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Distinct manifestations of executive dysfunction in aged rats

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ABSTRACT

Different components of executive function such as working memory, attention, and cognitive flexibility can be dissociated behaviorally and mechanistically; however, the within-subject influences of normal aging on different aspects of executive function remain ill-defined. To better define these relationships, young adult and aged male F344 rats were cross-characterized on an attentional set-shifting task that assesses cognitive flexibility and a delayed response task that assesses working memory. Across tasks, aged rats were impaired relative to young; however, there was significant variability in individual performance within the aged cohort. Notably, performance on the set-shifting task and performance at long delays on the delayed response task were inversely related among aged rats. Additional experiments showed no relationship between aged rats' performance on the set-shifting task and performance on a hippocampal-dependent spatial reference memory task. These data indicate that normal aging can produce distinct manifestations of executive dysfunction, and support the need to better understand the unique mechanisms contributing to different forms of prefrontal cortical-supported executive decline across the lifespan.

1. Introduction

Across species, aging is accompanied by a decline in neurocognitive functions, including learning and memory mediated by medial temporal lobe structures and executive functions mediated by prefrontal cortex (PFC; Alexander et al., 2012; Bizon et al., 2012; Buckner, 2004). Research in animal models has made considerable strides in understanding the neural basis of age-related decline in learning and memory (Burke et al., 2012; Engle and Barnes, 2012; Foster et al., 2012); however, there has been less progress in understanding the neural mechanisms that contribute to impaired executive functioning across the lifespan. This relative paucity of data stems in part from the complexity in defining the distinct cognitive processes that are subserved by the PFC and our still limited understanding of how these processes integrate to effectively organize and guide behavior. Executive functions have been operationalized in a variety of ways but can include attention, planning, cognitive flexibility, working memory, inhibitory control, and decision-making (Fuster, 2000; Glisky, 2007; Kesner and Churchwell, 2011; Miller and Cohen, 2001; Robbins, 1996). Among these processes, age-related decline in working memory and cognitive flexibility are particularly well described. Though many definitions for working memory exist, this term is most often used in reference to the maintenance of a representation "in mind" of a stimulus that is no longer present in the environment (e.g., Goldman-Rakic, 1996). In contrast, cognitive or behavioral flexibility refers to the ability to effectively update internal representations and shift behavioral responses to accommodate changes in environmental contingencies (e.g., Dias et al., 1996).

Cognitive flexibility can be assessed in primates and rodents using "set-shifting" tasks. The prototypical set-shifting task, designed for human subjects, is the Wisconsin Card Sorting task (Berg, 1948), in which subjects are required to sort a deck of cards that contain multiple stimulus features (e.g., shape and color). Subjects must initially learn through trial and error which stimulus feature governs the correct choice (e.g., red indicates correct choice, ignore shape). After acquisition of this rule, an unsignaled 'shift' occurs such that the external contingencies are altered and the subjects must now inhibit the initial rule and shift their response strategies to accommodate the new contingencies (e.g., ignore color, square signals correct choice). Analogues of the Wisconsin Card Sorting task have been developed for use in nonhuman primates and rodents, and across species, damage to the dorsolateral PFC or its rodent homologue, medial PFC (mPFC), does not affect acquisition of the initial rule but selectively impairs the ability to set-shift (Birrell and Brown, 2000; Bissonette et al., 2008; Darrah et al., 2008; Demakis, 2003; Dias et al., 1996;

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Floresco et al., 2008; Owen et al., 1991; Ragozzino, 2007; Ragozzino et al., 1999; Uylings et al., 2003). Working memory tasks are generally designed such that to-be-remembered information varies across trials, requiring active resistance to proactive interference and distraction. Working memory is commonly assessed using delayed response tasks in which subjects are required to remember information about a spatial location over some delay interval, and then to accurately recall that information in a choice setting. As with set-shifting tasks, performance on working memory tasks is impaired after damage to primate dorsolateral or rodent medial PFC, and such lesions tend to disproportionately affect performance at long delays (Floresco et al., 1997; Freedman and Oscar-Berman, 1986; Goldman and Rosvold, 1970; Mishkin, 1957; Ragozzino et al., 1998).

Previous work in humans, nonhuman primates, and rodents has shown that cognitive flexibility and working memory decline across the lifespan. Notably, however, there is considerable variability among aged subjects, such that some perform on par with young whereas others demonstrate varying degrees of impairment (Barense et al., 2002; Bizon et al., 2009; Gallagher et al., 1993; Glisky, 2007; Morrison and Baxter, 2012; Park, 2000; Robbins et al., 1998). Despite such well-documented individual differences, the relationship between the presence and severity of impairment on tasks that assay these different components of executive function is not well defined. The fact that PFC damage impairs working memory and set-shifting performance, and that both functions are compromised in disease states such as schizophrenia, suggest that age-related impairments in working memory and cognitive flexibility are mediated by common neural mechanisms and might be expected to covary (Chudasama and Robbins, 2006). However, these processes can also be dissociated using a variety of PFC manipulations that include modulation of dopaminergic and GABAergic signaling, both of which can be compromised at advanced ages (Cools and D'Esposito, 2011; Durstewitz and Seamans, 2008; Enomoto et al., 2011; Floresco and Magyar, 2006; Li et al., 2010; McQuail et al., 2012). A primary goal of the current study was to determine the relationship between age-related impairments in cognitive flexibility (assessed using a set-shifting task) and working memory (assessed using a delayed response task) in aged Fischer 344 rats. In addition, performance on the set-shifting task was compared with performance on the Morris water maze, an assay that is sensitive to medial temporal lobe-mediated mnemonic dysfunction in aged rats (Bizon et al., 2009; Frick et al., 1995; Gallagher et al., 1993).

2. Methods

2.1. Subjects

Young (5 months old; $n = 20$) and aged (22 months old; $n = 25$) male Fischer 344 rats were obtained from the National Institute on Aging colony (Taconic Farms, Hudson, NY, USA) and housed in the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited vivarium facility in the McKnight Brain Institute Building at University of Florida in accordance with the rules and regulations of the University of Florida Institutional Animal Care and Use Committee and National Institutes of Health guidelines. The facility was maintained at a consistent temperature of 25 °C with a 12-hour light/dark cycle (lights on at 8:00 AM) with free access to food and water except as otherwise noted. Rats were tested in 3 cohorts, each including young and aged subjects. After completing the set-shifting protocol, subsets of these rats were subsequently trained on the working memory ($n = 9$ young and $n = 11$ aged) or water maze ($n = 10$ young, $n = 13$ aged) tasks. Note that not all rats tested in experiment 1 were tested in experiments 2 and 3.

2.2. Experimental procedures

2.2.1. Experiment 1: effects of aging on set-shifting

2.2.1.1. Apparatus. Testing in the set-shifting and working memory tasks was conducted in 8 identical standard rat behavioral test chambers (30.5 × 25.4 × 30.5 cm; Coulbourn Instruments, Whitehall, PA, USA) with metal front and back walls, transparent Plexiglas side walls, and a floor composed of steel rods (0.4 cm in diameter) spaced 1.1 cm apart. Each test chamber was housed in a sound-attenuating cubicle, and was equipped with a recessed food pellet delivery trough located 2 cm above the floor in the center of the front wall. The trough was fitted with a photobeam to detect head entries and a 1.12 W lamp for illumination. Food rewards consisted of one 45-mg grain-based food pellet for each correct response (PJAI; Test Diet, Richmond, IN, USA). Two retractable levers were located to the left and right of the food trough (11 cm above the floor), and a 1.12 W cue lamp was located 3.8 cm above each lever. An additional 1.12 W house light was mounted near the top of the rear wall of the sound-attenuating cubicle. A computer interfaced with the behavioral test chambers and equipped with Graphic State 3.01 software (Coulbourn Instruments) was used to control experiments and collect data.

2.2.1.2. Shaping. The design of the set-shifting task was based on that used by Floresco et al. (2008). Before the start of behavioral testing, rats were reduced to 85% of their free feeding weights over the course of 5 days and maintained at this weight for the duration of the experiments involving food restriction. Rats progressed through 4 stages of shaping before the onset of the set-shifting task, with new stages beginning on the day immediately after completion of the previous stage. On the day before shaping stage 1, each rat was given five 45-mg food pellets in its home cage to reduce neophobia to the food reward used in the task. Shaping stage 1 consisted of a 64-minute session of magazine training, involving 38 deliveries of a single food pellet with an intertrial interval of 100 ± 40 seconds. Shaping stage 2 consisted of lever press training, in which a single lever (left or right, counterbalanced across groups) was extended and a press resulted in delivery of a single food pellet. After reaching a criterion of 50 lever presses in 30 minutes, rats were then trained on the opposite lever using the same procedures.

Shaping stage 3 consisted of 90 trials that were designed to train rats to press the levers after their insertion into the test chamber. Each 20-second trial began with illumination of the house light and insertion of a single lever (either left or right, randomly selected within each pair of trials) into the test chamber where it remained for a maximum of 10 seconds. A response on the lever within this time window resulted in retraction of the lever, delivery of a single food pellet, and continued illumination of the house light for an additional 4 seconds. If a rat failed to respond on the lever within 10 seconds, the lever was retracted and the house light turned off, and the trial was scored as an omission. Rats received at least 4 daily sessions in this stage, and were trained until reaching criterion performance of fewer than 10 omissions out of the 90 trials.

Shaping stage 4 was designed to determine each rat's side bias (i.e., preference for one lever over the other). Each trial consisted of multiple phases. In the first phase of a trial, the house light was illuminated and both levers were inserted into the test chamber. A response on either lever resulted in retraction of both levers and delivery of a single food pellet. In the second phase of a trial, both levers were again inserted, but only a response on the lever opposite to the one chosen in the first phase resulted in food delivery. A response on the same lever chosen in the first phase (i.e., "incorrect") resulted in the levers being retracted and in the houselight being extinguished. After a "correct" response on this second phase of a trial, a new trial was initiated, whereas after an

"incorrect" response, the second phase of the trial was repeated. The second phase was repeated until rats made a "correct" response. In cases in which 5 or more of the initial 7 first phase choices were confined to a single side, that side was considered the rat's biased side. However, in cases in which neither side was initially chosen a disproportionate number of times (<5), the side associated with the greatest number of total responses across this stage of testing was considered the rat's biased side.

2.2.1.3. Visual cue discrimination. After the side bias determination session, rats were trained to press the lever associated with a visual cue (light). In this discrimination (Fig. 1A), the illumination of a cue light over a lever signaled the correct response, irrespective of the spatial location (left or right) of the cue. Each 20-second trial began with illumination of 1 of the cue lights (left or right, randomly selected in each pair of trials). After 3 seconds, the house light was illuminated and both levers were inserted into the chamber (the cue light remained illuminated while the levers were extended). A response on the lever corresponding to the cue light (a correct response) resulted in the house light remaining on for 4 seconds, during which time the levers were retracted, the cue light was extinguished, and a single food pellet was delivered. A response on the opposite lever (an incorrect response) or failure to respond within 10 seconds (omission) resulted in retraction of both levers and all lights being extinguished. Rats were considered to have acquired the task on reaching criterion performance of 8 consecutive correct trials (and at least 30 total trials, excluding omissions), with the maximum number of trials per session set at 120. If rats failed to acquire the task within a single session, they received additional sessions on subsequent days.

2.2.1.4. (Set-shift) left/right discrimination. After reaching criterion performance on the visual discrimination task, rats were tested the next day in the set-shift condition, in which the task contingencies were altered. In this new condition, rats were required to ignore the light cue and instead to consistently choose the left or right lever (whichever was not their biased side as determined in shaping stage 4). Hence, accurate performance required rats to "shift" their attention away from the visual cue and toward the left/right position of the lever. Beyond the shift in reward contingencies, trials were identical in presentation to those in the visual cue discrimination (i.e., on each trial, both levers were presented, with the cue light illuminated over one lever). As in the visual cue discrimination, the location of the illuminated cue light was randomized (left or right) in each pair of trials. Rats were considered to have acquired the task on reaching criterion performance of 8 consecutive correct trials, excluding omissions. The maximum number of trials per session was set at 120 and if rats failed to acquire the task within a single session, they received additional sessions on subsequent days.

2.2.1.5. Set-shifting statistical analysis. Raw data files were exported from Graphic State software and compiled using a custom macro written for Microsoft Excel (Dr Jonathan Lifshitz, University of Kentucky). Statistical analyses were conducted using SPSS 19.0. The total numbers of trials and errors required to achieve criterion on the visual discrimination and on the set-shift were used as the indices of performance. Considering that the task design involved explicit presentation of the same set of stimuli during the initial discrimination learning and the set-shift, the nature of the errors was also examined. Specifically, age comparisons were performed separately for errors that involved responses corresponding to previously-reinforced choices (the cue light was incongruent with the correct lever location and the rat chose based on the previous visual discrimination rule) and for errors that had never been reinforced (the cue light and spatial location were congruent and the rat's choice was not correct according to the rule in either type of discrimination; Floresco et al., 2008; Ragozzino et al., 2002).

Comparisons between age groups in the set-shifting task (trials to criterion and errors to criterion) were conducted using *t* tests. A Levene's test was conducted to test for differences in variance in individual performance between age groups. For analyses in experiments 2 and 3, in which performance was compared across cognitive tasks, aged rats were subgrouped into "cognitive groups" on the basis on their set-shifting performance using the trials-to-criterion measure. Aged rats were subdivided into "aged shifting-impaired" (rats in which trials to criterion on the set-shift was greater than 1 standard deviation from the mean of young rat performance) and "aged shifting-unimpaired" (all other rats). A one-factor analysis of variance (ANOVA) was used to compare set-shifting performance among these cognitive groups with Tukey post hoc tests performed when warranted. For this and all subsequent analyses, *p* values less than 0.05 were considered significant.

2.2.2. Experiment 2: effects of age on working memory and relationship to set-shifting performance

Both set-shifting and working memory task performance depend on the integrity of PFC systems and decline with age, but the nature of the relationship between age-related decline across these two distinct components of executive function is unknown. To investigate this relationship, subsets of young and aged rats characterized on the set-shifting task in experiment 1 were subsequently tested on a delayed response working memory task.

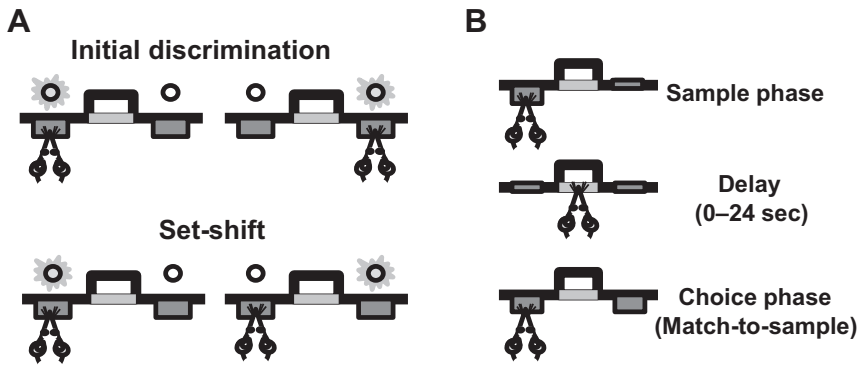

Fig. 1. Schematic diagrams of the set-shifting and working memory tasks. (A) The set-shifting task. Rats were initially trained in a visual discrimination in which they had to press the response lever illuminated by a small lamp, irrespective of its left/right location. On reaching criterion performance, they were shifted to a left/right discrimination in which they had to press the response lever in a particular location, irrespective of whether that lever was illuminated by the lamp. (B) The delayed response working memory task. Rats had to press a lever when extended during the sample phase, then, after a variable delay, press that same lever during the choice phase to earn a food reward.

2.2.2.1. Delayed response working memory task procedures. The delayed response working memory task was conducted in the same testing chambers used for the set-shifting task. Because the rats were already familiar with pressing the retractable levers to earn food rewards, they did not require additional shaping procedures. The design of the task was based on Sloan et al. (2006). Each session was 40 minutes in duration, and the house light was illuminated throughout the entire session except during timeout periods. A trial began with insertion of a single lever (the "sample" lever) into the chamber (Fig. 1B). The left/right position of this lever was randomly selected within each pair of trials, and a lever press caused it to retract and started the delay period timer. During the delay, rats were required to nosepoke into the food trough to initiate the "choice" phase, and the first such nosepoke emitted after the delay timer expired caused both levers to extend. During this choice phase, a response on the same lever pressed during the sample phase (a correct response) resulted in both levers being retracted and the delivery of a single food pellet. Entry into the food trough to collect the food pellet initiated a 5-second intertrial interval, after which the next trial was initiated. A response on the opposite lever from that chosen during the sample phase (an incorrect response) resulted in both levers being retracted and initiation of a 5-second "timeout" period during which the house light was extinguished, followed immediately by the start of the next trial.

During initial sessions in this task, there were no delays between the sample and choice phases, and a correction procedure was employed such that the sample lever was repeated on the same side after an incorrect response to prevent development of side biases. When rats reached a criterion of 80% correct choices across a session for 2 consecutive sessions, this correction procedure was discontinued and a set of 7 delays was introduced. The presentation of delay durations was randomized within each block of 7 trials, such that each delay was presented once. On establishing greater than 80% correct performance across 2 consecutive sessions, delays were systematically increased (set 1: 0, 1, 2, 3, 4, 5, and 6 seconds; set 2: 0, 2, 4, 8, 12, and 16 seconds; set 3: 0, 2, 4, 8, 12, 18, and 24 seconds). Rats were tested for 10 consecutive sessions on the delays in set 3.

2.2.2.2. Delayed response statistical analyses. Performance (percent correct at each delay) was averaged across 10 test sessions to provide a measure of mean accuracy at each delay. Group comparisons in the working memory task were conducted using 2-factor (age or cognitive group by delay) repeated measures ANOVAs. Comparisons of individual performance across the set-shifting (trials and errors to criterion) and working memory tasks (mean percent accuracy at each delay) were conducted using bivariate correlations performed separately for young and aged rats.

2.2.3. Experiment 3: relationship between set-shifting and spatial learning performance in aging

A decline in spatial memory is well-described in normal aging, and our laboratory and others have reported individual differences among aged rats with respect to the presence and degree of impairment on the Morris water maze task (Bizon et al., 2009; Gallagher et al., 1993; LaSarge et al., 2007). To determine the relationship between age-related decline in the PFC-dependent set-shifting task and in hippocampal-dependent spatial abilities, subsets of young and aged rats from experiment 1 were subsequently assessed on the water maze task.

2.2.3.1. Apparatus. The water maze consisted of a circular tank (diameter 183 cm, wall height 58 cm) painted white and filled with water (27 °C) made opaque with the addition of nontoxic white tempera paint. The maze was surrounded by black curtains to which were affixed large white geometric designs which provided extramaze visual cues. For the spatial reference memory (hidden platform) task, a retractable escape platform (12 cm diameter; HVS Image) was submerged 2 cm below the surface of the water in the center of the southwest quadrant of the maze. For the cued (visible platform) task, the platform was painted black and protruded 2 cm above the surface of the water, and was located in a different quadrant of the maze on each trial. A video camera mounted above the center of the maze was connected to a DVD recorder and computer, which were used for data storage and analysis using a video tracking system (Water 2020; HVS Image).

2.2.3.2. Spatial reference memory (hidden platform) task procedure. Spatial reference memory was assessed as described previously (Bizon et al., 2009). Briefly, on 8 consecutive days, rats received 3 trials per day with a 30-second intertrial interval. On each trial, rats were placed into the water facing the wall of the maze at 1 of 4 equally-spaced start positions (north, south, east, or west). The start positions were varied in a pseudo-random fashion, such that all rats started from each of the locations approximately the same number of times. On placement in the water, rats were allowed to swim until they found the hidden platform or until 90 seconds elapsed, at which time they were guided to the escape platform by the experimenter. Rats remained on the platform for 30 seconds and then were placed in a holding chamber for 30 seconds before the next trial. Every sixth trial (i.e., the third trial on days 2, 4, 6, and 8) was a probe trial in which the platform was lowered to the bottom of the maze for the first 30 seconds of the trial, after which it was raised to allow the rats to escape.

2.2.3.3. Cued (visible platform) task. After spatial reference memory training, rats were given a single session with 6 trials of cue training to assess sensorimotor abilities and motivation to escape the water. For this task, rats were trained to swim to a visible platform (painted black and protruding 2 cm above the water's surface). The start position and platform location were varied on each trial, making the extramaze cues explicitly irrelevant to the platform location. On each trial, rats were allowed to search for the platform for a maximum of 90 seconds and then were allowed to remain there for 30 seconds before a 30-second intertrial interval.

2.2.3.4. Water maze statistical analyses. For each task, data files were created using the Water 2020 software and were exported to Microsoft Excel and SPSS (version 19.0) for analysis. Training trial data in the spatial reference memory task were averaged into 4 blocks consisting of the 5 trials preceding each probe trial, and performance on these trials was analyzed using a "cumulative search error" measure. To calculate search error, a rat's distance from the platform was sampled 10 times per second and these distances were averaged into 1-second bins. Cumulative search error is the sum of these 1-second bins over the course of a training trial, minus the optimal path from the start location to the platform (Bizon et al., 2009; Gallagher et al., 1993). On probe trials, a "mean search error" measure was used. Mean search error is the sum of the 1-second bins (minus the optimal path from the start location to the platform), divided by the 30-second duration of the probe trials. Mean search error data from each probe trial were weighted and summed to provide a "spatial learning index" measure, which provides a single value reflecting overall spatial learning ability for each rat (Bizon et al., 2009; Gallagher et al., 1993). Comparisons between groups on training trials (in the hidden and visible platform tasks) were conducted using 2-factor ANOVA (age or cognitive group by training trial), with Tukey post hoc tests performed when warranted. Comparisons of individual performance across the set-shifting (trials and errors to criterion) and water maze (spatial

learning index) tasks were conducted using bivariate correlations performed separately for young and aged rats.

3. Results

3.1. Experiment 1: effects of aging on set-shifting

Young and aged rats required comparable numbers of sessions in the two stages of shaping which included a performance criterion (stage 2: mean [standard error of the mean (SEM)], young = 2.90 [0.22], aged = 2.64 [0.18], $t(43) = 0.93$, $p = 0.36$; stage 3: young = 5.40 [0.57], aged = 5.32 [0.46], $t(43) = 0.11$, $p = 0.93$). Likewise, there were no differences between young and aged rats in the number of trials required to reach criterion performance on the initial (visual) discrimination ($t(43) = 0.01$; $p = 0.99$; Fig. 2A). In contrast, aged rats were impaired relative to young adults on the set-shift task, requiring significantly more trials to reach criterion performance ($t(43) = 2.93$; $p < 0.05$; Fig. 2B). Similarly, aged rats also made significantly more errors before reaching criterion performance ($t(43) = 2.01$; $p < 0.05$; Fig. 2C) and, as expected, total trials and errors to criterion were strongly correlated in both young ($r = 0.71$; $p < 0.05$) and aged ($r = 0.86$; $p < 0.05$) rats.

A number of studies have suggested that age-related deficits in shifting tasks (e.g., Wisconsin Card Sorting task) are preferentially attributable to an inability to inhibit responding based on the previously reinforced rule, which manifests as a greater number of perseverative errors (Gamboz et al., 2009; Moore et al., 2006; Ridderinkhof et al., 2002). Because the current task design involved explicit presentation of the same set of stimuli during the initial discrimination learning and the set-shift, the nature of the age-related impairment could be further investigated using an error analysis. For each rat, errors to reach criterion on the set-shift were divided into those that involved responses that corresponded to previously reinforced choices (i.e., perseverative) and errors that had never been reinforced. As shown in Fig. 2C, aged rats made significantly more previously reinforced errors than young rats ($t(43) = 2.11$; $p < 0.05$). In contrast, there was no significant difference between age groups in the number of never-reinforced errors ($t(43) = 1.57$; not significant).

Significantly more heterogeneity in set-shifting performance was observed among aged in comparison with young adult rats (Levene's test, trials to criterion: $F = 22.98$, $p < 0.05$; errors to criterion: $F = 12.81$, $p < 0.05$). As shown in Fig. 3A, some aged rats performed as well as young adults and performance of others was well outside the range of young adult performance, demonstrating impairment. Subsequent experiments were designed to determine the extent to which this age-related set-shifting impairment predicted impairment in other components of executive function (experiment 2) and in other cognitive domains (experiment 3). For such comparisons, aged rats were subdivided into "aged shifting-impaired" (rats in which the number of trials to criterion on the set-shift was greater than 1 standard deviation from mean young rat performance) and "aged shifting-unimpaired" (all other rats). Using these criteria, $n = 12$ aged rats were classified as aged shifting-impaired and $n = 13$ aged rats were classified as aged shifting-unimpaired. Performance of these subgroups did not differ on the initial visual discrimination ($F(2,42) = 0.38$; $p = 0.68$) but differed significantly on the set-shift ($F(2,42) = 51.01$; $p < 0.05$). Post hoc analyses indicated that, as expected, aged shifting-impaired rats performed significantly worse than young and aged shifting-unimpaired rats on the set-shift ($p < 0.05$), and young and aged shifting-unimpaired subgroups did not differ from each other ($p = 0.80$). Fig. 3B and C show group set-shifting performance of the subsets of young and aged rats used in experiments 2 (young, $n = 9$; aged, $n = 11$) and 3 (young, $n = 10$; aged $n = 13$). Across both subsets of rats, performance reflected that of the overall age groups in that a main effect of age on set-shifting performance was evident, with aged rats requiring significantly more trials to reach criterion performance after the set-shift relative to young adults ($t(21) = 2.29$; $p < 0.05$). Moreover, aged shifting-impaired rats took significantly more trials to reach criterion on the set-shifting task relative to young and aged shifting-unimpaired rats (experiment 2: $F(2,19) = 17.06$, $p < 0.05$; Fig. 3B; and experiment 3: $F(2,22) = 20.78$, $p < 0.05$; Fig. 3C). In both experiments, post hoc analyses indicated that aged shifting-impaired rats were impaired relative to young and aged shifting-unimpaired rats ($ps < 0.05$) but that young rat performance did not differ significantly from aged shifting- unimpaired rats ($ps > 0.67$).

3.2. Experiment 2: effects of age on working memory and relationship to set-shifting performance

A subset of the rats tested in the set-shifting task in experiment 1 ($n = 9$ young, $n = 5$ aged shifting-unimpaired, and $n = 6$ aged shifting-impaired) was subsequently tested in the delayed response working memory task. There was no age difference in the number

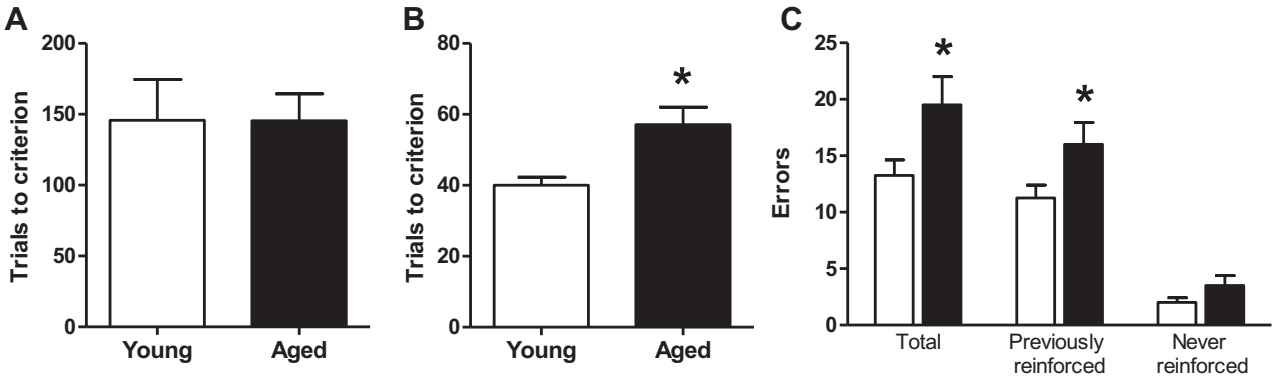

Fig. 2. Performance of young and aged rats on the set-shifting task. (A) Aged rats were no different from young in the number of trials required to reach criterion performance on the initial visual discrimination. (B) In contrast, after the set-shift, aged rats took significantly more trials to reach criterion performance on the left/right discrimination. (C) Aged rats made significantly more total errors than young rats in reaching criterion performance on the set-shift. Most of these errors involved responding according to the previously-reinforced (visual discrimination) response rule (e.g., if the left lever was correct during the left/right discrimination, a "previously-reinforced" error would involve responding on the right lever on trials in which the right lever was illuminated), and aged rats made significantly more previously-reinforced errors than young rats. In contrast, there were much fewer errors of the "never reinforced" type (e.g., in the previous example, responding on the right lever on trials in which the left lever was illuminated), and these did not differ between young and aged rats. * $p < 0.05$.

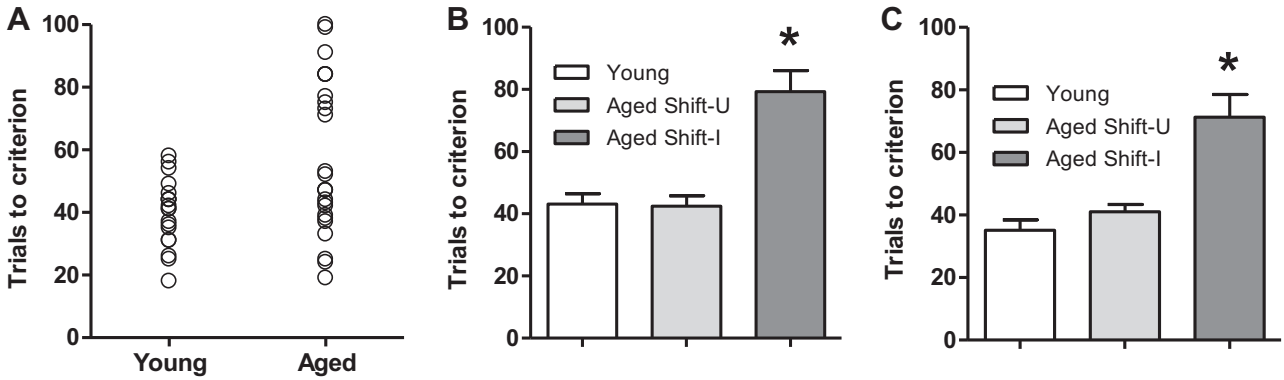

Fig. 3. Variability among aged rats on the set-shifting task. (A) There was significantly greater variability in the number of trials to criterion on the set-shift among aged compared with young rats (each point represents data from a single rat), such that performance of some rats fell within the range of young whereas performance of others fell outside this range, demonstrating impairment. Dividing the aged rats according to a criterion of greater or less than 1 standard deviation from the mean of the young group yielded 2 equally-sized subgroups of aged rats: an "aged shifting-unimpaired" (Aged Shift-U) subgroup that performed identically to young, and an "aged shifting-impaired" (Aged Shift-I) subgroup that performed significantly worse (greater number of trials to criterion) than the young and aged shift-unimpaired subgroups. (B) and (C) show data from the subsets of rats subsequently tested in the delayed response and water maze tasks, respectively. * $p < 0.05$.

of shaping sessions required to reach the longest set of delays in the working memory task (mean [SEM]: young = 13.56 [1.26], aged = 15.91 [0.81], $t(18) = 1.62$, not significant). In contrast, a 2-factor repeated measures ANOVA (age by delay) performed on data averaged across 10 sessions at the longest set of delays, revealed a significant main effect of age on the working memory task, such that across delays, aged rats performed significantly worse than young ($F(1,18) = 5.99$; $p < 0.05$; Fig. 4A). A main effect of delay ($F(6,108) = 140.99$; $p < 0.05$) was also evident such that performance declined as a function of delay duration, but there was no age by delay interaction ($F(6,108) = 0.40$; $p = 0.88$).

A considerably different pattern of results was evident when working memory performance was analyzed as a function of set-shifting performance. As shown in Fig. 4B, a 2-factor repeated measures ANOVA (cognitive group by delay) revealed a main effect of delay ($F(6,102) = 158.84$; $p < 0.05$) and a main effect of cognitive group ($F(2,17) = 9.65$; $p < 0.05$), and an interaction between delay and cognitive group ($F(12,102) = 1.90$; $p < 0.05$). Surprisingly, the main effect of cognitive group was carried by the fact that aged shifting-unimpaired rats were significantly impaired on the working memory task relative to young and aged shifting-impaired rats ($ps < 0.05$), whereas aged shifting-impaired rats performed comparably with young on the working memory task ($p = 0.65$). Hence, these data indicate an inverse relationship between age-related impairments on set-shifting and working memory tasks.

The nature of this inverse relationship was further tested using bivariate correlations conducted on individual performance measures of set-shifting (trials and errors to criterion) and working memory. Note that, for these analyses, performance at each delay used in the working memory task was expressed as "100 − mean percent correct at each delay" such that inverse relationships between performance on the 2 tasks would result in negative correlations. As shown in Table 1 and Fig. 4C, among aged rats, significant negative correlations were present between set-shifting and working memory performance at the 12-second ($r = -0.61$; $p < 0.05$) and 24-second ($r = -0.77$; $p < 0.05$) delays with a similar trend also evident at the 18-second delay ($r = -0.54$; $p = 0.09$). These correlations indicate that better set-shifting performance in aged rats was associated with worse working memory performance at long delays. Notably, in contrast to this inverse relationship at long delays, set-shifting performance (errors to criterion) in aged rats was positively associated with performance on the delayed response task at the 0-second delay ($r = 0.65$; $p < 0.05$). No significant relationships between performance on these tasks were observed among young rats at any delay, although trends toward positive correlations between set-shifting (trials and errors to criterion) and working memory performance were observed at 0- and 2-second delays ($rs > 0.6$; $ps < 0.1$; see Table 1).

3.3. Experiment 3: relationships between set-shifting and spatial learning performance in aging

A subset of the rats tested in the set-shifting task ($n = 10$ young, $n = 7$ aged shifting-unimpaired, and $n = 6$ aged shifting-impaired rats) was also tested in the Morris water maze task. As shown in Fig. 5A, aged rats were significantly impaired relative to young rats in their ability to locate a hidden platform in the water maze. A repeated measures ANOVA (age by trial block) revealed that all rats improved over the course of training (main effect of trial block, $F(3,63) = 9.66$; $p < 0.05$) but that aged rats had significantly greater search error associated with locating the hidden platform than did young adults, demonstrating impaired performance (main effect of age, $F(1,21) = 6.66$; $p < 0.05$). Similar to previous findings in this study population (Bizon et al., 2009; Murchison et al., 2009), these group age differences were not present on the first training trial ($t(21) = 0.004$; not significant), nor were there differences between young and aged rats in their ability to locate the visible platform during cue training (mean [SEM] path length: young = 242.38 [37.73], aged = 274.08 [27.62]; $t(21) = 0.70$; $p = 0.50$). Together, these data demonstrate that deficits associated with locating the hidden platform in aged rats were not because of impairments in sensorimotor function, motivation, or ability to learn the procedural aspects of the task.

Fig. 5B shows water maze performance plotted as a function of set-shifting ability among aged rats. A repeated measures ANOVA (cognitive group by trial block) revealed that all rats improved over the course of training (main effect of trial block, $F(3,60) = 9.68$; $p < 0.05$) but there was neither a significant main effect of cognitive group ($F(1,20) = 3.21$; $p = 0.06$) nor an interaction between cognitive group and trial block ($F(6,60) = 0.38$; $p = 0.89$). Nevertheless, because the main effect of cognitive group in this analysis was near statistical significance, post hoc tests were conducted to determine the relationships among the 3 cognitive groups. These tests revealed that although, in agreement with the main effect of age on performance previously described, there was a trend toward aged shifting-unimpaired and aged shifting-impaired groups differing from young ($ps = 0.13$ and 0.10, respectively), the 2 aged groups clearly did not differ from each other ($p = 0.97$).

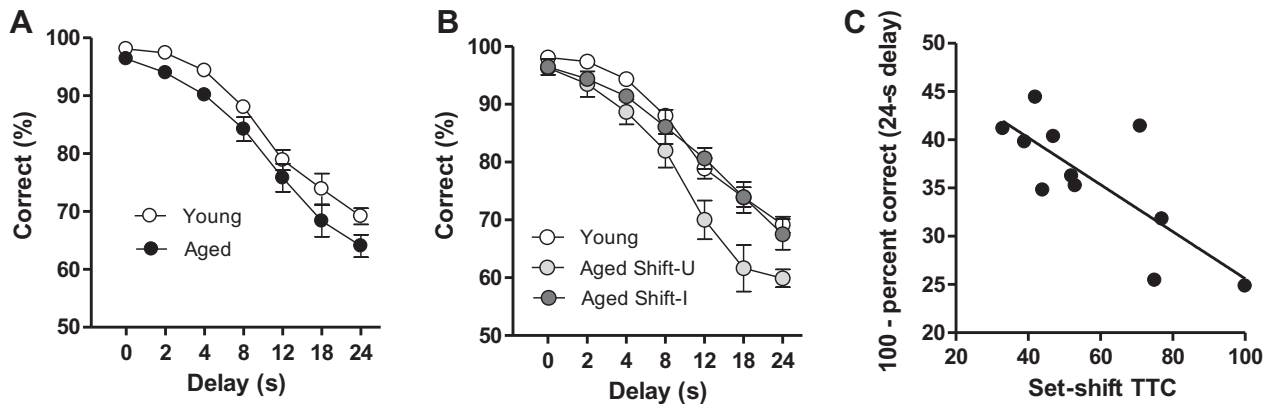

Fig. 4. Performance on the delayed response working memory task in young and aged rats, and relationships with set-shifting performance. (A) All rats showed delay-dependent decrements in performance on the delayed response task, but aged rats were impaired relative to young. (B) Aged shifting-impaired (Aged Shift-I) rats performed comparably with young on the delayed response task, whereas aged shifting-unimpaired (Aged Shift-U) rats performed worse than shifting-impaired and young cohorts. (C) Among aged rats, performance on the set-shifting task was significantly correlated with performance on the delayed response task at the 24-second delay, such that worse set-shifting predicted better working memory (TTC = trials to criterion).

To further compare performance across set-shifting and water maze tasks, an overall measure of spatial learning ability was derived for each rat using performance on the 4 interpolated probe trials. The spatial learning index was calculated as previously described (Bizon et al., 2009; Gallagher et al., 1993). These learning index scores have been associated with age-related changes in neurobiological substrates of spatial memory, and other aspects of cognition (Banuelos et al., 2013; Bizon et al., 2001; LaSarge et al., 2007; McQuail et al., 2012; Smith et al., 2000). As expected, an unpaired *t* test performed on the mean spatial learning indices in young and aged rats indicated that aged rats were significantly impaired (higher learning index scores) relative to young (young = 239.28 [9.73], aged = 277.47 [10.88]; $t(21) = 2.53$; $p < 0.05$). However, in agreement with the group comparisons in Fig. 5B, bivariate correlations performed separately on young (not shown) and aged (Fig. 5C) rats revealed no significant relationships between set-shifting and spatial memory performance among either young ($r = 0.33$, $p = 0.35$) or aged ($r = 0.06$, $p = 0.85$) rats. Hence, in agreement with previous work, these data indicate a lack of relationship between age-related impairments in measures of cognitive flexibility and spatial learning (Barense et al., 2002; Schoenbaum et al., 2002).

4. Discussion

The overarching goal of these experiments was to determine the relationship between age-associated performance deficits in 2 aspects of PFC-dependent executive functions: set-shifting and working memory. In agreement with previous population-based studies in humans, nonhuman primates, and rodents (Barense et al., 2002; Bizon et al., 2009, 2012; Glisky, 2007; Mizoguchi et al., 2009; Morrison and Baxter, 2012; Park, 2000; Ramos et al., 2003; Robbins et al., 1998; Segovia et al., 2008), aged rats in the current study were impaired relative to young in the set-shifting and working memory tasks. Among aged rats, however, there was a striking inverse relationship between individual performance measures across the 2 tasks such that aged rats impaired on the set-shifting task performed comparably with young on the working memory task, and aged rats impaired on the working memory task performed comparably with young on the set-shifting task. These data suggest that normal brain aging can result in distinct manifestations of executive dysfunction among individual subjects and support the need to better understand the mechanisms that might contribute to different forms of executive impairment that emerge at advanced ages.

Notably, this inverse relationship between set-shifting and delayed response performance was evident only in aged rats and was specific to long (>12 seconds) delays. It is possible that the absence of such a relationship in young rats reflects a statistical limitation rather than the influence of factors specific to advanced age. Indeed, there was significantly less variability in individual performance measures on both tasks within the young cohort, reducing the power to detect reliable cross-task correlations. However, contrary to this interpretation, it is notable that among young and aged rats, there was evidence of a positive relationship between performance at the 0-second delay on the delayed response task and performance on the set-shifting task. These different relationships that were evident at short and long delays argue against several other alterative explanations for the inverse relationship between set-shifting and long delay performance in the working memory task. First, they indicate that the inverse relationship is not carried by chance performance among aged rats (i.e., a "regression to the mean" across multiple behavioral measures). Second, they indicate that the inverse relationship at long delays is not attributable to performance strategies learned in the set-shifting task (e.g., "always press the left lever") carrying forward to influence delayed response performance, because such performance strategies would be expected to influence delayed response performance similarly at all delays. Indeed, performance measures in the delayed response task (Fig. 4) were obtained after several weeks of shaping in this task, and analyses of lever selection showed no evidence of side biases (not shown) as would be expected if set-shifting strategies directly influenced delayed response performance. Finally, it is important to note that the absence of

Table 1
Correlations between set-shifting and working memory performance

Set-shifting measure	Working memory (100 − percent correct at each delay)						
	0 s	2 s	4 s	8 s	12 s	18 s	24 s
Young							
Trials to criterion	0.63^{+}	0.63^{+}	0.53	−0.23	−0.52	−0.27	−0.29
Errors to criterion	0.52	0.63^{+}	0.68*	−0.36	−0.60^{+}	−0.28	−0.35
Aged							
Trials to criterion	0.36	0.08	0.01	−0.12	−0.61*	−0.54^{+}	−0.77*
Errors to criterion	0.65*	0.40	0.02	0.34	−0.42	−0.35	−0.55^{+}

$^{+}$ $p < 0.1$.
* $p < 0.05$.

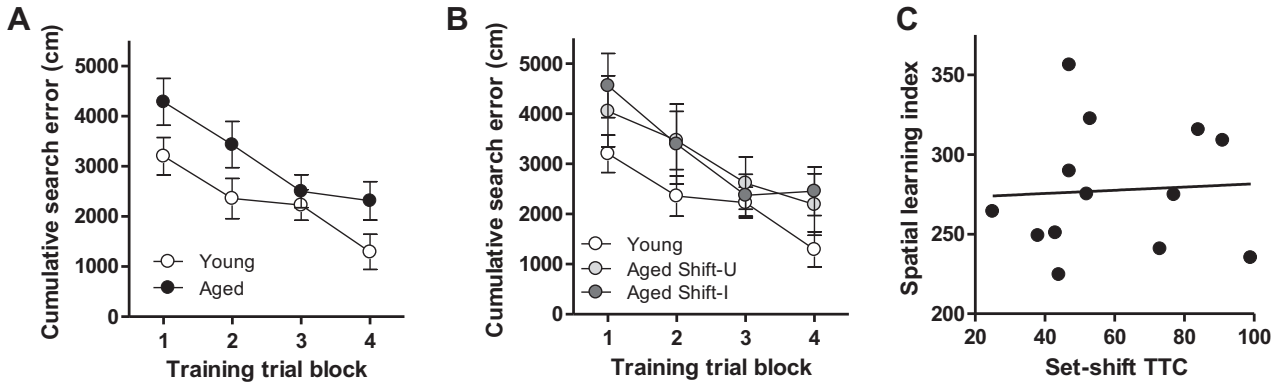

Fig. 5. Relationships between performance in the set-shifting and water maze tasks. (A) Aged rats performed significantly worse than young (greater cumulative search error) across the 4 blocks of training trials in the water maze. (B) Both the aged shifting-unimpaired (Aged Shift-U) and shifting-impaired (Aged Shift-I) subgroups were impaired relative to young rats (greater cumulative search error), but the 2 aged subgroups did not differ from each other. (C) Among aged rats, there was no correlation between performance on set-shifting measured by trials to criteria (TTC) and performance on the interpolated probe trials in the water maze (as assesed by the learning index measure; see text for details) (TTC = trials to criterion).

age-related deficits on the visual discrimination in the set-shifting task and at any stage of shaping strongly supports that age-related impairments in either task are not attributable to differences in motivation to press the levers for food or other nonspecific effects of age on appetitive operant behavior (see also Simon et al., 2010).

The opposing relationships between set-shifting and working memory across different delays suggest that somewhat different neurocognitive mechanisms mediate performance at short and long delays in the delayed response task. Notably, performance at the 0-second delay in the delayed response task would be expected to strongly tax the "updating" component of working memory and the ability to minimize proactive interference from immediately previous trials. Updating of the contingencies that signal the correct (rewarded) choice on the delayed response task involves focusing attention to new stimuli and inhibition of previously rewarded responses, both of which would also enable effective set-shifting. In contrast, performance at long delays might be mediated to a greater degree by the "maintenance" component of working memory or the ability to hold trial-specific information across the duration of the trial and minimize distraction during these extended delays. Indeed, because cognitive demands critical for maintenance likely involve minimizing attention to external stimuli, such maintenance processes might be viewed as functionally oppositional to those that enable effective updating and set-shifting. The current data suggest the intriguing possibility that aging disrupts the normal coordination of these updating and maintenance functions, such that some subjects show an impaired ability to hold information or representations stable across time (hereon referred to as impaired "representational stability") whereas others show an impaired ability to flexibly modify those representations as dictated by alterations in environmental contingencies (hereon referred to as impaired "cognitive flexibility").

It is possible that a single mnemonic deficit could mediate the inverse relationship between performance on working memory and set shifting tasks observed in the current study. Specifically, set-shifting might be expected to be facilitated in subjects unable to effectively recall the previously-learned rule (i.e., the visual discrimination). In this case, however, a strong relationship between learning of the initial (visual discrimination) and shifted (left/right discrimination) rules would be expected. Notably, no such relationships were observed in the current study among young or aged rats. Indeed, despite the fact that all rats required multiple sessions to acquire the visual discrimination rule, there were no age differences in trials-to-criterion to learn this rule as would be expected if between-session intervals were associated with age-related mnemonic deficits.

Across species, advanced age is associated with impairments in set-shifting and other tests of cognitive flexibility. In humans, aged individuals show impaired performance relative to young cohorts on the Wisconsin Card Sorting task (Ashendorf and McCaffrey, 2008; Robbins et al., 1998; Terry and Sliwinski, 2012; Volkow et al., 1998a) and similar set-shifting impairments have been observed in aged monkeys (Moore et al., 2005, 2006; although see Zeamer et al. 2011). The present findings in aged rats are consistent with these data, as well as with previous studies reporting set-shifting performance deficits in aged rats using a "digging task" in which rats are trained to shift between olfactory and tactile stimulus discriminations to obtain a food reward buried in a small pot (Barense et al., 2002; Nieves-Martinez et al., 2012). It could be argued that aged rats' impaired performance after the set-shift in the current study was attributable to the spatial nature of the post-shift discrimination problem, rather than an impairment in set-shifting per se. Despite well-documented spatial learning deficits in aged rats (Bizon et al., 2009; Foster et al., 2012; Gallagher et al., 1993), we believe this explanation to be unlikely for two reasons. First, although water maze navigation and spatial (i.e., left/right) discrimination might represent somewhat different cognitive operations, it is notable that no correlations were observed between individual measures of aged rat performance across set-shifting and spatial learning tasks (Fig. 5B and C). Second, and more importantly, considering that the task design involved explicit presentation of the same set of stimuli during the initial discrimination learning (visual discrimination) and during the set-shift (left/right discrimination), it was possible to determine whether errors committed during the left/right discrimination were consistent with perseveration on the initial visual cue response rule or instead reflected never-reinforced responses. If the increase in errors observed in aged rats after the set-shift were primarily because of an inability to perform the left/right discrimination, it would be expected that errors would be similarly distributed across previously- and never-reinforced categories (Enomoto et al., 2011; Floresco et al., 2008). Instead, in young and aged rats, most of the errors were of the previously-reinforced type. This pattern is consistent with the interpretation that errors across both ages largely reflected persistent responding to the initial rule and that the reliable increase in the number of perseverative errors in aged rats reflected an impaired ability of aged rats to shift their behavior in accordance with the new response rule.

Despite substantial evidence that domain-specific cognitive processes can be dissociated behaviorally and mechanistically, relationships between different aspects of age-related cognitive decline remain poorly delineated. Consistent with previous work in aged rats and as previously described herein, individual measures of aged rat performance on the set-shifting task in the current study were not related to performance measures on the water maze task (Barense et al., 2002). These data are consistent with the idea that age-related deficits in PFC-mediated executive functions emerge somewhat independently from deficits in medial temporal lobe-dependent mnemonic functions and suggest that unique mechanisms contribute to domain-specific cognitive dysfunction in aging (Bizon et al., 2012; Gallagher and Rapp, 1997; Glisky, 2007; Ramos et al., 2003; Schoenbaum et al., 2002). Compared with the spatial reference memory water maze task, working memory assessed by the delayed response task more heavily engages the same PFC circuitry that supports set-shifting. Indeed, many working memory tasks engage the PFC and the hippocampus, making it difficult to precisely define the neural substrates underlying impaired performance (Floresco et al., 1997; Shaw and Aggleton, 1993). An advantage of the task design used here is that performance is reportedly unaffected by lesions of the hippocampus (Sloan et al., 2006). In contrast, lesions of the mPFC produce pronounced performance deficits on this task, mimicking those observed in aged rats in the current study (Sloan et al., 2006). These data suggest that the age-related deficits observed in the working memory task are attributable to decline in PFC function, an interpretation which agrees with other findings linking age-related working memory impairments to alterations in PFC anatomy, physiology, and neurochemistry (Arnsten et al., 2012; Hara et al., 2012; Mizoguchi et al., 2009; Rapp and Amaral, 1989). It is important to note that the design of the delayed response task, wherein rats must nosepoke in the centrally-located food trough during the delay phase to initiate the choice phase, specifically discourages the use of nonmnemonic "mediating" strategies to solve the task (e.g., remaining stationary in front of the sample lever during the delay period). However, this design does result in some variation in the actual duration of the delays. Analysis of actual delay durations showed that they were on average several seconds longer than the programmed delays, although they were comparable in young and aged rats (e.g., the actual durations of the 24-second delays were: young = 27.3 seconds, aged = 28.7 seconds). Most importantly, the actual delays did not correlate with choice accuracy in either age group (not shown), indicating that the actual delay duration was not a significant mediator of individual differences in delayed response task performance.

A growing body of literature has shown that dopamine signaling in PFC-striatal circuits is important for the integration of representational stability and cognitive flexibility in normal behavior. Representational stability and cognitive flexibility are dependent on intact dopamine signaling, but perturbations in dopamine availability in frontostriatal circuits can differentially influence these two components of executive function (Brozoski et al., 1979; Crofts et al., 2001; Floresco and Magyar, 2006; Robbins and Arnsten, 2009). Specifically, dopamine signaling in the PFC has been heavily implicated in representational stability, possibly by maximizing signal-to-noise ratio and distracter resistance during delays (Arnsten et al., 2009; Sawaguchi and Goldman-Rakic, 1991; Vijayraghavan et al., 2007). This is evident in individuals with gene polymorphisms ($Val^{158}Met$) that regulate activity of the dopamine catabolic enzyme catechol-O-methyltransferase and presumptive dopamine availability in PFC. The Met allele is associated with the highest PFC dopamine levels and carriers consistently exhibit superior working memory performance relative to individuals carrying the Val allele (putatively low PFC dopamine). Interestingly, however, this enhanced working memory performance might occur at the expense of less cognitive flexibility. Met allele carriers perform worse than Val carriers on task-switching and reversal learning, both of which require flexible adaptation of established response rules (Colzato et al., 2010; Krugel et al., 2009).

Opposing roles for dopamine in representational stability and cognitive flexibility have also been suggested by the dual-state theory of PFC dopamine receptor signaling (Durstewitz and Seamans, 2008). This theory, which is based on empirical evidence from in vitro pharmacological and electrophysiological studies, suggests that a predominance of D1 receptor signaling promotes stable PFC network states which should facilitate information maintenance. In contrast, a predominance of D2 receptor signaling promotes switching between different PFC network states, which should favor cognitive flexibility. Notably, aging is accompanied by a range of alterations in dopamine signaling, including reductions in dopamine synthesis, release, and receptor availability (Backman et al., 2010; Segovia et al., 2008; Volkow et al., 1998b). Such alterations could result in a failure to rapidly adjust between "stable" and "flexible" modes of PFC operation, leading to dominance of one mode and impairment in the other. Beyond dopamine, there is evidence that PFC GABAergic signaling might differentially influence representational stability and cognitive flexibility. Specifically, in a recent study examining a battery of mPFC-dependent aspects of behavior in young adult rats, intra-mPFC administration of a GABA (A) receptor antagonist impaired set-shifting but not working memory (Enomoto et al., 2011), indicating that the two functions are neurochemically dissociable at least under some conditions (Floresco and Magyar, 2006). Determining how alterations in dopaminergic, GABAergic, and other neurochemical systems contribute to distinct manifestations of executive dysfunction in aging, and how such alterations develop in relation to changes in executive function over the lifespan, will be important avenues for future research.

Disclosure statement

The authors have no actual or potential conflicts of interest to disclose. All work was conducted in accordance with the rules and regulations of the University of Florida Institutional Animal Care and Use Committee and National Institutes of Health guidelines.

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References

Alexander, G.E., Ryan, L., Bowers, D., Foster, T.C., Bizon, J.L., Geldmacher, D.S., Glisky, E.L., 2012. Characterizing cognitive aging in humans with links to animal models. Front. Aging Neurosci. 4, 21.

Arnsten, A.F., Vijayraghavan, S., Wang, M., Gamo, N.J., Paspalas, C.D., 2009. Dopamine's influence on prefrontal cortical cognition: actions and circuits in behaving primates. In: Iversen, L., Iversen, S., Dunnett, S.B., Bjorklund, A. (Eds.), Dopamine Handbook. Oxford University Press, New York, pp. 230–248.

Arnsten, A.F., Wang, M.J., Paspalas, C.D., 2012. Neuromodulation of thought: flexibilities and vulnerabilities in prefrontal cortical network synapses. Neuron 76, 223–239.

Ashendorf, L., McCaffrey, R.J., 2008. Exploring age-related decline on the Wisconsin Card Sorting Test. Clin. Neuropsychol. 22, 262–272.

Backman, L., Lindenberger, U., Li, S.C., Nyberg, L., 2010. Linking cognitive aging to alterations in dopamine neurotransmitter functioning: recent data and future avenues. Neurosci. Biobehav. Rev. 34, 670–677.

Banuelos, C., Lasarge, C.L., McQuail, J.A., Hartman, J.J., Gilbert, R.J., Ormerod, B.K., Bizon, J.L., 2013. Age-related changes in rostral basal forebrain cholinergic and GABAergic projection neurons: relationship with spatial impairment. Neurobiol. Aging 34, 845–862.

Barense, M.D., Fox, M.T., Baxter, M.G., 2002. Aged rats are impaired on an attentional set-shifting task sensitive to medial frontal cortex damage in young rats. Learn. Mem. 9, 191–201.
Berg, E.A., 1948. A simple objective technique for measuring flexibility in thinking. J. Gen. Psychol. 39, 15–22.
Birrell, J.M., Brown, V.J., 2000. Medial frontal cortex mediates perceptual attentional set shifting in the rat. J. Neurosci. 20, 4320–4324.
Bissonette, G.B., Martins, G.J., Franz, T.M., Harper, E.S., Schoenbaum, G., Powell, E.M., 2008. Double dissociation of the effects of medial and orbital prefrontal cortical lesions on attentional and affective shifts in mice. J. Neurosci. 28, 11124–11130.
Bizon, J.L., Foster, T.C., Alexander, G.E., Glisky, E.L., 2012. Characterizing cognitive aging of working memory and executive function in animal models. Front. Aging Neurosci. 4, 19.
Bizon, J.L., Helm, K.A., Han, J.S., Chun, H.J., Pucilowska, J., Lund, P.K., Gallagher, M., 2001. Hypothalamic-pituitary-adrenal axis function and corticosterone receptor expression in behaviourally characterized young and aged Long-Evans rats. Eur. J. Neurosci. 14, 1739–1751.
Bizon, J.L., LaSarge, C.L., Montgomery, K.S., McDermott, A.N., Setlow, B., Griffith, W.H., 2009. Spatial reference and working memory across the lifespan of male Fischer 344 rats. Neurobiol. Aging 30, 646–655.
Brozoski, T.J., Brown, R.M., Rosvold, H.E., Goldman, P.S., 1979. Cognitive deficit caused by regional depletion of dopamine in prefrontal cortex of rhesus monkey. Science 205, 929–932.
Buckner, R.L., 2004. Memory and executive function in aging and AD: multiple factors that cause decline and reserve factors that compensate. Neuron 44, 195–208.
Burke, S.N., Ryan, L., Barnes, C.A., 2012. Characterizing cognitive aging of recognition memory and related processes in animal models and in humans. Front. Aging Neurosci. 4, 15.
Chudasama, Y., Robbins, T.W., 2006. Functions of frontostriatal systems in cognition: comparative neuropsychopharmacological studies in rats, monkeys and humans. Biol. Psychol. 73, 19–38.
Colzato, L.S., Waszak, F., Nieuwenhuis, S., Posthuma, D., Hommel, B., 2010. The flexible mind is associated with the catechol-O-methyltransferase (COMT) Val158Met polymorphism: evidence for a role of dopamine in the control of task-switching. Neuropsychologia 48, 2764–2768.
Cools, R., D'Esposito, M., 2011. Inverted-U-shaped dopamine actions on human working memory and cognitive control. Biol. Psychiatry 69, e113–e125.
Crofts, H.S., Dalley, J.W., Collins, P., Van Denderen, J.C., Everitt, B.J., Robbins, T.W., Roberts, A.C., 2001. Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cereb. Cortex 11, 1015–1026.
Darrah, J.M., Stefani, M.R., Moghaddam, B., 2008. Interaction of N-methyl-D-aspartate and group 5 metabotropic glutamate receptors on behavioral flexibility using a novel operant set-shift paradigm. Behav. Pharmacol. 19, 225–234.
Demakis, G.J., 2003. A meta-analytic review of the sensitivity of the Wisconsin Card Sorting Test to frontal and lateralized frontal brain damage. Neuropsychology 17, 255–264.
Dias, R., Robbins, T.W., Roberts, A.C., 1996. Dissociation in prefrontal cortex of affective and attentional shifts. Nature 380, 69–72.
Durstewitz, D., Seamans, J.K., 2008. The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-o-methyltransferase genotypes and schizophrenia. Biol. Psychiatry 64, 739–749.
Engle, J.R., Barnes, C.A., 2012. Characterizing cognitive aging of associative memory in animal models. Front. Aging Neurosci. 4, 10.
Enomoto, T., Tse, M.T., Floresco, S.B., 2011. Reducing prefrontal gamma-aminobutyric acid activity induces cognitive, behavioral, and dopaminergic abnormalities that resemble schizophrenia. Biol. Psychiatry 69, 432–441.
Floresco, S.B., Block, A.E., Tse, M.T., 2008. Inactivation of the medial prefrontal cortex of the rat impairs strategy set-shifting, but not reversal learning, using a novel, automated procedure. Behav. Brain Res. 190, 85–96.
Floresco, S.B., Magyar, O., 2006. Mesocortical dopamine modulation of executive functions: beyond working memory. Psychopharmacology (Berl.) 188, 567–585.
Floresco, S.B., Seamans, J.K., Phillips, A.G., 1997. Selective roles for hippocampal, prefrontal cortical, and ventral striatal circuits in radial-arm maze tasks with or without a delay. J. Neurosci. 17, 1880–1890.
Foster, T.C., Defazio, R.A., Bizon, J.L., 2012. Characterizing cognitive aging of spatial and contextual memory in animal models. Front. Aging Neurosci. 4, 12.
Freedman, M., Oscar-Berman, M., 1986. Bilateral frontal lobe disease and selective delayed response deficits in humans. Behav. Neurosci. 100, 337–342.
Frick, K.M., Baxter, M.G., Markowska, A.L., Olton, D.S., Price, D.L., 1995. Age-related spatial reference and working memory deficits assessed in the water maze. Neurobiol. Aging 16, 149–160.
Fuster, J.M., 2000. Executive frontal functions. Exp. Brain Res. 133, 66–70.
Gallagher, M., Burwell, R., Burchinal, M., 1993. Severity of spatial learning impairment in aging: development of a learning index for performance in the Morris water maze. Behav. Neurosci. 107, 618–626.
Gallagher, M., Rapp, P.R., 1997. The use of animal models to study the effects of aging on cognition. Annu. Rev. Psychol. 48, 339–370.
Gamboz, N., Borella, E., Brandimonte, M.A., 2009. The role of switching, inhibition and working memory in older adults' performance in the Wisconsin Card Sorting Test. Neuropsychol. Dev. Cogn. B Aging Neuropsychol. Cogn. 16, 260–284.
Glisky, E.L., 2007. Changes in cognitive function in human aging, in: Riddle, D.R. (Ed.), Brain Aging: Models, Methods, and Mechanisms. CRC Press, Boca Raton, pp. 3–19.
Goldman, P.S., Rosvold, H.E., 1970. Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. Exp. Neurol. 27, 291–304.
Goldman-Rakic, P.S., 1996. The prefrontal landscape: implications of functional architecture for understanding human mentation and the central executive. Philos. Trans. R. Soc. Lond. B Biol. Sci. 351, 1445–1453.
Hara, Y., Rapp, P.R., Morrison, J.H., 2012. Neuronal and morphological bases of cognitive decline in aged rhesus monkeys. Age (Dordr.) 34, 1051–1073.
Kesner, R.P., Churchwell, J.C., 2011. An analysis of rat prefrontal cortex in mediating executive function. Neurobiol. Learn. Mem. 96, 417–431.
Krugel, L.K., Biele, G., Mohr, P.N., Li, S.C., Heekeren, H.R., 2009. Genetic variation in dopaminergic neuromodulation influences the ability to rapidly and flexibly adapt decisions. Proc. Natl. Acad. Sci. U.S.A 106, 17951–17956.
LaSarge, C.L., Montgomery, K.S., Tucker, C., Slaton, G.S., Griffith, W.H., Setlow, B., Bizon, J.L., 2007. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. Neurobiol. Aging 28, 928–936.
Li, S.C., Lindenberger, U., Backman, L., 2010. Dopaminergic modulation of cognition across the life span. Neurosci. Biobehav. Rev. 34, 625–630.
McQuail, J.A., Banuelos, C., LaSarge, C.L., Nicolle, M.M., Bizon, J.L., 2012. GABA(B) receptor GTP-binding is decreased in the prefrontal cortex but not the hippocampus of aged rats. Neurobiol. Aging 33, 1124.e1–1124.e12.
Miller, E.K., Cohen, J.D., 2001. An integrative theory of prefrontal cortex function. Annu. Rev. Neurosci. 24, 167–202.
Mishkin, M., 1957. Effects of small frontal lesions on delayed alternation in monkeys. J. Neurophysiol. 20, 615–622.
Mizoguchi, K., Shoji, H., Tanaka, Y., Maruyama, W., Tabira, T., 2009. Age-related spatial working memory impairment is caused by prefrontal cortical dopaminergic dysfunction in rats. Neuroscience 162, 1192–1201.
Moore, T.L., Killiany, R.J., Herndon, J.G., Rosene, D.L., Moss, M.B., 2006. Executive system dysfunction occurs as early as middle-age in the rhesus monkey. Neurobiol. Aging 27, 1484–1493.
Moore, T.L., Schettler, S.P., Killiany, R.J., Herndon, J.G., Luebke, J.I., Moss, M.B., Rosene, D.L., 2005. Cognitive impairment in aged rhesus monkeys associated with monoamine receptors in the prefrontal cortex. Behav. Brain Res. 160, 208–221.
Morrison, J.H., Baxter, M.G., 2012. The ageing cortical synapse: hallmarks and implications for cognitive decline. Nat. Rev. Neurosci. 13, 240–250.
Murchison, D., McDermott, A.N., Lasarge, C.L., Peebles, K.A., Bizon, J.L., Griffith, W.H., 2009. Enhanced calcium buffering in F344 rat cholinergic basal forebrain neurons is associated with age-related cognitive impairment. J. Neurophysiol. 102, 2194–2207.
Nieves-Martinez, E., Haynes, K., Childers, S.R., Sonntag, W.E., Nicolle, M.M., 2012. Muscarinic receptor/G-protein coupling is reduced in the dorsomedial striatum of cognitively impaired aged rats. Behav. Brain Res. 227, 258–264.
Owen, A.M., Roberts, A.C., Polkey, C.E., Sahakian, B.J., Robbins, T.W., 1991. Extra-dimensional versus intra-dimensional set shifting performance following frontal lobe excisions, temporal lobe excisions or amygdalo-hippocampectomy in man. Neuropsychologia 29, 993–1006.
Park, D.C., 2000. The basic mechanisms accounting for age-related decline in cognitive function. In: Park, D.C., Schwartz, N. (Eds.), Cognitive Aging: A Primer. Psychology Press, Philadelphia, pp. 3–18.
Ragozzino, M.E., 2007. The contribution of the medial prefrontal cortex, orbitofrontal cortex, and dorsomedial striatum to behavioral flexibility. Ann. N. Y. Acad. Sci. 1121, 355–375.
Ragozzino, M.E., Adams, S., Kesner, R.P., 1998. Differential involvement of the dorsal anterior cingulate and prelimbic-infralimbic areas of the rodent prefrontal cortex in spatial working memory. Behav. Neurosci. 112, 293–303.
Ragozzino, M.E., Ragozzino, K.E., Mizumori, S.J., Kesner, R.P., 2002. Role of the dorsomedial striatum in behavioral flexibility for response and visual cue discrimination learning. Behav. Neurosci. 116, 105–115.
Ragozzino, M.E., Wilcox, C., Raso, M., Kesner, R.P., 1999. Involvement of rodent prefrontal cortex subregions in strategy switching. Behav. Neurosci. 113, 32–41.
Ramos, B.P., Birnbaum, S.G., Lindenmayer, I., Newton, S.S., Duman, R.S., Arnsten, A.F., 2003. Dysregulation of protein kinase a signaling in the aged prefrontal cortex: new strategy for treating age-related cognitive decline. Neuron 40, 835–845.
Rapp, P.R., Amaral, D.G., 1989. Evidence for task-dependent memory dysfunction in the aged monkey. J. Neurosci. 9, 3568–3576.
Ridderinkhof, K.R., Span, M.M., van der Molen, M.W., 2002. Perseverative behavior and adaptive control in older adults: performance monitoring, rule induction, and set shifting. Brain Cogn. 49, 382–401.
Robbins, T.W., 1996. Dissociating executive functions of the prefrontal cortex. Philos. Trans. R. Soc. Lond. B Biol. Sci. 351, 1463–1470. discusion, 70–71.
Robbins, T.W., Arnsten, A.F., 2009. The neuropsychopharmacology of fronto-executive function: monoaminergic modulation. Annu. Rev. Neurosci. 32, 267–287.
Robbins, T.W., James, M., Owen, A.M., Sahakian, B.J., Lawrence, A.D., McInnes, L., Rabbitt, P.M., 1998. A study of performance on tests from the CANTAB battery sensitive to frontal lobe dysfunction in a large sample of normal volunteers: implications for theories of executive functioning and cognitive aging. Cambridge Neuropsychological Test Automated Battery. J. Int. Neuropsychol. Soc. 4, 474–490.
Sawaguchi, T., Goldman-Rakic, P.S., 1991. D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251, 947–950.

Schoenbaum, G., Nugent, S., Saddoris, M.P., Gallagher, M., 2002. Teaching old rats new tricks: age-related impairments in olfactory reversal learning. Neurobiol. Aging 23, 555–564.

Segovia, G., Del Arco, A., de Blas, M., Garrido, P., Mora, F., 2008. Effects of an enriched environment on the release of dopamine in the prefrontal cortex produced by stress and on working memory during aging in the awake rat. Behav. Brain Res. 187, 304–311.

Shaw, C., Aggleton, J.P., 1993. The effects of fornix and medial prefrontal lesions on delayed non-matching-to-sample by rats. Behav. Brain Res. 54, 91–102.

Simon, N.W., LaSarge, C.L., Montgomery, K.S., Williams, M.T., Mendez, I.A., Setlow, B., Bizon, J.L., 2010. Good things come to those who wait: attenuated discounting of delayed rewards in aged Fischer 344 rats. Neurobiol. Aging 31, 853–862.

Sloan, H.L., Good, M., Dunnett, S.B., 2006. Double dissociation between hippocampal and prefrontal lesions on an operant delayed matching task and a water maze reference memory task. Behav. Brain Res. 171, 116–126.

Smith, T.D., Adams, M.M., Gallagher, M., Morrison, J.H., Rapp, P.R., 2000. Circuit-specific alterations in hippocampal synaptophysin immunoreactivity predict spatial learning impairment in aged rats. J. Neurosci. 20, 6587–6593.

Terry, C.P., Sliwinski, M.J., 2012. Aging and random task switching: the role of endogenous versus exogenous task selection. Exp. Aging Res. 38, 87–109.

Uylings, H.B., Groenewegen, H.J., Kolb, B., 2003. Do rats have a prefrontal cortex? Behav. Brain Res. 146, 3–17.

Vijayraghavan, S., Wang, M., Birnbaum, S.G., Williams, G.V., Arnsten, A.F., 2007. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nat. Neurosci. 10, 376–384.

Volkow, N.D., Gur, R.C., Wang, G.J., Fowler, J.S., Moberg, P.J., Ding, Y.S., Hitzemann, R., Smith, G., Logan, J., 1998a. Association between decline in brain dopamine activity with age and cognitive and motor impairment in healthy individuals. Am. J. Psychiatry 155, 344–349.

Volkow, N.D., Wang, G.J., Fowler, J.S., Ding, Y.S., Gur, R.C., Gatley, J., Logan, J., Moberg, P.J., Hitzemann, R., Smith, G., Pappas, N., 1998b. Parallel loss of presynaptic and postsynaptic dopamine markers in normal aging. Ann. Neurol. 44, 143–147.

Zeamer, A., Decamp, E., Clark, K., Schneider, J.S., 2011. Attention, executive functioning and memory in normal aged rhesus monkeys. Behav. Brain Res. 219, 23–30.