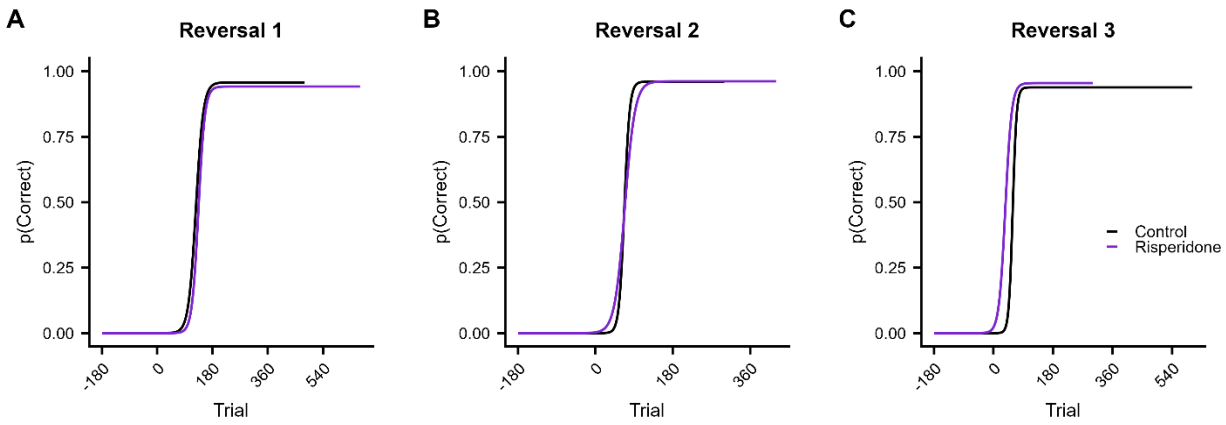

Figure 3: Results of the adolescent-exposed SDR. A show the first reversal, B shows the second reversal, and C shows the third reversal

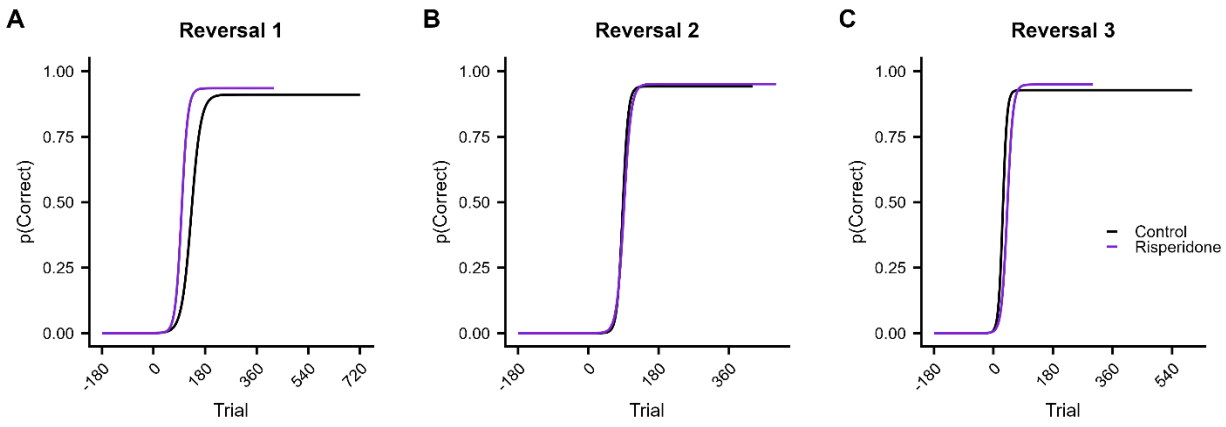

Figure 4: Results of the adult-exposed SDR experiment. A shows the results of reversal 1, B shows the results of reversal 2, and C shows the results of reversal 3.

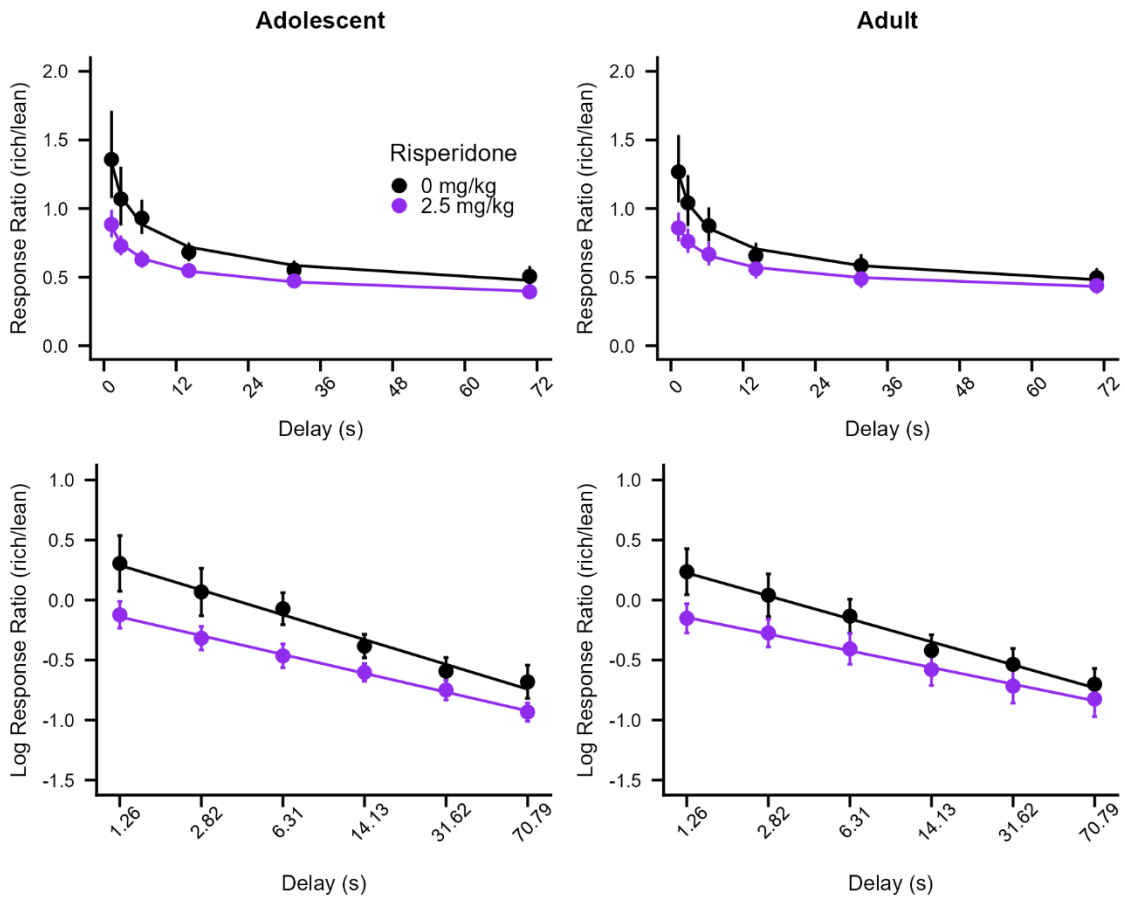

Figure 5: Results of both Adult- and Adolescent-exposed Delay discounting experiments. The top two panels show the response ratio of the Larger-Later (LL) reward as a function of delay, and the bottom two panels show this information presented as a linear function of the log of the response ratios.