Published in final edited form as:

Behav Processes. 2015 September ; 118: 59–70. doi:10.1016/j.beproc.2015.06.005.

Spatial Discrimination Reversal and Incremental Repeated Acquisition in Adolescent and Adult BALB/c Mice

Andrew Nathanael Shen^{a,*}, Derek A. Pope^a, Blake A. Hutsell^{a,b}, and M. Christopher Newland^{a,*}

^aDepartment of Psychology, Behavioral Toxicology Lab, Auburn University

^bDepartment of Pharmacology and Toxicology, Virginia Commonwealth University

Abstract

Adolescence is characterized by neural and behavior development that includes increases in novel experiences and impulsive choice. Experimental rodent models can characterize behavior phenotypes that typify adolescence. The present experiment was designed to characterize differences between adolescent (post-natal day (PND) 34 - 60) and adult (PND 70 - 96) BALB/c mice using a response-initiated spatial discrimination reversal (SDR) and incremental repeated acquisition of response chains (IRA) procedures. During SDR, adolescents omitted more trials and were slower to initiate trials than adults, but the age groups did not differ on accuracy and perseveration measures. During IRA, adolescents displayed poorer overall performance (measured by progress quotient), lower accuracy at individual chain links, and completed fewer long response chains (>3 links) than adults. In both procedures (SDR and IRA), the poorer performance of adolescents appeared to be related to the use of a response device that was spatially removed from reinforcer delivery. These results indicate that SDR and IRA performance can be established during the brief rodent adolescent period but that these two age groups' performances differ. We hypothesize that adolescent behavior is more sensitive than adult behavior to the spatiotemporal distance between response device and location of reinforcer delivery.

Keywords

adolescent; BALB/c; incremental repeated acquisition; spatial discrimination reversal

1. Introduction

Adolescence is marked by increased risky and often impulsive decision-making (e.g. substance abuse, unprotected sex), the emergence of many psychiatric illnesses, and rapid development of academic skills (Moffit, 1993; Spear, 2000; Swendsen et al., 2012). Critical changes in the volume of cortical grey and white matter, receptor pruning, and development

*Corresponding authors at: Department of Psychology, 226 Thach Hall, Auburn University, AL 36849. anshen14@gmail.com, newlanc@auburn.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

of cortical connections occur throughout the brain, often between 12-18 years of age (Brenhouse & Andersen, 2011; Giedd et al., 1999; Jacobus et al., 2013). The development of the pre-frontal cortex (PFC) and subcortical structures such as the nucleus accumbens (NAc) are especially important during adolescence because they are among the last regions to mature (Giedd et al., 1999; Paolicelli et al., 2011). The PFC has been implicated in processes ranging from behavior inhibition, task switching, attentional selection, and resistance to disruption in studies with rodents (Dalley et al., 2004; 2008). Imbalances in the development of brain regions like the PFC and NAcc during development may leave adolescents increasingly vulnerable to developing maladaptive patterns of behavior (Adriani & Laviola, 2004; Galvan et al., 2006; Moffit, 1993; Pinkston & Lamb 2011). Evidence from clinical and experimental studies implicate dysfunctions in fronto-striatal pathways, connecting frontal cortices with the basal ganglia, in a range of disorders, including ADHD and autism spectrum disorders (Cherkasova & Hechtman, 2009; Jentsch & Taylor, 1999; Langen et al., 2012). Less is known about how adolescents and adults respond to challenging behavioral demands and these may exacerbate differences between age groups.

Experimental models of adolescent behavior often use rat and mouse strains because they are cost-effective and share similarities in anatomy and physiology with primates (Spear, 2000). Rodent adolescence “encompasses the week preceding the onset of puberty and the first few days thereafter (Macri et al., 2002)” and contains three sub-periods: early (also prepubescent or juvenile; PND 21-34), middle (sometimes “periadolescence,” PND 34-46), and late adolescence (sometimes young adulthood; PND 46-59) (Laviola et al., 2003). Several behavior assays with rodents model characteristic patterns of adolescent behavior, including behavior rigidity, disinhibition, and impulsivity (Evenden, 1999; Madden & Johnson, 2010). Recent investigations have adapted these procedures to meet the time constraints presented by the rapid progression of rodent development (e.g., Adriani et al., 2003; Andrzejewski et al., 2011; Pinkston & Lamb, 2011; Sturman et al., 2010). Relative to adults, adolescent rodents spend more time exploring novel environments, develop an exaggerated sensitization response to psychostimulants, and discount delayed and probabilistic reinforcers more steeply (Adriani et al., 1998; Laviola et al., 2001; Pinkston & Lamb, 2011; Zoratto et al., 2013). Also, adolescents learn to discriminate multiple stimuli more slowly (Spear & Brake, 1983) and display greater persistence under extinction compared to adults (Andrzejewski et al., 2011; Myslivecek & Hassmannova, 1979). The current study sought to extend the classes of behavior that can be studied during rodent adolescence by adapting two procedures: spatial discrimination reversal (SDR) and incremental repeated acquisition (IRA).

Discrimination reversal procedures first assess the acquisition of a simple discrimination and then reverse it such that the previously reinforced behavior is now under extinction while the previously unreinforced behavior now is reinforced. Discrimination reversal procedures have been used in humans and a range of non-human animals (Fellows & Farah, 2003; Pagani et al., 2004) and are sensitive to disruption of the orbitofrontal cortex in rodents, nonhuman primates, and humans (Dalley et al., 2004; Zald & Andreotti, 2010). The first reversal is especially sensitive to disruption of the orbitofrontal cortex by lesions, by altering neurotransmitter (especially dopamine) activity in this region (Dalley et al, 2004), and by gestational methylmercury (MeHg) exposure in rodents (Paletz et al., 2007; Reed et al.,

2006). SDR procedures present an opportunity to examine behavioral flexibility in adolescent mammals, a period marked by transient dopaminergic (DAergic) functioning and structural development. The SDR procedure used here required that a mouse initiate a trial by pressing a back lever, an approach taken to increase the likelihood that a mouse was on-task and to displace it from the choice levers at the beginning of a trial.

Repeated acquisition (RA) procedures, pioneered by Boren (1963), require subjects to acquire chains of responses within a session. Incremental repeated acquisition (IRA), an extension of RA, allows for the assessment of acquisition and performance in such a way that the length of the chain increases gradually, often according to some behavior criterion, within a single session (Cohn and Paule, 1995). IRA may be viewed as an apical procedure that captures many of the functions thought to be mediated by the prefrontal cortex and higher-order functioning (Cohn & Paule, 1995; Paule, et al., 2012). There is high correlation, $r=0.53$, between measures of IRA accuracy and IQ scores in human children (Paule et al., 1999) and improvement demonstrates a developmental time course (Baldwin et al., 2012). Specifically, the dependent measure progress quotient (PQ), directly related to the number of reinforcers earned at a given chain length, can vary over a dynamic range and serve as an independent, overall measure of performance (Bailey et al., 2010; Johnson et al., 2010). The IRA procedure has been used with a wide variety of species, including rats, mice, pigeons, and non-human primates (Cohn & Paule, 1995) and has garnered extensive use when evaluating acute drug effects and chronic exposure to environmental contaminants (Bailey et al., 2013; Paule & McMillan, 1984). A mastery-based approach allows task difficulty to adjust in real time according to the animal's performance, an approach that has led to the production of very long response chains by mice (Bailey et al., 2010; Johnson, et al., 2010). The dynamic, within-session adjustment of difficulty allows for identification of influential factors within individual animals.

The present study investigated middle to late adolescent (PND 34-60) and adult (PND 70-90) BALB/c mouse behavior in SDR and IRA procedures. These procedures allowed for age-based comparisons between a discrete-trials operant procedure and a more rate-based free operant procedure.

2. Materials and Methods

2.1 Subjects

Nineteen adolescent and 18 adult male BALB/c mice, purchased from Harlan Laboratories, arrived when they were 28 and 63 days old, respectively. Adolescent mice were group housed; five mice per cage in clear *Plexiglas*® cages with ash chip bedding. The adults, whom had been group-housed as adolescents at Harlan Laboratories, arrived at the vivarium individually housed and were kept separate for the duration of the experiment. In our experience, group-housed adult male BALB/c mice engage in aggressive barbering and fighting which can result in grievous injury and sometimes death. Adult mice were singly housed in cages that contained a clear *Plexiglas*® divider that prevented physical contact but allowed visual, olfactory, and auditory interactions between two mice in the same cage. Adolescent mice weighed between 12-15g upon arrival and were initially given *ad libitum* access to standard rodent chow (Purina Mills Inc., St. Louis, MO). Upon the start of testing,

food was removed 4hr before experimental sessions and returned at the end of the session. Adult mice were maintained at a weight 23-25g by feeding approximately 2.5-3.0g standard rodent chow per day. Autoshaping (see section 2.3) began on PND 34 for adolescent mice and PND 70 for adult mice.

Mice were housed in an AAALAC-accredited vivarium, temperature and humidity-controlled, and maintained on a 12-h light-dark cycle (lights on at 6:00 am). All mice had ad libitum access to water. Prior to the start of autoshaping, mice were acclimated to a sweetened condensed milk and water solution (3:1 water to milk) over a two-day period (4 and 8hr per day). All procedures were approved by the Auburn University IACUC.

Following autoshaping, adolescent and adult mice were randomly assigned to either the IRA or the SDR group with the constraint that the groups acquired lever-pressing similarly during autoshaping. The IRA group included 10 adolescent and 11 adult mice and the SDR group included nine adolescent and seven adult mice. Subjects were exposed to either the IRA or SDR procedure for the duration of the study.

2.2. Apparatus

Daily experimental sessions for both IRA and SDR procedures were conducted in 10 Med Associates operant conditioning chambers (Med Associates Inc., product #ENV-007). Each chamber, measuring 30.5 cm L $\times$ 24.1 cm W $\times$ 29.2 cm H, contained two stainless steel front and back walls and two Plexiglas[®] side walls. Mounted on the front wall were two retractable levers (product #ENV-307W), separated by a dipper reservoir. One non-retractable lever (product #ENV-307A) was mounted in the center of the back wall. Dipper presentation allowed 4-s access to 0.1cc solution of sweetened condensed milk and water (3:1 ratio). Pressing the levers with 2g of force registered a response. A single 2.8-W house light was located near the ceiling of the chamber on the back wall, a Sonalert[™] tone generator was located in the top left, and a white noise generator was located in the top right of the back wall. The operant chambers were enclosed in sound-attenuating cabinets, with a fan for ventilation and masking noise.

2.3 Autoshaping

Following seven days of habituation to home cages, adolescent (PND 34) and adult (PND 70) subjects began autoshaping of lever pressing at three spatially distinct locations. The autoshaping procedure has been described in detail previously (see Reed et al., 2006; Paletz et al., 2007) and was implemented without modification with the adolescents and adults. Generally, adults completed one autoshaping session per day (training on one lever). However, due to time constraints, adolescents sometimes received multiple (2-3) autoshaping sessions per day (training on more than one lever), but total training duration never exceeded eight hours per day.

2.4 Spatial Discrimination Reversal (SDR)

2.4.1. Chain training—To train the mice to perform the response-initiated SDR procedure, adolescent (PND 38) and adult subjects (PND 73) acquired a short, two-response chain comprising a trial-initiation response on the (non-retractable) back lever followed by a

choice response on one of two retractable front levers. Each trial commenced with presentation of a 56 kHz tone and illumination of the house-light. A response on the back lever within 15-s caused one of two front levers to extend (left or right, counterbalanced across subjects). A response on the extended front lever within 15-s produced 4-s access to the dipper, followed by a 10-s intertrial interval (ITI) during which the house light remained off and responses on the back lever reset the 10-s ITI. Omission of a response during either 15-s limited hold ended the trial and began the 10-s ITI. Sessions consisted of 60 trials, and chain training continued until 50 correct chains occurred within one session.

2.4.2. SDR—Following chain training, adolescents (PND 42) and adults (PND 76) immediately moved to the complete SDR procedure, consisting of an original discrimination (OD) and a subsequent discrimination reversal. A response on the back, trial-initiation lever caused both the left and right retractable levers to extend into the chamber. A response on the lever designated “correct” produced access to the dipper for 4s while a response on the alternate lever designated “incorrect” led directly to the 10-s ITI. The lever producing dipper access remained constant throughout the session. As in chain training, subjects received 15-s for trial-initiation and choice responses, respectively. The criterion for a discrimination reversal was three consecutive sessions with at least 51 correct responses out of 60 total trials (85% correct, including omission and commission errors). Additionally, subjects progressed to the reversal if, after 10 sessions, accuracy was at least 85% for three consecutive sessions (i.e. sessions 8, 9, and 10) when counting only correct responses and commission errors, i.e., exclusive of omissions. The latter, more lenient, criterion was necessary because adolescent mice omitted many trials. When a subject met the reversal criterion, the lever that produced a reinforcer switched to the alternate lever (e.g. if a left lever press was correct during OD, a right lever press was correct during the discrimination reversal). After a reversal, a response on the lever that produced dipper presentation during OD led to the 10-s ITI and a response on the alternative lever produced access to the dipper. Adolescent subjects completed sessions until they reached PND 60, while adults completed two reversals.

2.5 Incremental repeated acquisition (IRA)

Adolescent (PND 38) and adult (PND 73) mice responded on three distinct response levers under an IRA procedure in which a response chain incremented from a one to a maximum of a six-link chain using backward chaining (Bailey, et al., 2010). Unlike the SDR procedure, response levers inserted into the chamber at the start of session never retracted until the session terminated. Backward chaining adds a new link before (or, in front of) the previously experienced link(s). For example, the 6-link response chain “RLBLBR” (R=right lever; L= left lever, B=back lever, reinforcer delivery = Rf) was trained as follows: $R \rightarrow Rf$, $B - R \rightarrow Rf$, $L - B - R \rightarrow Rf$, and so on up to a 6-link response chain. The links are numbered beginning with the reinforcer, so the link numbers would be $6,5,4,3,2,1 \rightarrow Rf$. Chain length was incremented according to mastery-based criterion that required six consecutive correct responses without errors in the first link of the chain to move to a two-link chain. All additional increases in chain length required three consecutive correct response chains to be completed. A distinct auditory stimulus was associated with each link in the chain. Correct chains resulted in 4-s access to 0.1cc of the sweetened condensed milk

mixture. Incorrect lever presses resulted in a 2-s ITI during which the house light was illuminated. To decrease responding during the ITI, each lever-press during this period increased the ITI by 1-s. Following the ITI, the chain reset to the largest link of the current chain (e.g. link 5 in a 5-link chain). Sessions ended after 1h or after 50 correct 6-link chains were completed. Adolescent and adult subjects completed 15 sessions IRA sessions, which occurred before the adolescents reached PND 60.

2.6 Data Analysis

The primary dependent measures of the SDR procedure were correct responses, commission and omission errors, trial-initiation latency, choice-response latency, and overall accuracy (corrects/corrects + commissions).

In the IRA procedure, the primary dependent measures were progress quotient (PQ, see below), maximum chain length (MCL), response rate, and reinforcer rate. Secondary measures included accuracy by chain link and interresponse times (IRTs) from two distributions; those IRTs between individual correct responses and those between correct and incorrect responses. Maximum chain length represented the longest chain length reached in a session. PQ was measured as

$$PQ = \frac{\left(\sum_{i=1}^6 i R_i \right)}{R_t} \quad (\text{Eq.1})$$

and assesses IRA performance by weighting reinforcers according to the link in which they occurred. Here, i is a weighting factor given by the chain length, R_i is the number of reinforcers earned in a given link, and R_t is the total number of reinforcers earned within a session. Thus, reinforcers earned following longer chains (e.g. 5-link) receive more weight than those earned following shorter chains (e.g. 2-link). An alternative way to view PQ is that it is the weighted number of responses that comprise correctly executed chains divided by the number of total correct chains.

Primary dependent measures for SDR and IRA procedures were averaged over the first two sessions and the last two sessions. Two-way repeated measures analysis of variance (RMANOVA) with Age (adolescent and adult) and Session (first two and last two) as factors were conducted (significant at $p < 0.05$). For commission and omission errors (SDR), relatively low counts led to negative (uninterpretable) standard deviations. Thus, raw data for both types of errors were transformed accordingly: first, 0.5 was added to the data (to account for zeroes); second, the logarithm (base 10) was taken.

Mantel-Cox survival analysis was used to determine differences in the number of sessions needed to complete the original discrimination and the first reversal under the SDR. It was also used to determine differences in the number of sessions needed to reach chains consisting of 2-5 links. Inverse survival curves were plotted for ease of viewing. When post-hoc tests were necessary, Tukey's correction was applied (shown in parentheses). Statistical analyses and graphing were done using SigmaPlot® v.12.5 (San Jose, CA).

3. Results

3.1 Autoshaping

One-way ANOVA revealed no differences in the number of reinforcers required to autoshape lever-pressing at all three lever locations (left, right, and back) [$F(1,36) = 2.66$, $p=0.11$]. On average, adolescents and adults required 124.2 and 137.1 reinforcers, respectively, to acquire stable lever-pressing.

3.2 Spatial Discrimination Reversal

3.2.1. Chain training—Adolescents and adults took between 2-4 sessions to complete chain training. Subjects that finished early did not start SDR until all animals from both age groups finished chain training. One-way ANOVA revealed no difference in the number of reinforcers required to finish chain training [$F(1,15) = 2.587$, $p=0.13$]. There was a trend that adolescents required more reinforcers to finish chain training than adults, but this was largely driven by one adolescent mouse whose total number of reinforcers required was 1.5 SD above the mean (not a statistical outlier and included in all analyses).

3.2.2 Original discrimination—Figure 1 shows representative data from one adolescent and one adult subject during the SDR procedure. Both the adolescent (top) and adult (bottom) subjects emit very few commission errors (open squares) but the adolescent mouse consistently omits (open triangles) many more trials than the adult (the vertical dashed line separates original discrimination from first reversal). This trend of high omission errors during original discrimination delayed adolescents' reaching the first reversal relative to adults. Inverse survival curves show the proportion of mice in each group meeting the stricter advancement criterion of at least 51 correct trials. More adults (7 of 7) met this strict criterion and began to do so earlier than adolescents (3 of 9; Fig. 2A, $p<0.001$). All seven adults but only three out of nine adolescents met reversal criterion after 10 sessions.

The RMANOVAs revealed a main effect of Session for all dependent measures (performance improved from the first to the last two sessions for both age groups). A main effect of Age is reported only if there was no Age X Session interaction. For accuracy (Fig. 2B), there was no Age X Session interaction [$F(1,14) = 0.00006$, $p=0.98$]. Thus, there was no difference in accuracy (when excluding omission errors) between adolescents and adults at any time during original discrimination sessions. For commission errors (Fig. 2C), there was no Age X Session interaction according to conventional criteria [$F(1,14) = 3.43$, $p=0.08$]. For omission errors (Fig. 2D), there was an Age X Session interaction [$F(1,14) = 19.72$, $p<0.001$]. Omission errors decreased for both age groups ($p's<0.001$) across sessions while both age groups omitted a relatively large proportion of trials during the first two sessions, adolescents omitted significantly more trials during the last two sessions than adults ($p<0.001$). Thus, omission, but not commission, errors slowed acquisition of the original discrimination by adolescent mice.

For trial-initiation latency (Fig. 2E), there was a significant Age X Session interaction [$F(1,14) = 12.71$, $p=0.003$], but for choice latency (Fig. 2F) there was no Age X Session interaction by conventional criteria [$F(1,14) = 4.04$, $p=0.06$] and the magnitudes of the

group differences were small. While trial-initiation latencies (Fig. 2E) significantly decreased for both age groups from the first to the last two sessions, adult latencies were significantly shorter than adolescents' were during the last two sessions ($p=0.01$). Choice latency (Fig. 2F) did not differ between adolescent and adult mice during the first or last two sessions.

3.2.3 First Reversal—Figure 1, to the right of the vertical dashed lines, shows representative data from the first reversal; the adolescent subject continued to omit more trials than the adult during first reversal but there was little difference in the number of commission errors. Similar to original discrimination, inverse survival curves for adults and adolescents (Fig. 3A) during first reversal were significantly different ($p<0.001$). That is, more adults (7 of 7) met the stricter first-reversal criterion (51 correct trials) than adolescents (5 of 9) and began doing so after fewer sessions. Despite the disparity in the number of subjects completing first reversal, RMAONVA revealed that for accuracy (Fig. 3B), commission errors (Fig. 3C), trial-initiation latency (Fig. 3E), and choice latency (Fig. 3F), there was no significant Age X Session interaction (p 's=0.64, 0.1, 0.1, and 0.7, respectively) for the first reversal.

For omission errors (Fig. 3D), there was a significant Age X Session interaction [$F(1,14) = 7.65$, $p=0.01$]. Tukey's test revealed that adolescents omitted more trials than adults during the last two sessions of first reversal ($p=0.002$). Adolescents, on average, omitted 8.6 more trials than adults during the last two sessions of first reversal, preventing most adolescents from meeting criteria for another reversal. Given that a trial-initiation response occurred, there was no difference in the latency or accuracy of choice between adolescents and adults, consistent with the original discrimination.

3.3 Incremental Repeated Acquisition

There were no age-related differences in autoshaping. Adolescent and adult IRA performance was examined by comparing PQ, MCL, response rate, and reinforcer rate for the first and last two sessions. Additional analyses included accuracy by chain-link and survival analysis of the number of session needed reach different chain lengths (plotted as inverse survival curves). Finally, IRT analyses of adolescent and adult responding provided support for inferences regarding the nature of age-related differences in the primary dependent measures of the IRA procedure. There were main effects of Age and Session for PQ, MCL, response rate, and reinforcer rate; only significant interactions are detailed below.

Figure 4A-D shows PQ, MCL, response rate, and reinforcer rate across the 15 sessions of IRA, respectively. Visual inspection shows that the rate at which performance improved was greater for adults than adolescents. This is confirmed in Figure 5, which shows these dependent measures for the first and last two sessions. Two-way RM ANOVAs revealed Age X Session interactions on PQ [$F(1,19) = 10.846$, $p=0.004$], MCL [$F(1,19) = 48.30$, $p<0.001$], response rate [$F(1,19) = 16.4$, $p<0.001$], and reinforcer rate [$F(1,19) = 27.696$, $p<0.001$]. During the first two sessions, there were no age-related differences for PQ, MCL, response rate, and reinforcer rate (p 's = 0.44, 0.93, 0.09, and 0.54, respectively). During the last two sessions, PQ, MCL, response rate, and reinforcer rate were significantly higher for

adults than adolescents (all p 's < 0.001). Reinforcer rate (Fig. 5B) was the only measure that was stable between the first and last two sessions for adolescents ($p = 0.66$), while increasing for adults ($p < 0.001$).

Adults reached increasingly longer chains faster than adolescents. Fig. 6 shows inverse survival curves for IRA: the proportion of subjects reaching a given chain length by session. Survival analyses revealed that adults reached 3-link ($p < 0.001$), 4-link ($p < 0.001$), and 5-link ($p < 0.001$), but not 2-link ($p = 0.56$), chains significantly faster than adolescents.

To identify problem links within the chain, accuracy was calculated at each chain link. Figure 7 shows accuracy by chain link as a function of session. Adolescent and adult accuracy was similar during initial sessions but adult accuracy in link one (R lever, closest to reinforcement) rapidly increased after two sessions and accuracy in link two (B lever) increased after eight sessions. After grouping accuracy by the first and last two sessions (Fig. 7, inset bar graphs) there was a significant Age X Session interaction for accuracy in the first link [$F(1,19) = 6.75, p = 0.02$] and second link [$F(1,19) = 21.1, p < 0.001$]; adults were more accurate than adolescents during the last two sessions (p 's < 0.001). While all adults reached three- and four-link chains and nine of 11 reached five-link chains, adolescents did not consistently reach longer chains (3-link, $n = 5$; 4-link, $n = 2$; 5-link, $n = 1$, Fig. 6), precluding RMANOVA on the longer chains.

To characterize differences between adolescent and adult behavior during the IRA procedure further, interresponse times (IRTs) from the last two sessions were analyzed and a quantile-quantile (Q-Q) plot was used to convey information about the microstructure of responding that IRT distributions contain (Newland, 1997). IRTs were collected and two distributions were created: IRTs between two correct responses and IRTs between a correct and incorrect response. Only those from two-link chains and longer were used because in a one-link chain every correct response was followed by reinforcement and every incorrect response was followed by a 2-s ITI. From these distributions, every decile was taken for an individual subject and the deciles were averaged by age group.

Figure 8 shows the distribution of adult IRTs (y-axis) plotted as a function of the distribution of adolescent IRTs (x-axis), averaged across the last two sessions. The major diagonal (dashed line, slope = 1.0, intercept = 0) shows what would occur if the distributions from adolescents and adults were identical. For IRTs between correct responses, the slope was not significantly different than 1.0 [$F(1,14) = 0.04, p = 0.83$] but the intercepts were significantly different [$F(1,14) = 93.0351, p < 0.001$]; the adult IRTs were consistently shorter than adolescent IRTs by approximately 2.8-s.

For IRTs between correct and incorrect responses, the slope was significantly less than 1.0 [$F(1,14) = 3.497, p < 0.001$] indicating greater variability for adolescents than for adults and a disproportionate increase in the length of those IRTs toward the right tail of the distribution (longer IRTs) for adolescents. For adults, incorrect responses were usually preceded by IRTs shorter than for correct responses. For adolescents, IRTs that fell in the longer half of the distribution (falling to the right of about 20") were more than twice as long as those produced by adults. For example, the 70th and 90th percentile for adolescents were about 30-

s and close to 55-s respectively while for the adults these durations were about 13-s and 25-s. It would appear that much incorrect responding by adolescents is related to long pauses between responses and that the conditions that led to long IRTs had a larger impact on adolescents than adults. Long IRTs indicate that mice were engaged in behavior incompatible with lever-pressing preceding errors and may be interpreted as a marker of distractibility.

4. Discussion

The objective of the present experiment was to characterize performance differences between middle to late adolescent (34-60) and adult PND 70-90) mice on two operant procedures; SDR and IRA. During the SDR procedure, adolescents consistently omitted more trial-initiation responses than adults, but accuracy of responding was very similar between the two ages. During the IRA procedure, adolescents responded more slowly, earned fewer reinforcers, and reached chains of shorter lengths than their adult counterparts. These results lend further support to a literature base demonstrating behavior and neurobiological differences between adolescent and adult rodents (for reviews, see Spear, 2000; Laviola et al., 2003; Wahlstrom et al., 2010; Sturman & Moghaddam, 2011).

4.1 Spatial discrimination reversal

The SDR procedure assesses discrimination learning and performance during the reversal phase, often considered an index of behavioral flexibility (Chudasama & Robbins, 2003). In the present study, adolescent mice required more trials to acquire the original discrimination than adults, but this was solely due to omission, not commission, errors. Thus, accuracy was not significantly different between age groups during original discrimination and the first reversal. Adult trial-initiation latencies decreased during original discrimination and the first reversal, while adolescent latencies remained stable and never decreased to adult levels (Figs. 2E & 3E). Thus, the impact of age on SDR performance was restricted to the latency and frequency of a trial-initiation response, but there was no evidence of differential perseveration by adolescent mice during the reversal. Newman & McGaughy (2011) and Johnson & Wilbrecht (2011) both used two-choice olfactory-driven digging tasks in mid- to late-adolescent and young-adolescent rats, respectively, finding that adolescents performed equally as well as adults during acquisition and an intradimensional shift, similar to the results reported here.

Adolescent rodents display greater novelty-seeking and locomotor activity than adults (Adriani et al., 1998; Adriani & Laviola, 2000; Douglas et al., 2003; Stansfield & Kirstein, 2006). Such off-task behavior would compete with trial-initiation responding in the SDR procedure, and may explain the larger number of omissions and longer latencies to initiate trials by adolescents. Similar to our finding of trial-initiation omissions, Zoratto et al. (2013) investigated probability discounting in adolescent and adult Wistar rats and found that adolescents consistently performed fewer trials than adults. It is worth noting that, in the current results, choice latency was very short relative to trial-initiation latency and adolescent omissions were limited to the trial-initiation and not the choice response. This

suggests that extraneous reinforcers such as novelty-seeking, can compete with trial-initiation responses to a greater degree than a short behavior chain already in progress.

The present study contrasts with a recent report by Simon et al. (2013) in which adolescents appeared more flexible than adults when transitioning from an operant (CRIT) to a Pavlovian procedure. Yet, adolescent omissions (~2× as many as adults) during CRIT (inhibition vs. response), when the “response period” changed from 10-s to 5-s, make it difficult to distinguish response inhibition from trial omission. This suggests that the “response inhibitory” tone had less control over adolescent than adult behavior. This is important because a key component of completing a reversal successfully is the inhibition of previously reinforced responses such that a new response can be acquired (Barkley, 1997; Newland et al., 2013). These differences highlight the importance of procedural considerations when making conclusions about an expansive construct like behavior flexibility. In the present study, in which trial initiations were separated from the choice response, adolescents and adults performed equally as well on both the original discrimination and reversal, but adolescents initiated fewer trials, which is indicative of greater distractibility (Spear, 2000; Wahlstrom et al., 2010).

4.2 Incremental repeated acquisition

Adolescent mice acquired long chains under the IRA procedure more slowly than adults (Fig. 4-7) and this may have been related to low response rates. These low response rates suggest that adolescents spent more time engaged in behavior incompatible with target behavior, e.g. novelty-seeking. Increased time spent engaging in such behavior would interfere with reinforcement of initial short chains, which are necessary to advancing chain length. Fig. 8 illustrates a fundamental difference between adults and adolescent responding by emphasizing the interresponse time between correct and incorrect responses (IRTs) by age. Long IRTs suggest engagement in alternative behavior between responses. The time between correct responses was consistently longer by about 2.8 s for adolescents than adults, but the spread of the distribution (the difference between the 10th and 90th percentile) was nearly identical. This stands in sharp contrast to the IRTs preceding incorrect responses (correct to incorrect). For adolescents, these IRTs were much longer than those produced by adults, and the distinction in the distribution was greater the longer the IRT. The differences were not subtle: at the 70th and 90th percentiles, for example, the differences were approximately 28-s and 30-s respectively. These differences suggest that adolescents' incorrect responses were preceded by extended periods of disengagement from the procedure.

The distinction between adolescents and adults in the distribution of IRTs also suggests that the determinant of errors may be different. For the adults, the spread of IRTs ending in a correct response (correct to correct) was not dramatically different from those that ended in an error (correct to error) but the medians were about 12-s and 10-s, respectively, so errors tended to be preceded by short IRTs (except for the highest two percentiles). This suggests that for adults, in contrast to adolescents, errors followed relatively short IRTs and may result from responding too quickly. Consistent with this idea, Zoratto et al. (2013) &

Sturman & Moghaddam (2010) found that adults emitted more inadequate or premature responses than adolescents, respectively (both studies refer to responses during an ITI).

There was a direct relation between response rate, the inverse of IRT, and the performance-based measures PQ and MCL. In part, this is because it is necessary to respond in order to advance the chain but it may also reflect the very long IRTs that preceded adolescent errors. Stated differently, lower response rates result in lower reinforcer rates and ultimately produce a positive feedback loop that constrains behavior output. The apparently off-task behavior may be analogous to off-task behavior in the SDR procedure inferred from adolescent omission errors. The differences seen with IRA contrast with studies reporting moderate or no differences between adolescents and adults on fear conditioning, water-maze performance, or acquisition of responding under variable interval schedules of reinforcement (Akers, et al., 2012; Broadwater & Spear, 2013; Kendig, et al., 2013).

Adolescent and adult response rates increased from the first two to the last two sessions of IRA, with a corresponding increase in PQ (Fig. 4 & 5). It is likely that reinforcer rates would be higher during shorter rather than longer chains, as fewer responses are necessary to earn reinforcement. Despite reaching longer and more difficult chain-lengths, adult reinforcer rate increased across sessions while adolescent reinforcer rate, during shorter links, remained constant across sessions. This suggests that the reinforcer captured lever-presses at chain links more temporally removed from reinforcement for adults than it did for adolescents, thus maintaining higher reinforcer rates despite the increase in response requirement as a function of chain length (Bizo & Killeen, 1997; Killeen & Sitomer, 2003). Stated another way, adolescent performance could be related to steepened delay-of-reinforcement gradients sometimes observed in adolescent rodents (Adriani & Laviola, 2003; Pinkston & Lamb, 2011). During IRA, there is a progressively increasing delay to reinforcement from the first link of the current chain as the number of links required to complete the chain increases. Adolescents did not reliably complete chains greater than three, suggesting diminished control by delayed reinforcers as the number of links, and thus delay to reinforcer delivery, increased.

Another, not unrelated possibility, is that links requiring back-lever presses required that subjects move farther from the pellet dispenser. A back lever-press was the second link in the chain, the penultimate response (L-B-R-L-B-R.). It is possible that chain links that decrease the spatial contiguity between response and reinforcer (i.e., the second-link) would be more disrupted relative to the other locations (Boakes, 1977; Killeen, 1974). Evidence for this can be found in Fig. 7, in which adolescent accuracy in link two (back lever response) was poor and showed little improvement across sessions compared to links one and three (front right and left responses, respectively). Insofar as adolescents demonstrate steepened delay of reinforcement gradients, such response demands have the potential to steepen those gradients (Reilly et al., 2012).

Adolescent omission errors during SDR and low accuracy at the second link of IRA may be related. That is, similar to the two-link chain of IRA, SDR required subjects to emit a short, static sequence of lever-presses commencing with the more distant back lever (back-to-front left or right). Given a steepened delay-of-reinforcement gradient, one might expect similar

distributions of errors across different procedures based on a lever's spatial or temporal distance from reinforcement. The present results suggest that sensitivity to effort and delay-of-reinforcement are important factors when characterizing adolescent behavior in response-initiated SDR and IRA procedures. The present findings may therefore extend those of recent studies using rodent models to quantify age-related differences in behavior (Adriani & Laviola, 2003; Pinkston & Lamb, 2011; Andrzejewski et al., 2011). Taken together, the rodent literature agrees with results from human adolescent studies of impulsivity using delay, probability, and time-based discounting paradigms (Green et al., 1994; Olson et al., 2008).

4.3 Limitations

The aim of the study was to compare responding of adolescent and adult mice under functionally similar conditions, but several factors aside from age may have differentiated the groups. Both adolescent and adult mice were shipped from a commercial supplier, and shipping-induced stress could have adversely affected the adolescent mice more than the adults. The length of habituation to allow measures of stress (e.g. corticosterone) to return to normal after shipping often varies from 2-7 days (Landi et al., 1982; Van Ruiven et al., 1998; Conour et al., 2006), and thus our habituation time of 7 days was relatively conservative.

Caloric restriction was undertaken in a different fashion for the two age groups, and this could have reduced the efficacy of the reinforcer maintaining responding in the adolescent mice relative to adults. Also, to facilitate response acquisition during the brief adolescent period, two autoshaping sessions were conducted daily for that age group. Yet, adolescents and adults did not differ in the rate of acquisition of lever-pressing (i.e. number of reinforcers necessary to finish autoshaping) nor did they differ in chain training for SDR; differences only emerged during experimental IRA and SDR sessions.

Finally, adolescent mice were group-housed, which is recommended for appropriate development, but adults were singly-housed. Brodtkin (2007) reported that adult BALB/c mice display highly aggressive behavior that can sometimes result in injury, and this is consistent with observations in our laboratory. Isolated male mice (C57 and DBA) display hyperactivity and a decreased stress response (forced swim test) compared to control group-housed mice (Vöikar et al., 2005), and these factors could have contributed to differences in response rate in the current study. However, Haluk & Wickman (2010) found no difference between isolated and group-housed adult male mice (C57) under fixed and progressive ratio schedules, and Arndt et al. (2009) found no difference between isolated and group-housed adult male mice (C57 and BALB/c) in the modified hole board test or hormone stress response (corticosterone).

Consistently, isolate housing during *adolescence* has been found to have detrimental effects on behavior in adulthood (Van den Berg et al., 1999; Würbel, 2001; Schrijver & Würbel, 2001), but in the current study both the adolescents (at Auburn University) and adults (housed at Harlan Laboratories prior to shipping) were group-housed during adolescent development. Taken together, the results presented here are most likely robust and indicative of age-related differences between the behavior of adolescent and adults BALB/c mice.

5. Conclusion

The procedures used here provided measures of rapid within-session acquisition of response chains that required simple and conditional discriminations (SDR and IRA, respectively). In an SDR procedure, adolescent mice omitted more trials relative to adults in both the original discrimination and first reversal phases, but did not differ on accuracy. In an IRA procedure, adolescent mice responded less frequently, earned fewer reinforcers, and emitted fewer long chains than adults, producing differences in PQ, a measure of productivity. Across procedures, a pattern of adolescent omissions emerged; during SDR adolescents omitted trial-initiation back lever responses and during IRA adolescents omitted correct back lever-presses in favor of incorrect front lever presses. These findings suggest that adolescent and adult BALB/c mice differ in sensitivity to delayed reinforcement. Both procedures were sensitive to age differences in BALB/c mice, increasing the tools available for studying operant behavior during the rodent period of adolescence.

Acknowledgments

Support for this research was provided by NIH grant ES003299.

References

- Adriani W, Chiarotti F, Laviola G. Elevated novelty seeking and peculiar d-amphetamine sensitization in periadolescent mice compared with adult mice. *Behavioral Neuroscience*. 1998; 112(5):1152–1166. [PubMed: 9829793]
- Adriani W, Laviola G. A unique hormonal and behavioral hyporesponsivity to both forced novelty and d-amphetamine in periadolescent mice. *Neuropharmacology*. 2000; 39(2):334–346. [PubMed: 10670429]
- Adriani W, Granstrem O, Macri S, Izykenova G, Dambinova S, Laviola G. Behavioral and neurochemical vulnerability during adolescence in mice: studies with nicotine. *Neuropsychopharmacology*. 2003; 29(5):869–878. [PubMed: 14666123]
- Adriani W, Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behavioural Pharmacology*. 2004; 15(5-6):341–352. [PubMed: 15343057]
- Akers KG, Arruda-Carvalho M, Josselyn SA, Frankland PW. Ontogeny of contextual fear memory formation, specificity, and persistence in mice. *Learning & Memory*. 2012; 19:598–604. [PubMed: 23161449]
- Andrzejewski ME, Schochet TL, Feit EC, Harris R, Mckee BL, Kelley AE. A comparison of adult adolescent rat behavior in operant learning, extinction, and behavioral inhibition paradigms. *Behavioral Neuroscience*. 2011; 125(1):93–16. [PubMed: 21319891]
- Bailey JM, Johnson JE, Newland MC. Mechanisms and performance measures in mastery-based incremental repeated acquisition: behavioral and pharmacological analyses. *Psychopharmacology*. 2010; 209(4):331–341. [PubMed: 20217054]
- Bailey JM, Hutsell BA, Newland MC. Dietary nimodipine delays the onset of methylmercury neurotoxicity in mice. *NeuroToxicology*. 2013; 37:108–117. [PubMed: 23583802]
- Barkley RA. Behavioral inhibition, sustained attention, and executive functions: constructing a unifying theory of ADHD. *Psychological Bulletin*. 1997; 121(1):65–94. [PubMed: 9000892]
- Bizo LA, Killeen PR. Models of ratio schedule performance. *Journal of Experimental Psychology: Animal Behavior Processes*. 1993; 23:351–367. [PubMed: 9206029]
- Boakes RA. Performance on learning to associate a stimulus with positive reinforcement. *Operant-Pavlovian Interactions*. 1977:67–97.
- Boren JJ. Repeated acquisition of new behavioral chains. *American Psychologist*. 1963; 17:421.

- Brenhouse HC, Andersen SL. Developmental trajectories during adolescence in males and females: a cross-species understanding of underlying brain changes. *Neuroscience and Biobehavioral Reviews*. 2011; 35(8):1687–1703. [PubMed: 21600919]
- Broadwater M, Spear LP. Age differences in fear retention and extinction in male Sprague-Dawley rats: effects of ethanol challenge during conditioning. *Behavioural Brain Research*. 2013; 252:377–387. [PubMed: 23810415]
- Brodtkin, Edward S. BALB/c mice: low sociability and other phenotypes that may be relevant to autism. *Behavioural Brain Research*. 2007; 176(1):53–65. [PubMed: 16890300]
- Cherkasova MV, Hechtman L. Neuroimaging in attention-deficit hyperactivity disorder: beyond the frontostriatal circuitry. *Canadian Journal of Psychiatry*. 2009; 54(10):651–664.
- Chudasama Y, Robbins TW. Dissociable contributions of the orbitofrontal and infralimbic cortex to Pavlovian autoshaping and discrimination reversal learning: further evidence for the functional heterogeneity of the rodent frontal cortex. *Journal of Neuroscience*. 2003; 23:8771–8780. [PubMed: 14507977]
- Cohn J, Paule MG. Repeated acquisition of response sequences: the analysis of behavior in transition. *Neuroscience & Biobehavioral Reviews*. 1995; 19(3):397–406. [PubMed: 7566741]
- Conour LA, Murray KA, Brown MJ. Preparation of animals for research—issues to consider for rodents and rabbits. *ILAR journal*. 2006; 47(4):283–293. [PubMed: 16963809]
- Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neuroscience and Biobehavioral Reviews*. 2004; 28(7):771–784. [PubMed: 15555683]
- Dalley JW, Mar AC, Economidou D, Robbins TW. Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. *Pharmacology, Biochemistry, and Behavior*. 2008; 90(2):250–260.
- Douglas LA, Varlinskaya EI, Spear LP. Novel-object place conditioning in adolescent and adult male and female rats: effects of social isolation. *Physiology & Behavior*. 2003; 80(2-3):317–325. [PubMed: 14637231]
- Evenden JL. Varieties of impulsivity. *Psychopharmacology*. 1999; 46(4):348–361. [PubMed: 10550486]
- Fellows LK, Farah MJ. Ventromedial frontal cortex mediates affective shifting in humans: evidence from a reversal learning paradigm. *Brain*. 2003; 126:1830–1837. [PubMed: 12821528]
- Galvan A, Hare TA, Parra CE, Penn J, Voss H, Glover G, Casey BJ. Earlier development of the accumbens relative to orbitofrontal cortex might underlie risk-taking behavior in adolescents. *Journal of Neuroscience*. 2006; 26(25):6885–6892. [PubMed: 16793895]
- Giedd JN, Blumenthal J, Jeffries NO, Castellanos FX, Liu H, Zijdenbos A, Paus T, Evans AC, Rapoport JL. Brain development during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience*. 1999; 2:861–863. [PubMed: 10491603]
- Green L, Fry AF, Myerson J. Discounting of delayed rewards: a life-span comparison. *Psychological Science*. 1994; 5(1):33–36.
- Haluk DM, Wickman K. Evaluation of study design variables and their impact on food-maintained operant responding in mice. *Behavioural Brain Research*. 2010; 207(2):394–401. [PubMed: 19879302]
- Jacobus J, Thayer RE, Trim RS, Bava S, Frank LR, Tapert SF. White matter integrity, substance use, and risk taking in adolescence. *Psychology of Addictive Behaviors*. 2013; 27(2):431–442. [PubMed: 22564204]
- Jentsch JD, Taylor JR. Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology*. 1999; 146(2):373–390. [PubMed: 10550488]
- Kendig MD, Boakes RA, Rooney KB, Corbit LH. Chronic restricted access to 10% sucrose solution in adolescent and young adult rats impairs spatial memory and alters sensitivity to outcome devaluation. *Physiology and Behavior*. 2013; 120:164–172. [PubMed: 23954407]
- Killeen PR. Psychophysical distance functions for hooded rats. *The Psychological Record*. 1974; 24(2):229–235.
- Killeen PR, Sitomer MT. MPR. *Behavioral Processes*. 2003:49–64.

- Landi MS, Kreider J, Lang C, Bullock L. Effects of shipping on the immune function in mice. *American journal of veterinary research*. 1982; 43(9):1654–1657. [PubMed: 7149414]
- Langen M, Leemans A, Johnston P, Ecker C, Daly E, Murphy D. Fronto-striatal circuitry and inhibitory control in autism: findings from diffusion tensor imaging tractography. *Cortex*. 2012; 48(2):183–193. [PubMed: 21718979]
- Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescence and animal models. *Neuroscience and Biobehavioral Reviews*. 1999; 23(7):993–1010. [PubMed: 10580313]
- Laviola G, Pascucci T, Pieretti S. Striatal dopamine sensitization to *d*-amphetamine in periadolescent but not in adult rats. *Pharmacology, Biochemistry, and Behavior*. 2001; 68:115–124.
- Laviola G, Macri S, Marley-Fletcher S, Adriani W. Risking-taking behavior in adolescent mice: psychobiological determinants and early epigenetic influence. *Neuroscience and Biobehavioral Reviews*. 2003; 27:19–31. [PubMed: 12732220]
- Macri S, Adriani W, Chiarotti F, Laviola G. Risk taking during exploration of a plus-maze is greater in adolescent than in juvenile or adult mice. *Animal Behaviour*. 2002; 65(4):541–546.
- Madden, GJ.; Johnson, PS. A delay-discounting primer. In: Madden, GJ.; Bickel, WK., editors. *Impulsivity: the behavioral and neurological science of discounting*. APA Books; Washington, DC: 2010. p. 11–37.
- Moffitt TE. Adolescence-limited and life-course-persistent antisocial behavior: a developmental taxonomy. *Psychological Review*. 1993; 100:674–701. [PubMed: 8255953]
- Myslivecek J, Hassmannová J. Ontogeny of active avoidance in the rat: learning and memory. *Developmental Psychobiology*. 1979; 12(2):169–186. [PubMed: 456753]
- Newland MC. Quantifying the molecular structure of behavior: separate effects of caffeine, cocaine, and adenosine agonists on interresponse times and lever-press durations. *Behavioural Pharmacology*. 1997; 8:1–16. [PubMed: 9832996]
- Newland MC, Hoffman DJ, Heath JC, Donlin WD. Response inhibition is impaired by developmental methylmercury exposure: Acquisition of low-rate lever-pressing. *Behavioural Brain Research*. 2013; 253(0):196–205. [PubMed: 23721962]
- Newland MC, Reed MN, LeBlanc A, Donlin WD. Brain and blood mercury and selenium after chronic and developmental exposure to methylmercury. *Neurotoxicology*. 2006; 27:710–720. [PubMed: 16824603]
- Newman LA, McGaughy J. Adolescent rats show cognitive rigidity in a test of attentional set shifting. *Developmental Psychobiology*. 2011; 53(4):391–401. [PubMed: 21365641]
- Olson EA, Hooper CJ, Collins P, Luciana M. Adolescents' performance on delay and probability discounting tasks: contributions of age, intelligence, executive functioning, and self-reported externalizing behavior. *Personality and Individual Differences*. 2007; 43(7):1886–1897. [PubMed: 18978926]
- Pagani JH, Brown KL, Stanton ME. Contextual modulation of spatial discrimination reversal in developing rats. *Developmental Psychobiology*. 2005; 46(1):36–46. [PubMed: 15690387]
- Paletz EM, Day JJ, Craig-Schmidt MC, Newland MC. Spatial and visual discrimination reversals in adult and geriatric rats exposed during gestation to methylmercury and n-3 polyunsaturated fatty acids. *Neurotoxicology*. 2007; 28:707–719. [PubMed: 17582499]
- Paolicelli RC, Bolasco G, Pagani F, Maggi L, Scianni M, Gross CT. Synaptic pruning by microglia is necessary for normal brain development. *Science*. 2011; 333(6048):1456–1458. [PubMed: 21778362]
- Paule MG, McMillan DE. Incremental repeated acquisition in the rat: acute effects of drugs. *Pharmacology, Biochemistry, and Behavior*. 1984; 21(3):431–439.
- Paule MG, Forrester TM, Maher MA, Cranmer JM, Allen RR. Monkey versus human performance in the NCTR operant test battery. *Neurotoxicology and Teratology*. 1990; 12:503–507. [PubMed: 2247039]
- Paule MG, Chelonis JJ, Buffalo EA, Blake DJ, Casey PH. Operant test battery performance in children: correlation with IQ. *Neurotoxicology & Teratology*. 1999; 21(3):223–230. [PubMed: 10386825]

- Paule MG, Green L, Myerson J, Alvarado M, Bachevalier J, Schneider S, Schantz SL. Behavioral toxicology of cognition: extrapolation from experimental animal models to humans: behavioral toxicology symposium overview. *Neurotoxicology and Teratology*. 2012; 34(4):263–273. [PubMed: 22311110]
- Pinkston JW, Lamb RJ. Delay discounting in C57BL/6J and DBA/2J mice: adolescent-limited and life-persistent patterns of impulsivity. *Behavioral Neuroscience*. 2011; 125(2):194–201. [PubMed: 21463022]
- Reed MR, Paletz EM, Newland MC. Gestational exposure to methylmercury and selenium: effects on a spatial discrimination reversal in adulthood. *Neurotoxicology*. 2006; 27:721–732. [PubMed: 16759706]
- Reilly MP, Posadas-Sanchez D, Kettle LC, Killeen PR. Rats (*Rattus norvegicus*) and pigeons (*Columba livia*) are sensitive to the distance to food, but only rats request more food when distance increases. *Behavioural Processes*. 2012; 91:236–243. [PubMed: 22989930]
- Schrijver NC, Würbel H. Early social deprivation disrupts attentional, but not affective, shifts in rats. *Behavioral neuroscience*. 2001; 115(2):437. [PubMed: 11345968]
- Simon NW, Gregory TA, Wood J, Moghaddam B. Differences in response initiation and behavioral flexibility between adolescent and adult rats. *Behavioral Neuroscience*. 2013; 127(1):23–32. [PubMed: 23398439]
- Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. *Developmental Psychobiology*. 1983; 16(2):83–109. [PubMed: 6339302]
- Spear LP. The adolescent brain and age-related behavioral manifestations. *Neuroscience and Biobehavioral Reviews*. 2000; 24:417–463. [PubMed: 10817843]
- Stansfield KH, Kirstein CL. The effects of novelty on behavior in adolescent versus adult rats. *Developmental Psychobiology*. 2006; 48(1):10–15. [PubMed: 16381024]
- Sturman DA, Mandell DR, Moghaddam B. Adolescents exhibit behavioral differences from adults during instrumental learning and extinction. *Behavioral Neuroscience*. 2010; 124:16–25. [PubMed: 20141277]
- Swendsen J, Burstein M, Case B, Conway KP, Dierker L, He J, Meriangas KR. Use and abuse of alcohol and illicit drugs in US adolescents: Results of the national comorbidity survey – adolescent supplement. *Archives of General Psychiatry*. 2012; 69(4):390–398. [PubMed: 22474107]
- Van den Berg CL, Pijlman FTA, Koning HAM, Diergaarde L, Van Ree JM, Spruijt BM. Isolation changes the incentive value of sucrose and social behaviour in juvenile and adult rats. *Behavioural Brain Research*. 1999; 106(1–2):133–142. [PubMed: 10595429]
- Van Ruiven R, Meijer GW, Wiersma A, Baumans V, Van Zutphen LFM, Ritskes-Hoitinga J. The influence of transportation stress on selected nutritional parameters to establish the necessary minimum period for adaptation in rat feeding studies. *Laboratory animals*. 1998; 32(4):446–456. [PubMed: 9807759]
- Voikar V, Polus A, Vasar E, Rauvala H. Long-term individual housing in C57BL/6J and DBA/2 mice: assessment of behavioral consequences. *Genes, Brain and Behavior*. 2005; 4(4):240–252.
- Wahlstrom D, White T, Luciana M. Neurobehavioral evidence for changes in dopamine system activity during adolescence. *Neuroscience and Biobehavioral Reviews*. 2010; 34(5):631–648. [PubMed: 20026110]
- Wenger GR, Schmidt C, Davisson MT. Operant conditioning in the Ts65Dn mouse: learning. *Behavioral Genetics*. 2004; 34(1):105–109.
- Würbel H. Ideal homes? Housing effects on rodent brain and behaviour. *Trends in neurosciences*. 2001; 24(4):207–211. [PubMed: 11250003]
- Zald DH, Andreotti C. Neuropsychological assessment of the orbital and ventromedial prefrontal cortex. *Neuropsychologia*. 2010; 48(12):3377–3391. [PubMed: 20728457]
- Zoratto F, Laviola G, Adriani W. Gambling proneness in rats during the transition from adolescence to young adulthood: A home-cage method. *Neuropharmacology*. 2013; 67(0):444–454. [PubMed: 23220407]

Highlights

- Adolescent and adult BALB/c responded under spatial discrimination reversal (SDR) and incremental repeated acquisition (IRA) procedures.
- Adolescent mice omitted more trials than adults during SDR, but there was no effect of age on accuracy.
- Adolescent mice were less productive (i.e. lower progress quotient) than adults during IRA.
- Adolescents appeared to engage in more off-task behavior than adults on both tasks.
- These procedures increase the tools available for studying operant behavior during rodent adolescence.

Figure 1.

Correct responses (closed circles), commission errors (open squares), and omission errors (open triangles) for one adolescent and one adult subject. The vertical dashed lines denote the transition from original discrimination to first reversal. Characteristically, adolescent omissions persisted throughout original discrimination and the first reversal, though there was little difference in accuracy (excluding omissions) between the two age groups.

Figure 2.

Dependent measures for adolescent and adult mice during the original discrimination of SDR. Inverse survival curves (A) for adolescents (filled circles, solid line) and adults (open circles, dashed line) show the number of sessions required to complete the original discrimination (#: $p < 0.05$). Mean accuracy (B), commission errors (C), omission errors (D), trial-initiation latency (E), and choice latency (F) for adolescent (filled bars) and adults (open bars) were averaged over the first and last two sessions of the original discrimination (#: $p < 0.05$). Vertical bars represent $\pm$ standard error of the mean (SEM).

Figure 3.

Dependent measures for adolescent and adult mice during the first reversal of SDR. Inverse survival curves (A) for adolescents (filled circles, solid line) and adults (open circles, dashed line) show the number of sessions required to complete the first reversal (A). Mean accuracy (B), commission errors (C), omission errors (D), trial-initiation latency (E), and choice latency (F) for adolescent (filled bars) and adults (open bars) were averaged over the first and last two sessions of the first reversal (#: $p < 0.05$). Vertical bars represent $\pm$ SEM.

Figure 4. Progress quotient (A), max chain length (B), response rate (C), and reinforcer rate (D) as a function of session from the IRA procedure. Vertical bars represent $\pm$ SEM.

Figure 5. Mean progress quotient (A), max chain length (B), response rate (C), and reinforcer rate (D) for adolescent (filled bars) and adult (open bars) mice averaged over the first and last two sessions (#: $p < 0.05$). Vertical bars represent $\pm$ SEM.

Figure 6.

The inverse survival graph shows the number of sessions necessary for adolescent (left) and adult (right) subjects to reach a given chain length, starting with a 2-link chain. Adults reached longer chains faster, while adolescents struggled to move past 2 and 3-link chains.

Figure 7.

Line-graphs: Mean accuracy for links one (top left), two (top right) and three (bottom left) by session for adolescent (filled circles) and adults (open circles). Dashed lines indicate chance accuracy for each chain length. Bar-charts: Mean accuracy by chain link averaged over the first and last two sessions for adolescent (filled bars) and adults (open bars). Vertical bars represent $\pm$ SEM.

Figure 8.

Q-Q plot of IRTs between two correct responses (open circles) and a correct and incorrect response (filled circles) for adolescent (x-axis) and adult (y-axis) subjects during the last two session of the IRA procedure. The leftmost data point in each series represents the location of the 10th percentile. Subsequent data points to the right represent the 20th, 30th... 90th percentile. Straight lines were compared against the dashed line (slope of 1, y-intercept of 0) using least squares regression (see text for details).