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Adolescents exhibit behavioral differences from adults during instrumental learning and extinction

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Abstract

Adolescence is associated with the development of brain regions linked to cognition and emotion. Such changes are thought to contribute to the behavioral and neuropsychiatric vulnerabilities of this period. We compared adolescent (P28-42) and adult (P60+) rats as they performed a simple instrumental task and extinction. Animals were trained to poke into a hole for a food-pellet reinforcer. After six days of training, animals underwent extinction sessions in which the previously rewarded behavior was no longer reinforced. During extinction we examined the effects of continued presentation of a cue light and food restriction. Adults and adolescents exhibited similar performance during training, although adolescents made more task-irrelevant pokes, consistent with increased exploration. Adults made more premature pokes, which could indicate a more exclusive focus on the task. During extinction, adolescents made more perseverative (previously reinforced) pokes than adults. This behavior was strongly modulated by the combination of motivational factors present (food restriction and cue light), indicating that adolescents were differentially sensitive to them. Furthermore, food restriction induced greater open-field activity in adolescents but not adults. Thus, as the neural circuitry of motivated behavior develops substantially during adolescence, so too does the behavioral sensitivity to motivational factors. Understanding how such factors differently affect adolescents may shed light on mechanisms that lead to the development of disorders that are manifested during this period.

Keywords

Adolescence; instrumental learning; extinction; open field; motivation

Introduction

Adolescence is a major transitional period between childhood and adulthood. It encompasses puberty, a time of reproductive development, and is characterized in humans and rodents by numerous non-reproductive socio-behavioral changes (Spear, 2000). It is during adolescence that the symptoms of several psychiatric disorders typically arise, including depression, eating disorders, and schizophrenia (Pine, 2002; Sisk & Zehr, 2005; Volkmar, 1996). Characteristic adolescent behavioral changes include elevated social interaction (Csikszentmihalyi, Larson, & Prescott, 1977) and increased novelty-seeking and risk-taking behavior (Adriani, Chiarotti,

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& Laviola, 1998; Macrì, Adriani, Chiarotti, & Laviola, 2002; Spear, 2000; Stansfield & Kirstein, 2006; Steinberg, 2008). These latter behaviors correlate with drug and alcohol use (Andrucci, Archer, Pancoast, & Gordon, 1989), and adolescence is often considered a period of increased addiction vulnerability (Adriani & Laviola, 2004; Chambers, Taylor, & Potenza, 2003). Furthermore, as in human adolescents, adolescent mice exhibit greater impulsivity than adults, as measured by reduced preference for larger delayed food reinforcers over more immediate smaller ones (Adriani & Laviola, 2003).

Along with these behavioral changes, the adolescent brain undergoes extensive remodeling (McCutcheon & Marinelli, 2009), with neurogenesis (Pinos *et al.*, 2001), axonal growth (Benes, Taylor, & Cunningham, 2000; Cunningham, Bhattacharyya, & Benes, 2002), myelination (Benes, Turtle, Khan, & Farol, 1994), apoptosis (Nunez, Sodhi, & Juraska, 2002), and synaptic and receptor pruning (Andersen, Thompson, Rutstein, Hostetter, & Teicher, 2000; Meyer, Ferres-Torres, & Mas, 1978; Teicher, Andersen, & Hostetter, 1995) accompanying shifts in white-matter density and cortical grey-matter volume (Benes *et al.*, 1994; Giedd *et al.*, 1999; Juraska & Markham, 2004; Paus, 2005; Paus *et al.*, 2001). The mesocortical dopaminergic circuitry, considered broadly relevant to motivated behavior [for reviews see Cools (2008), Salamone & Correa (2002), Berridge (2007), and Floresco & Magyar (2006)] also undergoes considerable development during adolescence (Chambers *et al.*, 2003; Ernst & Fudge, 2009; Lewis, 1997; Spear, 2000).

Much of the literature on adolescence has focused on drug-related behavioral differences such as ethanol or psychostimulant sensitivity differences (Badanich, Maldonado, & Kirstein, 2008; Bolanos, Glatt, & Jackson, 1998; Little, Kuhn, Wilson, & Swartzwelder, 1996; Moy, Duncan, Knapp, & Breese, 1998; Pautassi, Myers, Spear, Molina, & Spear, 2008; Spear & Brake, 1983; Varlinskaya & Spear, 2006). For example, adolescents tend to exhibit less amphetamine- and cocaine-induced locomotor stimulation and stereotypy (Bolanos *et al.*, 1998; Laviola, Adriani, Terranova, & Gerra, 1999; Mathews & McCormick, 2007; Spear & Brake, 1983). Conversely, adolescent rats show greater sensitivity to the cataleptic effects of neuroleptics like haloperidol (Shalaby & Spear, 1980; Spear & Brake, 1983). Less is known about developmental differences in motivated behavior more generally, such as how various non-pharmacological factors might affect adolescents differently than adults. The aim of this study was to characterize several similarities and differences in adolescent motivated behavior during an instrumental learning task and extinction. While motivation can be a somewhat elusive concept, others have defined it as “the set of processes through which organisms regulate the probability, proximity and availability of stimuli” (Salamone & Correa, 2002). Here we use the term “motivational factor” to identify elements that increase behavioral manifestations of such processes in an organism. We tested adolescent and adult rats in a simple paradigm where they learned to pair a particular action (nose poke) with a desired outcome (food pellet), and followed the training period with extinction, at which point the action-outcome association was no longer reinforced. During extinction we examined how continued food restriction and presentation of a task cue may differently affect adolescents. We further compared the impact of one of these motivational factors, food restriction, on adolescent and adult open-field activity. Studying such differences may inform our understanding of how adolescent neurodevelopment leads to typical age-specific behavioral propensities and disease processes

Methods

Subjects

Adolescent (P28-42; $n = 42$) and adult (P60+; $n = 42$) male Sprague-Dawley rats (Harlan, Frederick, MD) were used. Pre-adolescent juvenile rats (P21) and adults were received four days before beginning handling and operant box habituation. Training on the instrumental task

began immediately after habituation, corresponding to one week after arrival (P28 for adolescents). All subjects were housed in pairs under 12 hour light/dark cycle conditions (lights on at 7pm), and testing was performed during animals' active phase. Food restriction was imposed during the habituation period, at which time pre-adolescents received 5g and 8g chow on consecutive days and then were maintained at 10g chow/day on the final day of habituation and throughout training. This level of food-restriction was chosen after observing that not all food was consumed from the previous day in some cages prior to testing when early adolescents were fed 5g, 8g, and then sustained at 12g chow/day. Adults were given 15g chow/day during habituation and training. All rats had *ad-libitum* access to water except during testing. Experimental protocols were approved by the University of Pittsburgh Animal Care and Use Committee.

Instrumental Task

Operant chambers (Coulbourn Instruments, Allentown, PA) were equipped with a house light that illuminated the chamber during the task, three nose-poke holes, a food trough, and a food pellet delivery system. Nose-poke holes were arrayed horizontally on the wall opposite the food trough. Entries into the nose-poke holes or the food trough were detected by infrared photosensors. A PC-based controller and *Graphic State* software (Coulbourn Instruments) were used to run the task and record the rats' behavior.

During the first day of habituation, rats were placed in the operant chamber for 20 min with the house light on. During the second and third days of habituation, rats were placed in the operant box for 20 min with the house light on and food pellets (fortified dextrose pellets, 45 mg, Bio-Serv, Frenchtown, NJ) were delivered into the food trough every 30 sec.

Rats were then trained on a reinforcement schedule in which a single instrumental nose poke was reinforced with the delivery of a single food pellet. The house light was continuously illuminated for the duration of each session. Trials began with the illumination of a light cue in the center (and only the center) nose-poke hole. After an animal poked into that hole, the light turned off and one pellet was delivered to the food trough, along with the illumination of a food trough light. The trial-onset cue would remain illuminated until the rat performed the nose poke (instrumental response). In order for the next trial to begin, the rat was required to poke into the food trough to retrieve the pellet, which turned off the food-trough light, and then wait for a fixed 5-sec inter-trial interval (ITI). Animals received daily training sessions over six consecutive days. Each session was terminated upon the delivery of 99 pellets or the passage of 30 min. Nose pokes into either of the non-illuminated (left and right) modules were not reinforced, although this behavior was recorded and categorized as "task-irrelevant pokes." Video cameras allowed behavior to be monitored by the experimenter during testing. Rats that did not learn the instrumental task after three days were hand-shaped to the center hole and performed the task for three full sessions after this. These animals ($n = 5$) were excluded from all instrumental training analyses. However, as their extinction and open-field behavior was not statistically different from that of their peers, these data were combined with their corresponding groups.

Extinction

The day after the completion of the last training session, rats began one of four extinction paradigms, during which the instrumental behavior was no longer reinforced. In the first group (adult $n = 18$; adolescent $n = 18$) animals remained food-restricted. Additionally the trial-onset cue that was previously associated with the beginning of each trial during training continued to be presented during extinction. A poke to the illuminated hole would turn off the cue light as before, but no food pellet was delivered. If the animal then poked in the food trough, after a 5-sec ITI the cue light was presented again. If it did not poke into the food trough, the cue

light would reappear after a 15-sec delay. In a second group (adult $n = 6$, adolescent $n = 6$) animals underwent extinction exactly as in the first group, except that these animals were given *ad libitum* access to food in their home cages each day (beginning immediately after the last training session). In a third extinction group (adult $n = 12$; adolescent $n = 12$) food restriction was maintained; however, no cue light was presented to the animals during extinction. In a final group (adult $n = 6$; adolescent $n = 6$) animals were given *ad libitum* access to food in their home cages and no cue was presented during extinction sessions.

Open Field

After operant-box testing during the second and third days of extinction, a subset of food-restricted and *ad lib* adult and adolescent rats (adult $n = 24$; adolescent $n = 24$) were placed in the center of an open field arena ($1\text{ m} \times 1\text{ m}$, divided into 25 squares) under normal white lighting and given 5 minutes to explore while being videotaped. An experimenter, blinded to the food-restriction status of the animals, rated the number of total square entries and entries into the central grids. A square entry was counted when a rat's hind legs passed from one square into the next.

Statistical Analysis

To delineate age-related differences in task performance during training, age (between) $\times$ session (within) repeated measures ANOVAs were performed on total trials per session, task-irrelevant pokes (left- and right-hole pokes), latency from cue onset to instrumental poke, and latency from instrumental poke to food-trough poke. During extinction, repeated-measure ANOVAs were used to examine the effects of age, food-restriction status, cue-light presence, and their interactions on perseverative and task-irrelevant pokes. These models were broken up to more easily interpret potential 3-way between-factor interactions. The following analyses were performed separately on animals for which a cue was or was not presented during extinction: age $\times$ food-restriction status (between) $\times$ session (within); on food-restricted and *ad lib* animals: age $\times$ cue-light presence (between) $\times$ session (within); and for adults and adolescents: food-restriction status $\times$ cue-light presence (between) $\times$ session (within). To assess open-field behavior, age $\times$ food-restriction status ANOVAs were performed on total grid entries and central grid entries. When necessary, significant ANOVA results were supplemented with Fisher LSD post-hoc tests. In all repeated-measure ANOVAs for which the assumption of sphericity was violated, the Lower-Bound correction was used for a maximally conservative degrees of freedom adjustment. Pearson's correlation and analysis of covariance (ANCOVA; used in models with fixed factors such as age or food-restriction status) were used to determine the relationship between continuous variables (e.g. total trials performed during training, total perseverative pokes during extinction), as well as to test for potential relationships within each age group between body weight and training trials, task-irrelevant pokes, and perseveration during extinction. We also used Pearson's correlation to examine the relationship between task-irrelevant pokes (during training and during extinction) and open-field behaviors, training trials, and perseveration during extinction. To avoid the potential problem of singular poking events registering as multiple pokes (e.g. several pokes within 1 sec as an animal retrieves a food pellet), a distinct poke was defined as one that was not immediately preceded by a previous poke within 1 sec. The exception to this was counting an instrumental response as such even after immediately following a premature poke.

Results

A significant interaction effect was observed for the total number of trials performed by adults versus adolescents across training sessions $F(1,1)=7.05$, $p = 0.01$. This was due to adults performing a significantly greater number of trials in sessions 3-6, but not sessions 1-2, when most of the initial learning took place. Total trial performance was generally stable for

adolescents and adults beginning in sessions 3-4 (Figure 1A). Examination of the average cumulative trials performed over time within sessions demonstrated that the difference between adolescents and adults in total trials in sessions 3-6 was associated with an early drop in the rate of adolescent trial performance after initially being similar to that of adults (Figure 1B). The rate of adolescent trial performance in the first session was slightly faster (a steeper line) than that of adults. This suggests that on average, adolescents either learned the task slightly earlier or were simply slightly more active during this period (Figure 1B).

A significant age $\times$ session interaction was found for the mean latency from cue onset to instrumental poke $F(1,1)=5.64$, $p < 0.05$. While the latency from trial-onset cue to center-hole poke was initially lower for adolescents than adults, in sessions 4-6 adults had a shorter average latency (Figure 2A). Because adolescents more readily reduced their response rate in the latter portion of most training sessions (Figure 1B), the age-related latency differences in sessions 4-6 could be due to this effect. We therefore examined this latency during the first 5 minutes of each session, when adolescents and adults performed trials at the highest rate. A significant age difference was still present $F(1,77)=9.03$, $p < 0.01$, however there was no significant difference in latency for sessions 3-6 (Figure 2B). There was also no significant main effect or interaction for the average latency from instrumental poke to food-trough entry $F(1,77)=3.37$, $p > 0.05$ and $F(1,1)=2.49$, $p > 0.05$, although adolescents appear to exhibit a shorter latency during the first training session (Figure 2C). In spite of these latency similarities (and even slightly shorter cue-to-poke latencies for adolescents in early sessions), adults consistently performed more premature pokes across sessions, defined as pokes prior to the trial-onset cue during the ITI $F(1,77)=21.72$, $p < 0.001$ (Figure 3A). Conversely, adolescents consistently performed more task-irrelevant (left and right) hole pokes than adults during training $F(1,77)=191.31$, $p < 0.001$ (Figure 3B).

During extinction sessions when the trial-onset cue was present, food-restricted adolescents performed significantly more perseverative (previously reinforced center hole) pokes than adults $F(1,33)=33.16$, $p < 0.001$. No age $\times$ session interaction was present $F(1,1)=1.28$, $p > 0.05$ (Figure 4A). In food-restricted rats for whom no cue light was presented during extinction, a main effect of age was still observed $F(1,22)=32.14$, $p < 0.001$ and again no interaction was present $F(1,1)=0.535$, $p > 0.05$ (Figure 4B). To test the hypothesis that the difference in perseverative pokes between food-restricted adolescents and adults was larger when the cue was present than when it was absent, we ran an age $\times$ cue-light presence (between) $\times$ session (within) repeated-measures ANOVA. This model indicated via a significant age $\times$ cue-light presence interaction that food-restricted adolescents performed disproportionately more perseverative pokes than food-restricted adults when the cue light was present than when it was absent $F(1,55)=4.41$, $p < 0.05$ (Figure 5A). Rats not food-restricted during extinction performed significantly fewer perseverative pokes than food-restricted rats when the cue was present $F(1,43)=35.07$, $p < 0.01$. No differences were observed between *ad lib* adolescents and adults when the cue light was present $F(1,10)=1.73$, $p > 0.05$ (Figure 4C) and no significant age $\times$ cue light presence interaction was present in *ad lib* animals $F(1,20)=1.95$, $p > 0.05$ (Figure 5B). An age $\times$ food-restriction status interaction was observed $F(1,43)=6.15$, $p < 0.05$, indicating that among animals to whom the cue was presented (Figures 4A,4C), food restriction more strongly increased perseverative pokes in adolescents than adults (Figure 5C). When the cue light was absent (Figures 4B, 4D), food-restricted animals still performed more perseverative pokes than *ad lib* ones $F(1,32)=11.57$, $p < 0.01$. However, no age $\times$ food-restriction status interaction was observed when the cue light was absent $F(1,32)=0.148$, $p > 0.05$, indicating that food restriction did not have a stronger effect on adolescents than adults when the cue was absent (Figure 5D). When rats were no longer food-restricted and the cue was omitted, adults performed fewer perseverative pokes than adolescents $F(1,10)=39.79$, $p < 0.001$ (Figure 4D). We performed a cue-presence $\times$ food-restriction status (between) $\times$ session (within) ANOVA separately on adolescents and adults. Adolescents exhibited a significant

food-restriction status $\times$ cue-presence interaction $F(1,38)=11.96$, $p = 0.001$ (Figure 5E), indicating that cue presence interacted with food restriction to further increase perseveration in these younger animals. In adults, however, no such interaction was observed $F(1,37)=2.43$, $p > 0.05$ (Figure 5F). Total trials performed during training was a significant covariate for adults and adolescents predictive of total perseverative pokes during extinction $F(1,75)=11.49$, $p = 0.001$. This indicates a positive linear relationship between training trials and perseveration within age groups, although adolescents tended to perform fewer total trials during training but more perseverative pokes during extinction than adults. No statistically significant relationship was observed for trial performance, task-irrelevant poking, or perseverative pokes during extinction as a function of the covariate body weight when food-restriction status was included as a fixed factor ($p > 0.05$; data not shown).

Adolescents continued to perform significantly more task-irrelevant pokes than adults during extinction $F(1,76)=124.31$, $p < 0.001$ (Figure 6A). A main effect of food restriction on total task-irrelevant pokes across extinction sessions was observed $F(1,80)=7.25$, $p < 0.01$. However, post-hoc comparisons indicated that while *ad lib* adults performed significantly fewer total task-irrelevant pokes than food-restricted adults, this effect was not significant for adolescents (Figure 6B). There was no statistically significant correlation between task-irrelevant pokes and open-field grid entries, total training trials, or perseveration during extinction for adolescents or adults ($p > 0.05$; data not shown).

Food-restricted adolescents gained weight throughout the experiment. On the first day of testing food-restricted adolescents (P28) weighed (mean $\pm$ SD) 74.9 ± 6.1 . By the first day of extinction, food-restricted adolescents (P34) weighed 110.4 ± 7.2 g. On the fifth day of extinction food-restricted adolescents (P38) weighed 116.9 ± 8.2 g.

In the open field, adolescents performed more total grid entries than adults $F(1,43)=90.48$, $p < 0.001$, and an age $\times$ food-restriction status interaction was significant $F(1,43)=5.55$, $p < 0.05$. Food restriction increased adolescents' total grid entries, but adults were unaffected (Figure 7A). The effect was similar for central grid entries, with adolescents entering the central grids more than adults $F(1,43)=25.38$, $p < 0.001$, and an age $\times$ food-restriction status interaction was observed $F(1,43)=5.03$, $p < 0.05$. As with total grid entries, food restriction increased central grid entries in adolescents but not adults (Figure 7B).

Discussion

In the present study we compared the behavior of adolescent and adult rats during a simple instrumental task and extinction. There was no age-related difference in the total number of trials performed during the first two sessions, although the within-session rate of trial performance and the latencies from the trial-onset cue to the instrumental response may have been slightly faster in adolescents during those sessions. By sessions 3-4 adults and adolescents reached a stable maximum in the total number of trials performed, with adults performing more total trials from session 3 onward. We also found that the rate of trial performance was similar in the early portions of these sessions and an absence of latency differences indicate similar performance once the task was well learned. We did observe persistent differences in task-irrelevant pokes (performed more by adolescents) and premature pokes (performed more by adults). During extinction we found that adolescents tended to perform more perseverative pokes than adults, although the presence and extent of this difference was modulated by the combination of food-restriction and continued cue presentation. Finally, we observed that adolescents were more active in an open field than adults generally, and that this activity was increased by food restriction in the younger animals only.

Adolescents and adults perform different behaviors during the ITI. The increased task-irrelevant pokes by adolescents may be consistent with greater exploration and general activity in adolescents, described by others (Shalaby & Spear, 1980; Spear, 2000) and observed in our open-field experiment. It is notable, however, that we found no significant predictive relationship among individual animals between open-field activity and task-irrelevant poking. As others have interpreted premature pokes in a different task as a measure of impulsivity (Carli, Robbins, Evenden, & Everitt, 1983; Robbins, 2002), we were initially surprised to observe this behavior more in adults than adolescents. One important difference between those studies and this one is that in the present study there was no penalty for premature poking, and so rats had no reason to resist a desire to poke early. Thus, the behavior probably does not reflect poor impulse control in the present task. Both adolescents and adults increased premature poking across training sessions; this suggests that premature pokes do not indicate a weaker cue-action association on the part of adults, since we would expect such an association to improve over time. The observed age-related differences in these behaviors could represent a greater exclusive focus on the task at hand by adults whereas adolescents are more inclined to shift their attention to the task-irrelevant holes during that period. It is noteworthy that once the trial-onset cue was presented, adolescents were at least as quick to respond as adults. Thus, premature poking may indeed reflect the single-mindedness of task-performing rats, with adolescents tending to divert themselves more often (although to a lesser extent over time) to explore task-irrelevant holes, although this propensity does not impair task performance.

During extinction adolescents tended to perform more perseverative (previously reinforced) pokes. Others have demonstrated that adolescents exhibit resistance to extinction of cocaine-seeking behavior in a conditioned place preference paradigm (Brenhouse & Andersen, 2008); we observe this pattern in the context of a natural (food) reinforcer. While this may suggest behavioral inflexibility or cognitive impairment on the part of adolescents, it appears unlikely, as the extent of this age-related difference was modulated by motivational factors (food-restriction status and cue presence). When animals remained food restricted and the cue light continued to be presented (i.e. the same circumstances as training) the difference between adolescents and adults in perseverative pokes was the greatest. If only one of these factors was present the difference was either small (e.g. when animals remained food-restricted but no cue was presented) or absent altogether (when animals had *ad lib* home cage food access but the cue was presented during extinction). When neither factor was present adolescents performed more perseverative pokes than adults, but this was due to a reduction in adult perseveration; adolescents with one motivational factor perseverated to a similar degree as those with neither motivational factor. This pattern of results suggests that adolescents have a higher ceiling for behavioral activity when these motivational factors are present and a higher floor when they are absent. Conversely, adults have a lower ceiling when these factors are present and a lower behavioral floor when they are absent. Similarly, *ad lib* adults performed even fewer task-irrelevant pokes during extinction than their food-restricted counterparts; *ad lib* adolescents exhibited no significant reduction in this behavior. Thus, motivational factors, separately and in combination, differently affect the extinction behavior of adolescents and adults.

We observed greater total open-field grid entries among adolescents, which is consistent with the majority of studies that compare adolescent and adult locomotion and novelty-induced activity (Darmani, Shaddy, & Gerdes, 1996; Spear & Brake, 1983; Stansfield & Kirstein, 2006); however see Philpot, (2008). We also observed greater central grid entries by adolescents regardless of food-restriction status. As central grid entries are thought to be a function of an animal's anxiety state, these results are consistent with adolescents spending more time exploring the open arms of an elevated plus maze (Macrì *et al.*, 2002) [however see Doremus-Fitzwater *et al.* (2009b)], and displaying a greater propensity for either risk-taking behavior (Ernst, Pine, & Hardin, 2006; Steinberg, 2008) or exploration and novelty-seeking (Darmani *et al.*, 1996; Philpot & Wecker, 2008; Spear & Brake, 1983; Stansfield & Kirstein,

2006). Interestingly, food restriction increased total grid entries and central grid entries in adolescents while it had no effect on adult open field activity. Even in this novel environment not previously associated with food reinforcement, food restriction has an activating effect on adolescents. Food restriction is known to increase dopamine receptor signaling and the rewarding and motor activating effects of drugs (Carr, Tsimberg, Berman, & Yamamoto, 2003); it also increases motivation to work for food, which is why food-reinforced instrumental tasks such as ours employ it. It is possible that the novelty of the open field environment maximally activated adults, such that food restriction did not further increase activity. In addition to exhibiting a higher baseline of open-field activity, the increased food-restriction-induced activity of adolescents may be due to a higher potential behavioral ceiling in these younger animals.

We cannot be certain that the level of food restriction and its motivational effects were equal between age groups. This is particularly difficult because adolescents gain a great deal of weight (and must do so to be healthy, even under food restriction) while adults lose weight. Although we cannot equate adolescent and adult food-restriction, our lack of observed latency differences and the similar early trial-performance rate in sessions 3-6 indicate that whatever baseline motivational differences might exist, they were not large enough to cause differences in these behavioral measures once the task was well learned. The exception to this is the within-session drop in trial-performance rate by adolescents, which could reflect earlier satiety by these animals. Such differences, however, do not adequately explain the sustained disparities in task-irrelevant poking and perseveration during extinction. Similarly, behavioral differences in the open field could partially reflect unequal food restriction. If this were to entirely account for the age-related differences we might still expect to observe adult open field activity to be affected to a lesser or greater extent. The lack of any change in adult open field behavior suggests that the age-related behavioral differences are not solely due to differences in the degree of food-restriction severity. Finally, the pattern of perseveration during extinction depended strongly on the combination of both food restriction and cue presence. In fact, those adolescents that were *ad lib* while the cue was presented, were food-restricted with the cue absent, or lacked both the cue and food restriction all exhibited similar levels of perseveration. It was only the combination of food restriction and cue presence that substantially increased adolescent perseveration. Thus, age-related differences in the motivational consequences of food restriction appear insufficient to account for all of these results, although we acknowledge that this is a difficulty in using any task that food restricts adolescents and adults.

While care was taken to expose adolescents and adults to similar conditions prior to and during behavioral testing, there is always the possibility that adolescents could respond differently to housing, shipping, or other conditions differently and in such a way that might affect measures of behavioral performance. For example, adolescents and adults were shipped four days prior to handling. If their transport was stressful, our findings could reflect age-related differential effects of shipping stress on performance. Similarly, food restriction reduces the rate of normal adolescent weight-gain. The weights of our adolescent animals were within the normal to low-normal free-feeding range of adolescent Sprague-Dawley rats at corresponding ages described by McCutcheon and Marinelli (2009). Nevertheless, it is possible that reduced growth rate or various external factors could differently affect adolescent physiology and alter behavior.

The connection between adolescent neurodevelopmental and behavioral changes are of great clinical relevance, especially in light of the associated increased risk taking (Spear, 2000; Steinberg, 2008) and addiction vulnerability of this period (Chambers *et al.*, 2003; Compton, Thomas, Conway, & Colliver, 2005; Khuder, Dayal, & Mutgi, 1999). Significant components of the circuitry that underlies motivated, goal-directed behavior undergo substantial changes during adolescence (Doremus-Fitzwater, Varlinskaya, & Spear, 2009a; Spear, 2000). These regions subserve aspects of instrumental learning and extinction. The dorsal striatum is critical

to the expression of action-outcome associations of the sort formed during instrumental learning (Balleine, Liljeholm, & Ostlund, 2009). The prefrontal cortex (PFC) mediates the initial encoding of action-outcome learning, and plays a central role in cognitive flexibility, such as during extinction (Corbit & Balleine, 2003; Jung, Baeg, Kim, Kim, & Kim, 2008). The amygdala and extended amygdala are thought to provide valence information necessary for learning the initial rewarding or anti-rewarding contingencies of an operant behavior, and for allowing flexibility when contingencies change (Koob, 2009). Finally, the nucleus accumbens (NAc), which receives a convergence of information from the prefrontal cortex (PFC), amygdala, thalamus, and other regions, is critical to incentive-motivated behavior, instrumental learning, and food intake (Kelley, 2004; Mogenson, Jones, & Yim, 1980). These crucial brain regions undergo numerous changes during adolescence. There is increasing dopaminergic innervation of the PFC during adolescence (Benes *et al.*, 2000; Rosenberg & Lewis, 1994). Dopamine D1, D2, and D4 receptor expression peaks during adolescence in the dorsal striatum (Seeman *et al.*, 1987; Tarazi & Baldessarini, 2000; Teicher *et al.*, 1995; Teicher, Krenzel, Thompson, & Andersen, 2003) and PFC (Andersen *et al.*, 2000) before being pruned to lower adult levels. Others have also found a similar pattern for the NAc (Tarazi & Baldessarini, 2000) although this has not always been observed (Teicher *et al.*, 1995). During adolescence there is also increasing glutamatergic connectivity from the basolateral amygdala to the PFC (Cunningham *et al.*, 2002; Cunningham, Bhattacharyya, & Benes, 2008) and from the PFC to the NAc among D1-expressing neurons, before this is reduced in adulthood (Brenhouse, Sonntag, & Andersen, 2008).

Recently, the triadic node hypothesis has been proposed to explain the elevated risk-taking behavior of adolescents in terms of underlying neurodevelopment (Ernst & Fudge, 2009; Ernst *et al.*, 2006). This hypothesis posits that in adolescents, NAc-mediated approach is out of balance with amygdala-mediated avoidance. The third “node” of this model is the PFC, which normally maintains equilibrium between these countervailing forces. This intriguing framework may account for adolescent proclivities to both take risks and behave impulsively. The idea that these circuits associated with reward and motivation are shifted in their sensitivities during adolescence is consistent with age-related regional neural activation differences in anticipation, receipt, and omission of reward observed in fMRI studies (Galvan *et al.*, 2006; Van Leijenhorst *et al.*, 2009) and greater adolescent preferences for natural (Douglas, Varlinskaya, & Spear, 2004; Wilmoth & Spear, 2009) and drug reinforcers (Badanich, Adler, & Kirstein, 2006; Brenhouse & Andersen, 2008; Brenhouse *et al.*, 2008; Philpot, Badanich, & Kirstein, 2003; Shram, Funk, Li, & Le, 2006; Vastola, Douglas, Varlinskaya, & Spear, 2002). Thus both the neural circuitry of motivated behavior and the sensitivity and preference for reinforcing stimuli is altered during adolescence. This framework is consistent with our finding that adolescent perseveration during extinction is differentially affected by the presence of certain motivational factors.

Human adolescence is often considered a period of “storm and stress” because of tendencies toward heightened interpersonal conflict, emotional reactivity, and risk behavior (Arnett, 1999). While the majority of adolescents do not experience psychiatric problems, it is at this time that problems often arise (Pine, 2002; Sisk & Zehr, 2005; Spear, 2000; Volkmar, 1996). The changing cognitive and affective milieu of the developing brain may cause adolescents to process and react to internal and external stimuli differently. This in turn could be relevant to their increased neuropsychiatric vulnerabilities and tendencies toward risk behavior. We observed that while certain measures of cognitive performance were similar among adolescent rats performing an instrumental learning task, persistent differences were observed during training (e.g. task-irrelevant and premature pokes) and extinction, with adolescents exhibiting more perseverative behavior and more sensitivity to the activating effects of internal and external motivational factors. By studying how adolescents respond to such stimuli differently

we may learn more about the unique propensities and neuropsychiatric vulnerabilities of the period.

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Figure 1.

Adolescent and adult trial performance across training sessions. A) Adolescents perform similar total trials during the first two training sessions. From session 3 onward adults perform more total trials than adolescents (* indicates significant difference between adolescents and adults). B) Within-session average cumulative trial performance over time plus and minus standard error (dashed lines). Sessions 1 and 2 indicate similar or slightly faster performance (steeper slope) by adolescents. The rate of performance is nearly equal during the early portion of sessions 3-6. The drop in trial performance rate after about 5-10 min into those sessions by adolescents contributes to their lower total trials, and may reflect earlier satiety.

Figure 2.

Evidence of slightly faster adolescent task acquisition followed by parity of performance. A) The mean latency from trial onset cue to central poke (instrumental response) was shorter in the first training session. During sessions 4-6 this latency became shorter in adults than adolescents. B) Taking only the first 5 minutes of each session, prior to the drop off in within-session cumulative trial performance seen in Figure 1B, we see that adolescent latencies are still shorter in early sessions, but there were no significant latency differences in later sessions. C) There was also no significant age difference in the mean latency from the central poke to the retrieval of the food pellet. (* indicates a significant difference between age groups)

Figure 3.

Adolescents and adults perform different behaviors between trials. A) Adults consistently performed more premature (pre-cue) pokes during the ITI than adolescents. In both age groups the number of premature pokes increased, but this increase was more pronounced in adults than adolescents. B) Adolescents consistently performed more task-irrelevant (left- and right-hole) pokes than adults. (* indicates significant age-related differences)

Figure 4.

Age-related differences in perseverative (previously reinforced) poking behavior were modulated by the presence of motivational factors. A) When animals remained food restricted and the cue light continued to be presented during extinction, adolescents consistently performed more perseverative pokes than adults. B) When the cue light was omitted but animals were still food restricted, adolescents still perseverated more but this difference was smaller than in A. C) When the animals had *ad lib* access to food outside of the task and the cue was still presented there were no perseveration differences between adolescents and adults. D) When animals were neither food restricted nor presented the cue, adults performed fewer perseverative pokes. Adolescents in this condition were more similar in their perseveration to their counterparts exposed to a single motivational factor as in B and C. (* indicates significant age-related differences)

Figure 5.

Interaction plots demonstrating differences between adolescents and adults in their sensitivities to the presence of motivational factors in different combinations collapsed across extinction sessions. A) Among food-restricted animals a significant age $\times$ cue presence interaction was observed. Adolescents perseverated more than food-restricted adults generally, but importantly the presence of the cue had a stronger activating effect on adolescents than adults. B) There was no age $\times$ cue presence interaction in *ad lib* animals. C) A significant age $\times$ food-restriction status interaction was observed for animals presented the cue. Among these animals only the food-restricted adolescents perseverated more than adults. C) When animals were not presented the cue there was no age $\times$ food-restriction status interaction. E) In adolescents, the combination of both motivational factors interacted to further increase in perseveration. A single or no motivational factor led to similar lower levels. B) Unlike adolescents, in adults the combination of motivational factors did not interact synergistically to further increase perseverative pokes. († indicates significant interactions)

Figure 6.

Task-irrelevant poking behavior during extinction. A) Adolescents consistently performed more task-irrelevant pokes during extinction. B) A main effect of food-restriction status was observed, although only *ad lib* adults performed significantly fewer task-irrelevant pokes. ([‡] indicates a significant difference in task-irrelevant pokes as a function of food-restriction status within an age group)

Figure 7.

Adolescents exhibited more open field activity which was modulated by food-restriction status. A) Adolescents performed more total grid entries than adults. This behavior was increased in food-restricted adolescents. There was no significant difference in total grid entries as a function of food-restriction in adult rats. B) Adolescents entered the central grid area more than adults and again food restriction increased this behavior in the younger animals. Adult central-grid entries were unaffected by food-restriction status. (* indicates a significant age-related difference; ‡ indicates a significant difference as a function of food-restriction status within an age group)