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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