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Enhanced performance of aged rats in contingency degradation and instrumental extinction tasks

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Abstract

Normal aging in rats affects behavioral performance on a variety of associative learning tasks under Pavlovian conditions. There is little information, however, on whether aging also impacts performance of instrumental tasks. Young (9–12 mo) and aged (24–27 mo) Fisher 344 rats were trained to press distinct levers associated with either maltodextrin or sucrose. The rats in both age groups increased their lever press frequency at a similar rate, suggesting that the initial acquisition of this instrumental task is not affected by aging. Using a contingency degradation procedure, we then addressed whether aged rats could adapt their behavior to changes in action-outcome contingencies. We found that young and aged rats do adapt, but that a different schedule of reinforcement is necessary to optimize performance in each age group. Finally, we also addressed whether aged rats can extinguish a lever press action as well as young rats, using two forty minute extinction sessions on consecutive days. While extinction profiles were similar in young and aged rats on the first day of training, aged rats were faster to extinguish their lever presses on the second day, in spite of their performance levels being similar at the beginning of the session. Together these data support the finding that acquisition of instrumental lever press behaviors is preserved in aged rats, and suggest that they have a different threshold for switching strategies in response to changes in action-outcome associations. This pattern of result implies that age-related changes in the brain are heterogeneous and widespread across structures.

Keywords

operant learning; effort; reinforcer devaluation; reward; cost

Introduction

While taking a purposeful action in order to reach a goal is a common daily experience, the components that make up such goal-directed behavior can be quite complex. For example, to successfully reach a goal, behaviors require continual updating of the cost/benefit value of

each action and outcome and the flexibility to adapt behavior to changing circumstances. It is known that older adults have difficulty adapting their behavior to situational changes and in making decisions when outcome information is uncertain (Eppinger, Hämmerer, & Li, 2011; Marschner, Mell, Wartenburger, Villringer, Reischies, & Heekeren, 2005; Mohr, Li, & Heekeren, 2010). As with older adults, aged rats also display reduced behavioral flexibility in tasks that require them to adapt, such as in altering behavior in response to changes in action-outcome associations (Bevilaqua, Rossato, Bonini Myskiw, Clarke, Monteiro, Lima, Medina, Cammarota, & Izquierdo, 2008; Mizoguchi, Shoji, Tanaka, & Tabira, 2010; Schoenbaum, Nugent, Saddoris, & Gallagher, 2002), and when outcomes are uncertain, old rats make less advantageous decisions (Simon, Gilbert, Mayse, Bizon, & Setlow, 2009). Most of the studies to date, however, have focused on age-related deficits in associative learning using Pavlovian tasks that pair stimuli with either positive or negative reinforcement. Associative learning, however, also includes procedures that utilize instrumental conditioning. Because this form of learning has been comparatively overlooked in the aging literature, this study was designed to examine how aging impacts associations made between behavioral actions and positive reinforcement.

One benefit of using positive reinforcement procedures is that it allows associative learning to be assessed in a relatively non-stressful environment. Stress is known to produce greater deficits in performance of certain tasks in older compared to younger rats, including tasks such as the elevated plus maze (Pêgo, Morgado, Cerqueira, Almeida, & Sousa, 2006) and extinction from fear conditioning (Bevilaqua et al., 2008; Oliveira-Silva, Pinto, Pereira, Ferraz, Barbosa, Coelho, Gualberto, Souza, Faleiro, Franco, & Ribeiro, 2007). In contrast, when using the nausea inducing compound lithium chloride (LiCl), which is a different type of stressor, older rats show facilitated learning compared to younger rats in the conditioned taste aversion task (Gámiz & Gallo, 2011). Because stress itself can potentially affect the results of a given test, in either a positive or a negative way depending on the stressor, this may have contributed to the variable results obtained from older rats in previous tests. The present experiment was designed to investigate associative learning in aged rats in the absence of the potential confound of stress, thereby enabling us to compare our rats' performance to earlier aging studies using aversive outcomes.

A series of instrumental tasks were performed in this study to better characterize the performance of aged rats in this form of associative learning. Because the current literature is contradictory with respect to whether acquisition of lever press behavior is slower (Port, Murphy, & Magee, 1996; Stephens, Weidmann, Quartermain, & Sarter, 1985) or does not change with age (Gilbert, Mitchell, Simon, Banuelos, Setlow, & Bizon 2012; Simon et al., 2009), the present experiment first sought to examine the behavioral performance of aged rats during initial lever press acquisition, a cognitive domain known to rely on NMDA receptor activation in the prefrontal cortex (PFC) and striatum (Baldwin, Holahan, Sadeghian, & Kelley, 2000; McKee, Kelley, Moser, & Andrzejewski, 2010). Next we examined how well aged rats could flexibly modify their behavior after devaluation of a reward through satiation. This form of learning is known to depend on numerous structures, including the prelimbic cortex (Corbit & Balleine, 2003), nucleus accumbens core (Corbit, Muir, & Balleine, 2001), basolateral complex of the amygdala (BLA; Coutureau, Marchand, & Di Scala, 2009) and dorsal striatum (Yin, Ostlund, Knowlton, & Balleine, 2005). The

purpose of this test was to compare our results with those of a previous study which used LiCl, a nausea-inducing compound, to devalue a reward (Singh, Jones, McDannald, Haney, Cerri, & Schoenbaum, 2011). Aged rats are known to have difficulties reversing a learned action (i.e., Nomura, Izaki, Takita, Tanaka, & Hori, 2004; Stephens et al., 1985), but it is not known whether they are also impaired when an action-outcome association is degraded, rather than fully reversed. Thus a contingency degradation test was also administered to evaluate the ability of aged rats to alter their behavior following this procedure. This task is known to rely on the activity of the prelimbic cortex (Corbit & Balleine, 2003), dorsal striatum (Yin et al., 2005) and BLA (Balleine, Killcross, & Dickinson, 2003). Aged rats are known to be impaired in learning to extinguish freezing behavior following fear conditioning (Bevilaqua et al., 2008), however extinction of an instrumental behavior to rewards has never been fully assessed in aging. Thus the final cognitive domain examined was the ability to extinguish lever press actions once these are no longer rewarded, a form of learning known to depend on the ventral medial prefrontal cortex and its relationship with the nucleus accumbens shell (Ghazizadeh, Ambroggi, Odean, & Fields, 2012). The results of the experiments reported here suggest that the impact of aging on instrumental learning is different than that previously reported using Pavlovian tasks, and also reveal conditions under which aged rats use different strategies to optimize their performance than do their young counterparts.

Method

Subjects

A total of 32 young (9–12 mo) and 41 aged (24–27 mo) male Fischer 344 (the National Institute of Aging colony at Charles River) rats participated in this study. All rats performed the Morris swim task (see below) and were then assigned to one of four groups; an aged rat group ($n = 16$), a young group ($n = 17$) that was tested in parallel with the aged rat group, a separate control young rat group ($n = 12$) and a separate aged control group ($n=9$). An additional 13 aged animals were unable to complete the experiment due to health-related issues, and were not included in any part of the analysis. Also, 3 aged rats showed a strong preference for pressing one of the two levers during contingency degradation training, and for that reason, these animals and their young pairs were eliminated from the study (6 animals total).

Each rat was housed individually within a Plexiglas guinea pig container, in a facility maintained on a reversed light-dark cycle, with food and water available *ad libitum* up until the completion of the spatial memory task. Following testing on the Morris swim task, all rats continued to be housed in the same manner, but were gradually food deprived (over ten days) until they reached 85% of their original body weight. For the duration of the behavioral experiments, the rats were weighed daily and fed accordingly to maintain the reduced body weight. All rats were screened regularly for health issues such as cataracts, nutrition intake, appearance of tumors, and removed from the experiment at the first signs discomfort or distress. All animal procedures were conducted in accordance with the United States National Institutes of Health *Guide for the Care and Use of Laboratory Animals* and approved by the University of Arizona Institutional Animal Care and Use Committee.

Apparatus, operant chamber

Testing was conducted in rectangular stainless steel (15" × 10.5") chambers. Each test chamber was held within a sound-attenuating shell, along with speakers delivering white noise. A 1.5" × 2.5" food cup area equipped with an infrared beam (Med-Associates, St. Albans, VT) to record head entry (i.e., nose poke), was located at the base of one wall of the chamber and was flanked by two retractable levers (Med-Associates). Liquid food rewards were delivered to the food cup area by a metal cannulae and a Tygon tube linked a liquid reservoir to the cannulae. The amount of food reward delivered was controlled by solenoids (Parker Hannifin Corporation, Fairfield, NJ). The solenoids were located outside the sound attenuating chambers and were covered by their own sound attenuating shell, to minimize, if not completely abolish these sounds from reaching the rat. For the behavioral testing the reward delivered was one of two types: 10% sucrose solution (C & H, Domino Foods, Yonkers, NY) or 15% maltodextrin (Carbo Gain) solution. Control of the experimental procedures and data collection was carried out by a computer interfaced with the behavioral testing chambers using Basic X software (NetMedia Inc., Tucson, AZ).

Behavioral training Procedures

Spatial Memory Testing—After arrival, rats were handled for approximately 5–10 minutes per day, for three days or until the animal stopped vocalizing and defecating during the handling process. Following this, rats were trained on two versions of the Morris swim task (Morris, 1984), as a standard test of hippocampal-dependent (spatial) and hippocampal-independent (cue) learning. Procedures for this task have been previously described in detail (Shen & Barnes, 1996). Briefly, over the course of 4 consecutive days all animals in the experiment were given 6 trials of spatial testing. During each trial, animals were released from one of seven different starting locations at the perimeter of a circular water tank. The tank contained an escape platform hidden by water made opaque from nontoxic powdered paint (Sargent Art, Inc., Hazleton, PA). The order of the release locations was pseudo-randomized such that no rat was released from the same starting location on two consecutive trials. Daily sessions consisted of three sets of two successive trials followed by a minimum of 20 minutes rest. During training, rats were held in a warm incubator to maintain body temperature between sessions. Immediately after the training session on day 4, animals received a probe trial, in which the escape platform was removed. Following the probe trial, two days (6 trials per day) of cued visual testing was performed. In these trials the escape platform was above the water and clearly visible and marked. The platform was moved to a different location between each trial.

Each rat's performance on the task was recorded and analyzed offline (ANY-maze, Wood Dale, IL). Since each animal performing the task was released at different locations, each with a different swimming velocity, a corrected integrated path length (CIPL) was calculated to ensure comparability of the rats' performance across release locations (Gallagher, Burwell, & Burchinal, 1993). Any rat that failed to find the platform (during the visual trials) was excluded from the study.

Instrumental training—Figure 1 illustrates the experimental timeline following food deprivation, which was followed by the young and aged rat groups. One day before the start

of the experiments, rats were given food reward samples in their home cage to help reduce effects of food neophobia during training. The training phase of the experiment began with three days of magazine training in the operant chambers (Figure 1), with both levers retracted and food rewards delivered in response to a nose poke into the food cup area. Rewards were delivered at a pseudo-random time interval between 15 and 25s, and the order of reward presentation was randomized. There was one magazine training session per day. For the remainder of the behavioral experiments, rats were tested twice daily unless otherwise noted. There was at least one hour interval between sessions, during which rats were returned to their home cage.

After this initial training, rats' behavior was shaped by the experimenter on a single lever at a time, until the animal could press independently on each lever for 50 consecutive times (Figure 1). The shaping procedure involved placing food reward onto the lever itself to encourage the rat to press. On average, each rat was given two 20 minute sessions a day until the criterion was successfully met. During training, rats gradually came to associate each lever with a specific type of food reward, either sucrose or maltodextrin solution. The pairing between each lever and food reward was counterbalanced between rats.

Over the course of twelve days (Lever training; Figure 1), rats pressed levers at a pre-defined probability schedule with appropriate outcomes (sucrose or maltodextrin) being delivered accordingly. The reward schedule employed here approximated a random ratio (RR) reinforcement schedule, gradually increasing from 1 to 5. Specifically, days 1–3 were performed at RR1, days 4–6 at RR2, days 7–8 at RR3, days 9–10 at RR4 and days 11–12 at RR5. Each training session lasted until the rat reached 70 rewards or 40 minutes. Because aged rats took 30 minutes and young 27 minutes on average to press for 70 rewards on the last day of training, we used a cut-off of 40 minutes to ensure that most rats obtained 70 rewards in all sessions.

Outcome devaluation and reward discrimination—Outcome devaluation testing began the day following instrumental training (Figure 1). The procedure consisted of pre-feeding rats with one of two outcome rewards (either sucrose or maltodextrin solution) in their home cage for 45 minutes. Allocation of outcomes was counterbalanced across rat groups for both devalued action (left or right lever) and outcome (sucrose or maltodextrin). Immediately after satiation treatment, rats were brought to their operant chambers for a 5 minute lever preference test. In this test, both levers were present and rats were free to press either lever. The test was performed in two different conditions, one where no reward was delivered (unrewarded extinction test) and one where reward was delivered as a result of pressing either lever (rewarded control test). Because a reward was delivered for every lever press in the rewarded control test, the amount of food reward was decreased from 0.2 to 0.1 mL to minimize the potential effect of satiation during test. Each test type was performed twice; one per reward satiation session. Tests were separated by one retraining day (day 24, 26, 28), during which rats performed two training sessions (one per lever) at RR5.

As an additional control, a group of 9 aged rats was separately tested to assess whether old rats can discriminate between sucrose and maltodextrin. After testing on the Morris swim task, these animals were food deprived to 85% of their original body weight. Rats were then

introduced to each food reward consecutively in their home cage for one hour each, for 4 days, alternating the order of presentation every day. The food rewards were offered in gravity fed water bottles. On day 5 and 6, rats were satiated on one of the food rewards for 45 minutes, and this was immediately followed by a 5 minute presentation of both food rewards. The amount of reward consumed was measured using a graduated cylinder.

Contingency degradation—Following reinforcer devaluation, rats received one day of retraining at RR5 (day 30), for each lever-reward pair (2 sessions). Subsequently, rats were trained for four days on a contingency degradation task (Figure 1). In this task, the contingency between a lever and its associated reward is weakened by also delivering that reward independently of lever presses during that session. For the other session, the lever-reward association remained intact and rewards were delivered at RR5. This procedure effectively degrades the association between rewards and actions (Balleine et al., 2003). Rats underwent 2 training sessions per day, one for each lever-outcome association, which lasted until rats obtained 70 rewards or until 40 minutes had passed. Sessions were separated by about 2 hours and the session order (degraded, regular) was alternated daily. One of these sessions (regular session) was identical to the last training session, using a RR5 schedule of reinforcement. In the other session (degraded session), rats obtained a reward at RR5 for each lever press, but also received noncontingent rewards. These noncontingent rewards were given at a probability of $p = 0.05$ after every second that the rat did not lever press. On the fifth day, responses to both levers were examined using a 5 minute extinction test.

Because our results on this task showed that only aged rats adapted their behavior to the degradation in a lever press – reward association, an additional control group of young rats ($n=12$) was included in this study. Specifically, these rats were trained to a RR20 schedule of reinforcement, to assess whether this schedule would restore behavioral performance to levels consistent with the literature in young rats. These rats first performed contingency degradation training with lever presses reinforced using a RR20 for contingent rewards and a probability of $p=0.05$ for noncontingent rewards. Unexpectedly, they showed poor lever press performance during training at RR20 and during the contingency degradation task. Thus they were retrained for 3 days at RR8.3 ($p = 0.12$) schedule, to increase the probability of reward delivery. This reinforcement schedule effectively re-established lever press performance, and contingency degradation was tested a second time using RR8.3 for contingent rewards and a probability of $p = 0.05$ for noncontingent rewards (see Table I). The data presented in the results section is from the second training session.

Extinction—Following contingency degradation, 13 young and 16 old rats were re-trained for one day on the lever-reward pair that was not degraded in the contingency degradation test (day 37; Figure 1). Then, on the next two days, rats were tested for their ability to extinguish lever pressing using 2 forty minute sessions (1 per day) where the appropriate lever was presented, but no reward outcome was delivered.

Data Analysis

The raw data were exported from Basic X software and analyzed using MATLAB (Natick, MA). Statistical tests were performed using R (R Development Core Team, 2011). Mixed-

effects models were used to analyze the effect of age, days (or time) or interaction on behavioral performance. Mixed-effects models extend the standard linear framework by adding a random slope and a random intercept for each rat. We used the lme4 package to perform our mixed-effect model analysis in R (Bates, Maechler, & Bolker, 2011). Our starting model for most analysis was the following:

$$Y_{it} = \alpha + \beta_1 \text{Age}_{it} + \beta_2 \text{Day}_{it} + a_i + b_i \text{Day}_{it} + \varepsilon_{it}$$

In this model, Y_{it} represents the dependent variable; CIPL for watermaze data, frequency of lever presses or nose pokes during initial and contingency degradation training, nose poke durations during initial training and lever press counts during extinction learning, for animal i at time (or day) t . α represents the average dependent variable start value (baseline performance level). $\beta_1 \text{Age}_{it}$ and $\beta_2 \text{Day}_{it}$ represent the average age and day effect on the slope of the model. a_i and $b_i \text{Day}_{it}$ represent the random variation in the intercept and slope of the model, thus taking into account the rat differences in baseline performance and learning curves. ε_{it} represent the residuals. All random variables were assumed to have a zero mean and a Gaussian distribution. The model used to fit the results from the extinction experiment also included a Day^2 term due to the quadratic nature of the learning curve in young and aged rats. Only the first ten minutes were included in the statistical analysis of the extinction data, because there are very few lever presses beyond that time point.

The contribution of the parameters age, day and interaction were assessed using the maximum likelihood method. This technique creates a likelihood ratio, known to approximate a Chi-square distribution that can be used to assess if a first model is significantly different from a second model that does not contain one of the parameters. This allows one to assess whether a given parameter is an essential contributor to a particular model. In this manuscript, the t and p values reported are taken from the simplest model, and the reported p values are conservative as they were estimated from the parameter's t values and the model's degrees of freedom. In mixed-effects models, the p value represents an upper bound approximation (Pinheiro & Bates, 2000). The likelihood ratio and its associated p value will be reported in situations where the p value of a parameter did not reach statistical significance on its own, but was a significant contributor to the model.

Normality of mixed-effect models was assessed using scatter and quantile-quantile plots of the models' residuals as compared to the fitted values. For the correlation analysis, a Pearson's product-moment correlation test was used. Where mixed-effect models were not appropriate, paired t -test, Wilcoxon Mann Whitney rank sum test and mixed effects ANOVAs (with lever as the within-subject factor and age as the between-subject factor) were performed and normality was assessed using a Shapiro Wilk test.

Because there can be numerous rewards delivered between each lever press during the degraded sessions of contingency degradation training, the calculation of the rat's latency to move from the food cup area to the lever was measured as the time between the first reward within a series of rewards and the following lever press. This latency measure is the one reported here for the degraded sessions of the contingency degradation test, because it

captures most effectively the aged rat's tendency to linger at the reward zone during this test.

Results

Morris swim task

In order to establish the baseline learning and memory performance of our aged rat cohort, all animals underwent training in two versions of the Morris swim task. Briefly, over the course of four days, rats learned to find a hidden submerged platform (spatial trials, 6 trials/day). Figure 2 shows the performance levels of both age groups on this task. We found a significant effect of day, suggesting that rats improved their performance over sessions ($t = 5.491$, $p < 0.01$). There was also a significant interaction effect (interaction effect; $t = 2.1$, $p < 0.05$), suggesting that young rats learned to find the platform at a faster rate than did aged rats. Finally, we found a significant age difference in CIPL values on day 4 (rank sum test, $p < 0.01$), thus supporting our conclusion that our aged rat group displayed impaired learning on the spatial trials of the Morris swim task.

In order to assess whether any rats exhibited visual impairments, rats underwent 12 trials in which the platform was above water, thus visible to the rat (visible trials, 6 trials/day). We found that all rats could find the visible platform, and even improved their performance as compared to the last day of the hidden platform trials. Indeed, we found that young and aged rats had significantly lower CIPL scores on the visible trials, as compared to the hidden platform trials of day 4 ($t = 6.865$, $p < 0.01$). Across the two days of trials with a visible platform, all rats were faster at finding the platform on the second day ($t = 3.489$, $p < 0.01$), with a trend for young rats to have lower CIPL values (age effect: likelihood ratio $\chi^2 = 3.7595$, $df=1$, $p = 0.053$; interaction effect: likelihood ratio $\chi^2 = 0.62$, $df=1$, $p > 0.05$). This trend is in part carried by aged rats that were slower to find the visible platform; however, all rats were able to find it at least 4 times on the second day of visible trials, thus confirming that they were able to see the platform.

Instrumental acquisition training

We were first interested in addressing whether aged rats could achieve lever press performance similar to that of their young counterparts. In the present study, two levers were used, each lever press potentially triggering the release of a specific reward (sucrose or maltodextrin). The lever-reward association was counterbalanced for each rat. Once rats could press independently on each of the two levers, the probability of reinforcement was gradually decreased from an RR1 to an RR5 schedule. This led to an increase in the frequency of lever presses (Figure 3A). During this training, the average frequency of lever presses and nose pokes of young and aged rats was similar (Figure 3A, B), with a significant effect of day (lever press $t = 9.5$, $p < 0.01$; nose pokes $t = 4.8$, $p < 0.01$), but no effect of age (lever press, likelihood ratio $\chi^2 = 2.35$, $df=1$, $p = 0.13$; nose pokes, likelihood ratio $\chi^2 = 0.6$, $df=1$, $p = 0.4$) nor a significant interaction (lever press, likelihood ratio $\chi^2 = 0.16$, $df=1$, $p = 0.7$; nose pokes, likelihood ratio $\chi^2 = 2.4$, $df=1$, $p = 0.1$). However, aged rats spent proportionally more time in the food cup area, as calculated by the proportion of time the infrared beam was interrupted in the area of the food reward, between the two levers (Figure

3C; age $t = 2.2$, $p < 0.05$; day $t = 3.01$, $p < 0.01$; interaction, likelihood ratio $\chi^2 = 1.93$, $df = 1$, $p = 0.17$). These results suggest that aged rats can learn to press levers as well as young rats, at least for the reinforcement schedule employed here (one reward for every five lever presses, RR5). These results also suggest that aged rats prefer to keep their head in the nose poke area, where rewards are delivered, as opposed to young rats who spent more time elsewhere in the chamber.

Outcome devaluation and reward discrimination tests

To assess whether the behavior of aged rats is sensitive to changes in the incentive value of the reward, we performed a reinforcer devaluation test. Specifically, rats were presented with one of the two rewards for 45 minutes in their home cage, followed immediately by transport to the operant chamber where both levers were extended and rats could press on either of them for 5 minutes. During this test, no rewards were delivered. The number of presses on each lever was recorded and normalized to the average lever press frequency from the last two days of training. This test was performed twice (once per food reward), separated by a day of retraining on both levers. This test was performed first in this experiment because the effects of selective satiation are short lasting, and would unlikely affect the rat's performance on subsequent tests.

We found that selective satiation did not influence lever choice under our experimental conditions in F344 rats (Figure 4, left panel: lever, $F_{(1,31)} = 3.4$, $p > 0.05$), with no age or interaction effects ($F < 1$). This result suggests that our rats may not have formed a full representation of each lever-reward association. To further confirm this interpretation, we performed the outcome devaluation task a second time, and removed the necessity to remember the lever-reward association by rewarding all lever presses. Under this "reward all lever press" situation, the rats did show an effect of selective satiation on lever choice (Figure 4, right panel: $F_{(1,31)} = 7.4$, $p < 0.05$), but no age ($F < 1$) or interaction effects ($F_{(1,31)} = 1.16$, $p > 0.05$). Because of the significant effect of selective satiation on lever choice in this control test, these results support the initial finding that with the procedure used here, neither young nor aged rats fully associated each lever with its food reward.

While these satiation data suggest that both young and old rats can discriminate between sucrose and maltodextrin, we further examined reward discrimination directly in aged rats. To this end, we tested a separate group of aged rats on a reward discrimination task, in which rats were satiated in their home cage for forty five minutes and immediately afterwards, were presented with both food rewards simultaneously in their home cage for five minutes. This test was performed twice, once per food reward type. We found that the present group of aged rats had a strong preference for sucrose as they drank twice as much sucrose after being satiated on either sucrose or maltodextrin (interaction effect $F_{(1,32)} = 9.0$, $p < 0.05$). Specifically, aged rats drank 2.2 ± 0.4 mL versus 1.9 ± 0.5 mL sucrose as compared to 1.0 ± 0.4 mL versus 1.2 ± 0.5 mL maltodextrin after satiation on sucrose and maltodextrin respectively. This is unlike the first batch of aged rats that performed the reinforcer devaluation task, who did not show an interaction effect between the choice of lever and the food that rats were satiated on ($F < 1$). This suggests that there can be strong inter-individual differences in groups of rats from the same breeder. This raises the important point that for

any given experiment, preference tests should be conducted and stimuli matched as much as possible to preference.

Contingency degradation

The performance of both age groups in response to selective degradation of one of the lever-reward associations was then examined. There were two phases to this task; a four day training phase followed by a test session on day five. There were two sessions per day in the training phase of this task, with each session type systematically alternated between days: the degraded session in which the value of one of the lever press-reward contingencies was degraded, and the regular session in which the lever press-reward association was maintained. This four day training phase has been used in previous studies (e.g., Corbit, Ostlund, & Balleine, 2002) and offers rats sufficient exposure to the new task condition so that they can adapt their behavior. There was a significant difference in the average frequency of lever presses during the regular versus the degraded sessions, within individual rat groups (Figure 5A1 old $t = 8.427$, $p < 0.01$; Figure 5B1 young $t = 5.674$, $p < 0.01$). However, there was no age difference in the frequency of lever presses for either session type (regular sessions, likelihood ratio $\chi^2 = 0.189$, $df=1$, $p>0.05$; degraded sessions, likelihood ratio $\chi^2 = 2.41$, $df=1$, $p>0.05$), with young and aged rats pressing at a similar frequency during regular sessions (old 14.6 ± 1.1 , young 14.9 ± 1.1 press/min, t-test $p>0.05$), and devalued sessions (old 9.3 ± 1.04 , young 11.9 ± 1.3 press/min, t-test $p>0.05$). However on day 5, in a five minute test session during which no reward was given, only aged rats showed significant discrimination between the degraded lever-reward pair and the non-degraded one (Figure 5A2 and B2; interaction $F_{(1,31)} = 4.4$, $p<0.05$; lever $F_{(1,31)} = 1.4$, $p>0.05$; age $F<1$). Thus, under this protocol, aged but not young rats adapted their behavior to the change in action-outcome contingencies.

To account for the possibility that the reinforcement schedule used here may have impacted young rats' ability to perform at levels previously reported in the literature (Braun & Hauber, 2012; Corbit & Balleine, 2003), a separate group of young rats was trained to an RR8.3 schedule of reinforcement ($p = 0.12$). This protocol is closer to the ones used for young rats in other studies, and forces rats to press more times to get rewards, thus increasing the effort cost. The delivery of noncontingent rewards was kept at a probability of $p=0.05$ for each second without a lever press. Using this protocol, young rats displayed a reduced lever press frequency to the degraded lever-reward pair, during contingency degradation training (Figure 5C1, lever choice $t = 21.11$, $p < 0.01$), and preferentially pressed on the non-degraded lever during the post-training test (Figure 5C2, paired t-test, $p < 0.05$). Taken together, these results confirm that young F344 rats can adapt their behavior during contingency degradation procedure, however they do so only when a different schedule of reinforcement is used. These results suggest the possibility that young and aged rats perceive lever press-reward associations differently, possibly because aged rats find lever presses more effortful. Thus using an RR5 schedule may have promoted the use of different behavioral strategies: young rats employed the active approach of pressing levers to obtain contingent rewards and aged rats the passive approach of waiting for noncontingent rewards. In contrast, young rats used the passive approach of waiting when under the more effortful RR8.3 schedule, as did older rats at RR5. This difference in strategy

also raises the possibility that there is an age effect on the choice of behavior in situations of response competition; namely when two or more responses can lead to reward.

Because it is possible that differences in motor speed between age groups may have contributed to the behavioral results reported above, we measured the rat's latency to move from the food cup area to the lever during contingency degradation training. Aged rats were slower to move away from the food cup area to the area of the levers after receiving a reward (10.3 ± 1.4 sec for old rats) than were young rats (7.3 ± 0.4 sec, t -test $p < 0.05$). For degraded sessions these measures were 17.6 sec and 11.7 sec, respectively for old and young rats (t -test $p < 0.05$). Similarly, we also found that aged rats spent a greater proportion of time in the food cup area during the regular ($t = 2.386$, $p < 0.05$) and the degraded sessions of contingency degradation training (likelihood ratio $\chi^2 = 11.089$, $df=1$, $p < 0.05$). Thus it is possible that the tendency of old rats to spend more time in the nose poke area facilitated learning that rewards were delivered independently of lever presses during degraded sessions, under the RR5 schedule of reinforcement.

Extinction

After being tested on the contingency degradation task, rats received one retraining session on the lever that was not previously degraded and extinction on that lever began the next day. Two extinction sessions lasting forty minutes each were performed on consecutive days, in which pressing the available lever did not lead to reward (Figure 6). We found no age differences on the first day of this task, suggesting that aged rats can learn to extinguish a lever press behavior previously associated with a food reward, at the same rate as do young rats (likelihood ratio $\chi^2 = 0.189$, $df=1$, $p > 0.05$). There was a strong effect of time as measured by the decrease in number of lever presses per minute ($t = 3.914$, $p < 0.001$ and $t = 2.72$, $p < 0.01$ for min and min² respectively). This suggests that aged rats can extinguish an instrumental action at the same rate as young rats, when previously paired with appetitive outcomes.

On day 2, all rats significantly decreased their lever press frequency over time ($t = 5.1$, $p < 0.001$), during the first 10 minutes of the session. However, there was a significant interaction effect suggesting that aged rats were faster to extinguish than were young rats on this second day ($t = 2.643$, $p < 0.01$). Indeed, young rats pressed on average 18 ± 2 times, compared to 11 ± 2 times for aged rats, during the first 5 minutes of the second extinction session. This result suggests that, consistent with the contingency degradation findings, aged rats can perform better on some aspects of instrumental tasks.

The above results suggest the hypothesis that aged rats may be more prone than young rats to refrain from lever pressing when it is not necessary or beneficial. This predicts that the behavioral performance of an aged rat during extinction would correlate with that rat's results during contingency degradation. Accordingly, we compared the rat's behavioral performance, as measured by the frequency of lever presses during the extinction task, with the frequency of lever presses during contingency degradation training. In fact, there was a positive correlation between lever press performance on the second day of extinction and performance on the last day of contingency degradation training (Figure 7; Pearson's $r = 0.45$, $p < 0.05$). This correlation was also significant when restricting the analysis to only

aged rats (Pearson's $r = 0.56$, $p < 0.05$). The time period used for this correlation was the first 5 min of extinction and the first 12 min of the fourth day of contingency degradation training because these were the periods when all rats were actively pressing on levers. Because three of the aged rats pressed very little on both of these measures, their performance levels on other task days were examined to confirm that their overall behavior was similar to the other aged rats. These three rats were found to perform within one standard deviation of the aged group mean for the first day of extinction and within two standard deviations for the regular session of contingency degradation training, thus there are no valid reasons to exclude them. Overall, these results suggest that the rats that pressed less frequently on the last day of contingency degradation training were also the ones that showed reduced lever presses on the second day of extinction. This further supports the hypothesis that aged rats have a greater tendency to produce behaviors that are consistent with a strategy that conserves effort.

Discussion

The primary novel findings of this study include the observations that aged rats are unimpaired in the initial acquisition and extinction of lever press actions for food rewards. Additionally, with the procedures used here, aged rats showed greater behavioral adaptability to changes in action-outcome contingencies and were faster to extinguish on the second extinction session. As will be discussed further below, the older rats appear to use behavioral strategies that minimize effort.

The acquisition of instrumental behaviors is preserved in aged rats

In line with a previous study, we did not find impairments in the initial acquisition of lever presses in aged rats (Nomura et al., 2004). With the schedule of reinforcement used here, aged rats pressed on average, at similar frequencies as did young rats. Thus the age-related decline in locomotor abilities that was reported in previous studies (Frutos, Pistell, Ingram, & Berthoud, 2012; Hebert & Gerhardt, 1998; Horner, Russ, & Biknevicius, 2011; Insel, Patron, Hoang, Nematollahi, Schimanski, Lipa, & Barnes, 2012) may not apply to instrumental tasks where the required responses are lever presses. However, we did find that aged rats were slower to move away from the nose poke area to a lever during the contingency degradation experiment. Thus the effects of aging on lever press performance are subtle and do not affect lever press acquisition.

Outcome devaluation using selective satiation in F344 rats

Results from the outcome devaluation task suggest that young and aged rats performed similarly following selective satiation. The results do suggest, however, that F344 rats in both age groups may not have formed a strong association between the lever-reward pairs. This pattern of results could also result from over-training. Based on previous literature, however, it is unlikely that the rats were over-trained because of the length of the training period, the schedule of reinforcement used (i.e. (Yin, Knowlton, & Balleine, 2004), and the fact that the aged rats tested here were sensitive to contingency degradation while over-trained rats are not (see Holmes, Marchand, & Coutureau, 2010). This leaves open the possibility that rats were, in fact, under-trained prior to being tested.

On the surface, the present results may seem in conflict with those of Singh and colleagues' (2011), who found a significant effect of LiCl injection on the behavioral performance of young and old rats in their outcome devaluation task. There are numerous differences between the present study and the one of Singh and colleagues (2011); they used a Pavlovian task, devaluation was achieved using an aversive solution and Long Evans rats were used. Any of these variables may have contributed to the differences found between our studies.

Changes in cost/benefit ratio promotes different strategies in young and aged animals

With aging, there is a loss of flexibility, such that learned behaviors may be more difficult to modify (Bizon, Foster, Alexander, & Glisky, 2012). Reversal learning, known to be impaired in aging, is an example of a behavior that requires flexibility in order to adapt the behavior to the change in stimulus-outcome or action-outcome associations (Nomura et al., 2004; Schoenbaum et al., 2002). Because of these results, our finding that aged rats are more sensitive to changes in action-outcome contingencies and as good at extinguishing their behavior is surprising. In fact, the results reported here suggest that aged rats can adapt their behavior as well as young rats or even outperform young rats in some situations. One possible explanation for this is that the perception of effort and outcome valuation may be different in aged animals leading to an altered cost/benefit ratio. The idea that aged rats may learn faster to refrain from performing an action when it is not needed to obtain a reward is supported by the observation of the faster extinction on day 2 in older animals. While it is possible that effort contributes to strategy selection under some conditions in aged rats, the fact that young and aged rats pressed at a similar frequency under RR5 does not support this hypothesis. Instead, the age effect found in the contingency degradation and extinction tasks may be the result of an age difference in how rats value changes in action-outcome contingencies, rather than how they value effort cost.

On top of the possibility of an altered cost/benefit ratio in old rats, they may also choose a different behavioral strategy when more than one response can be performed to obtain a reward. Specifically, the delivery of both contingent and non-contingent rewards in the contingency degradation task may have induced a response competition between pressing and staying by the reward zone (for a reviewed on response competition: Josephine E Haddon & Killcross, 2007). In support of this hypothesis, aged rats did spend more time at the reward zone than did young rats in the present experiment. Thus, in the presence of two competing responses aged rats appear to employ a different strategy than do young rats, potentially to minimize effort cost.

With aging, brain systems involved in cost/benefit decision making and behavioral strategy selection may be altered in a way that promotes the behavioral pattern reported here. For example, lesions of the orbitofrontal cortex are shown to be either beneficial or detrimental to performance on radial arm maze tasks, depending on how the contingencies are changed (Riceberg & Shapiro, 2012). The detection of response conflict, on the other hand, is a function primarily attributed to the anterior cingulate cortex in rodents, with the contextual control of response competition mediated by dopamine receptor activation in the prefrontal cortex (J E Haddon & Killcross, 2005; Josephine E Haddon & Killcross, 2011; Marquis,

Killcross, & Haddon, 2007). Aging is associated with lower dopamine transmission in areas that are involved in goal-directed behaviors, particularly the nucleus accumbens (Cruz-Muros, Afonso-Oramas, Abreu, Barroso-Chinea, Rodríguez, González, & González Hernández, 2007), but also in regions of the PFC (Mizoguchi, Shoji, Tanaka, Maruyama, & Tabira, 2009; Mizoguchi et al., 2010) and amygdala of rats (Míguez, Aldegunde, Paz-Valiñas, Recio, & Sánchez-Barceló, 1999; Nomura et al., 2004). Thus, age-related changes in dopamine transmission in select brain regions mediating goal-directed behaviors may be responsible for the aged rat's enhanced tendency to switch strategy or response choice as compared to young rats.

Dissociation of appetitive instrumental and noxious Pavlovian extinction tasks in aged rats

A number of studies suggest that aged rats are slower to extinguish learned responses (Bevilaqua et al., 2008; Oliveira-Silva et al., 2007; Schneider-Rivas, Paredes-Carbajal, Mascher, Angoa-Pérez, Jaramillo-González, Borgonio-Pérez, & Rivas-Arancibia, 2007; Schneider-Rivas, Rivas-Arancibia, Vazquez-Pereyra, Vázquez-Sandoval, & Borgonio-Pérez, 1995; Topic, Dere, Schulz, de Souza Silva, Jocham, Kart, & Huston 2005; Topic, Huston, Namestkova, Zhu, Mohammed, & Schulz, 2008). However in the current study, we found that aged rats were unimpaired at extinguishing their behavior in the initial extinction session and were even faster to extinguish during the subsequent session. Due to the differences in task design, there may be a number of explanations for these apparent discrepancies.

Aging may affect networks that are involved in Pavlovian conditioning differently from ones involved in instrumental conditioning. Indeed, the extinction studies that demonstrate age-related deficits were performed using Pavlovian conditioning. Not all brain structures or neuromodulators are involved in both Pavlovian and instrumental conditioning. For instance, neurons of the orbitofrontal cortex are required for Pavlovian, but not instrumental conditioning (Ostlund & Balleine, 2007). Also, blocking dopamine transmission can affect behavior using Pavlovian, but does not affect behavior on instrumental tasks (Lex & Hauber, 2010; Wassum, Ostlund, Balleine, & Maidment, 2011). Because dopamine transmission declines with aging, (Eppinger et al., 2011; Marschner et al., 2005; Mohr et al., 2010; Mora, Segovia, & del Arco, 2008), and because aging affects brain structures heterogeneously (Dickstein, Weaver, Luebke, & Hof, 2012; Morrison & Baxter, 2012), behavioral performance on Pavlovian tasks may be selectively compromised.

Additionally, aging may affect tasks using positive and negative reinforcers differentially. Whereas the same brain networks are thought to support appetitive and aversive extinction (Quirk & Mueller, 2008), behavior on these tasks can be selectively manipulated using molecular techniques. For instance, alterations of cannabinoid receptor 1 transmission selectively affects aversive, but not appetitive extinction learning (Harloe, Thorpe, & Lichtman, 2008). Thus aging may affect a brain system that is selectively involved in extinction learning of negative outcomes. Another possibility is that aversive extinction learning is not impaired in aged rats, but is compromised by stress and anxiety in negatively reinforced tasks. For example, it is suggested that aging is associated with a hyperactivity of

the hypothalamo-pituitary-adrenal (HPA) axis (e.g., Sapolsky, 1992; Segar, Kasckow, Welge, & Herman, 2009), with higher basal corticosterone levels, decrease in BDNF protein, BDNF mRNA, and TrkB mRNA in the hippocampus and amygdala (Goosens & Sapolsky, 2007; Sapolsky, Krey, & McEwen, 1983; Sapolsky, 1992; Shi, Shao, Yuan, Pan, & Li, 2010) and with a decrease in catecholamine release following stress (Cizza, Gold, & Chrousos, 1995; Segovia, del Arco, & Mora, 2009). Because stress is known to affect extinction learning, making rats slower to extinguish behaviors (Goswami, Cascardi, Rodríguez-Sierra, Duvarci, & Paré, 2010; Long & Fanselow, 2012), it is possible that these age-related neurochemical deregulations are responsible for the age impairments previously reported. The lack of a deficit in extinction in the present study may indicate that extinction is not impaired with aging *per se*, but is more susceptible to the effects of stress in aged rats.

In conclusion, our results suggest that under the right conditions, aged rats can learn and adapt instrumental behaviors as well as, or in some cases faster than, young rats. This is of interest because it favors the idea that aging does not affect brain systems homogeneously, and that some of these systems may be spared. Alternatively, instrumental behaviors that are relatively preserved may arise due to compensatory processes or altered behavioral strategies that allow rats to acquire and maintain goal-directed behaviors. These results contrast with the age-impairments found in tasks such as reversal learning and decision making under uncertainty, and suggests the possibility of altered cost/benefit ratios in aged rats. Our findings also suggest that aged rats can effectively employ different strategies to solve a task. Finally, aging may have a greater impact on structures involved in Pavlovian conditioning and stress regulation, thus accounting for the impairments previously reported in the rodent associative learning literature.

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Figure 1.

Experimental timeline. This experimental schedule began after all rats performed the Morris swim task and underwent 10 days of gradual food restriction. Note that days 24, 26, 28, 30 and 36 are retraining sessions at RR5.

Figure 2.

Morris swim task performance in young and aged rats. Corrected integrated path length (CIPL) score (X-axis) as a function of days of testing (Y-axis) indicates that young rats (solid lines) learned faster than old rats (dashed lines) to find a submerged platform, over a four day training period (spatial task). Once the water level was lowered and the platform made visible, young and aged rats (grey lines) were tested for two additional days and results show that path length to the visible platform was reduced over days, with no significant age difference in performance. Error bars represent ± 1 standard error of the mean.

Figure 3.

Behavioral performance during instrumental training. Over the course of 12 days (X-axis), young (solid lines) and aged (dashed lines) rats were trained to press levers for food rewards. As training went on, the schedule of reinforcement was modified such that increasing numbers of lever presses were required to obtain rewards. As a consequence of this change in reinforcement schedule, the lever press (A) and nose poke frequencies (B, Y-axis) increased with training, and the rate of change was similar for young and aged rats.

The proportion of time spent in the food cup area (C) did not change across training days, but was greater across days for aged rats. Error bars represent ± 1 standard error of the mean.

Figure 4.

Behavioral performance following outcome devaluation. Mean normalized lever presses during a 5 minute extinction test (Left) and a 5 minute control rewarded test (Right) following selective satiation of a reward in young and aged F344 rats. Results from these tests reveal an overall effect of satiation on lever choice in the control rewarded test only. Open bars represent the devalued food and the grey bar is the non-devalued (regular) food. Error bars represent ± 1 standard error of the mean.

Figure 5.

Instrumental performance during contingency degradation training and subsequent extinction testing. Over the course of 4 days (X-axis), aged (RR 5, A1), young (RR 5, B1) and young (RR 8.3, C1) rats underwent contingency degradation training, in which the contingency between a lever and reward pair was degraded by introducing rewards that were delivered independent of lever presses. The mean lever press frequency (Y-axis) for the contingent (solid lines, A1, B1, C1) and noncontingent lever-reward associations (dashed lines, A1, B1, C1) suggests that all rats press less on the lever during the degraded sessions (noncontingent). On day 5, a five min extinction test was performed, with both levers present (A2, B2, C2). The results reveal that only the aged and high-effort young rat groups modified their behavior to the degradation of one of the lever-reward pair and consequently, pressed less on that lever during the post test. Solid bars represent the regular lever press-reward association, and the open bars the degraded association. Error bars represent ± 1 standard error of the mean.

Figure 6.

Lever press performance during extinction. Average number of lever presses per minute (Y-axis) in young (solid line) and old (dashed line) rats, during two 40 minute sessions (Y-axis) in which no rewards were delivered. This test was performed on two consecutive days; day 1 (left) and 2 (right), and revealed that there was no difference in the rate that young and aged rats extinguished on day 1, but that aged rats were faster to extinguish on day 2. Error bars represent ± 1 standard error of the mean.

Figure 7.

Correlation between rate of lever pressing performance during extinction and contingency degradation training. The average lever press frequency (per min) during the second day of the extinction test (y axis) is significantly correlated with the average lever press frequency (per min) during the fourth day contingency degradation training (x axis), in young (open circles) and aged (closed circles) rats.

Table 1

Schedule of reinforcement of the contingent and noncontingent lever press – reward associations, for the young, old and control rat groups

Group	Contingent rewards Lever press	Noncontingent rewards	
		Lever press	Absence of lever press (per second)
Old & young	RR5	RR5	p = 0.05
Young (high-effort)	RR8.3	RR8.3	p = 0.05