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The Effects of Aging on Memory for Sequentially Presented Objects in Rats

Erin Hauser¹, Jerlyn C. Tolentino¹, Eva Pirogovsky², Erin Weston¹, and Paul E. Gilbert^{1,2}

¹Department of Psychology, San Diego State University, San Diego, CA

²San Diego State University-University of California San Diego, Joint Doctoral Program in Clinical Psychology, San Diego, CA

Abstract

The current study investigated memory for sequentially presented objects in young rats 6 months of age ($n=12$) and aged rats 24 months of age ($n=12$). Rats were tested on a task involving three exploratory trials and one probe test. During the exploratory trials, the rat explored a set of three sequentially presented object pairs (A-A, B-B, and C-C) for 5 min per pair with a 3 min delay between each pair. Following the exploratory trials, a probe test was conducted where the rat was presented simultaneously with one object from the first exploratory trial (A) and one object from the third exploratory trial (C). Results from the three exploratory trials showed no significant age-related differences in exploration, indicating that 24 month old rats explored the object pairs as much as 6 month old rats. Results from the probe test demonstrated that 6 month old rats spent significantly more time exploring object A compared to object C, indicating that young rats show intact memory for the temporal order of the exploratory trial objects. In contrast, 24 month old rats showed no preference for object A and spent a relatively equal amount of time exploring objects A and C. The results suggest that temporal order memory may decline as a result of age-related changes in the rodent brain. In addition, the findings may reflect differences in attraction to objects with different memory strengths. Since no significant age-related differences were detected during the exploratory trials, age-related differences on the probe trial were not due solely to decreased exploration, motivation, or locomotion in aged rats.

Keywords

Aging; Temporal Order; Sequence; Fischer 344 Brown Norway; Object Memory

Introduction

The ability to remember the temporal order of items or events in a sequence may be important for a variety of cognitive functions including episodic memory and executive function. Age-related impairment in temporal order memory has been well documented in older humans (Cabeza, Anderson, Houle, Mangels, & Nyberg, 2000; Daum, Graber, Schugens, & Mayes, 1996; Fabiani & Friedman, 1997; Golomb, Peelle, Addis, Kahana, & Wingfield, 2008;

Please address all correspondence to Dr. Paul Gilbert, SDSU-UCSD Joint Doctoral Program in Clinical Psychology, 6363 Alvarado Court, Suite 103, San Diego, CA 92120-4913. Tel: (619) 594-7409; Fax: (619) 594-3773; pgilbert@sciences.sdsu.edu.

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Hartman & Warren, 2005; Kahana, Howard, Zaromb, & Wingfield, 2002; Kessels, Hobbels, & Postma, 2007; Naveh-Benjamin, 1990; Old & Naveh-Benjamin, 2008; Parkin, Walter, & Hunkin, 1995; Schmitter-Edgecombe & Simpson, 2001). A recently published meta-analysis conducted by Old and Naveh-Benjamin (2008) examined 90 studies of episodic memory involving over 3000 older adults and found that temporal order memory is significantly impaired in older adults compared to young adults. However, other studies have reported normal temporal order memory in older adults (Sekuler, McLaughlin, Kahana, Wingfield, & Yotsumoto, 2006). Cabeza, Anderson, Joule, Mangels, and Nyberg (2000) used positron emission tomography (PET) to investigate brain activation of young and older adults during item retrieval and the retrieval of items in a temporal sequence. PET data revealed weak frontal activity in older adults compared to younger adults during temporal order retrieval. Despite well documented temporal order memory deficits in humans, few if any studies have examined temporal order memory in aged rodents. A number of researchers have emphasized the importance of the use of animal models to examine age-related changes in cognition and behavior (Brushfield, Luu, Callahan, & Gilbert, 2008; Driscoll, Howard, Stone, Monfils, Tomanek, & Brooks, 2006; Gallagher & Rapp, 1997; Schoenbaum, Nugent, Saddoris, & Gallagher, 2002; Solomon, Beal, & Pendlebury, 1988; Woodruff-Pak & Thompson, 1985). Therefore, the present study was designed to examine age-related deficits in temporal order memory in a rodent model of aging using a previously published rapidly administered exploration task (Hannesson, Howland, & Phillips, 2004a; Hoge & Kesner, 2007).

Fuster (2001) suggests that a primary function of the prefrontal cortex is in the temporal integration of information for the attainment of prospective behavioral goals. The prefrontal cortex may play a critical role in the organization of sequences of events due to its role in the mediation of cross-temporal contingencies (Fuster 2000, 2001). The prefrontal cortex may temporally organize fragments of information that are distributed spatially across cortical networks. Therefore, a critical function of the frontal lobes may be to support memory for the temporal order of items or events in a sequence. In humans, studies involving patients with frontal lobe damage (Daum & Mayes, 2000; Kesner, Hopkins, & Fineman, 1994; Lewinsohn, Zieler, Libet, Eyeberg, & Nielson, 1972; Milner, 1971; Milner, Petrides, & Smith, 1985; Petrides & Milner, 1982; Shimamura, 1995; Shimamura, Janowsky, & Squire, 1990; Szatkowska, Szymanska, & Grabowska, 2004) and neuroimaging studies involving healthy adults (Cabeza, Mangels, Nyberg, Habib, Houle, & McIntosh, 1997; Cabeza et al., 2000; Knutson, Wood, & Grafman, 2004; Oztekin, McElree, Staresina, & Davachi, 2009; Rowe & Passingham, 2001) have shown that the frontal lobes support temporal order memory. The frontal lobes also have been shown to play a role in temporal order memory in nonhuman primates (Inoue & Mikami, 2006; Ninokura, Mushiaki, & Tanji, 2003; Ninokura, Mushiaki, & Tanji, 2004) and rats (Barker, Bird, Alexander, Warburton, 2007; Chiba, Kesner, & Gibson, 1997; Hannesson, Vacca, Howland, & Phillips, 2004b).

The temporal lobes also may contribute to temporal order memory. In human studies, patients with relatively selective hippocampal damage demonstrate temporal order memory impairment (Downes, Mayes, MacDonald, & Hunkin, 2002; Hopkins, Kesner, & Goldstein, 1995a; Hopkins, Kesner, & Goldstein, 1995b; Mayes, Issac, Downes, Holdstock, Hunkin, & Montaldi, 2004). A recent functional magnetic resonance imaging study found extensive medial temporal lobe activity during the recall of sequences of scenes from a movie (Lehn, Steffenach, van Strien, Veltman, Witter, & Hayberg, 2009). Activation of the right hippocampus and bilateral parahippocampal cortices was related to retrieval of the temporal order of the scenes. Ekstrom and Bookheimer (2007) reported increased hippocampal activation during temporal order retrieval, whereas the parahippocampal cortex showed greater activation during spatial retrieval. Hippocampal lesions in rats result in impairments in temporal order memory for spatial, visual object, and olfactory stimuli (Agster, Fortin, & Eichenbaum, 2002; Chiba, Kesner, & Reynolds, 1994; DeCoteau & Kesner, 2000; Ergorul & Eichenbaum, 2006; Fortin,

Agster, & Eichenbaum, 2002; Gilbert, Kesner, & Lee, 2001; Hoang & Kesner, 2008; Hoge & Kesner, 2007; Howland, Harrison, Hannesson, & Phillips, 2008; Kesner, Gilbert, & Barua, 2002; Kesner, Hunsaker, & Gilbert, 2005; Lee, Jerman, & Kesner, 2005; Manns, Howard, & Eichenbaum, 2007). Memory for the temporal order of objects also is impaired in rats following a lesion of the perirhinal cortex (Barker et al., 2007). Studies have demonstrated that contralateral lesions or inactivations of the medial prefrontal cortex and the perirhinal cortex may produce substantial deficits in temporal order memory in rats (Barker et al., 2007; Hannesson et al., 2004a). Therefore, the aforementioned studies provide strong evidence that the frontal and temporal lobes play a critical role in memory for the temporal order of items or events in a sequence.

Age-related functional and structural changes have been documented in regions of the brain shown to play a role in temporal order memory including the frontal lobes and the temporal lobes. Magnetic resonance imaging studies in nondemented older adults demonstrated that increased age was associated with volumetric decreases in the frontal lobes (Coffey, Wilkinson, Parashos, Soady, Sullivan, Patterson, Figiel, Webb, Spritzer, & Djang, 1992; Jernigan, Archibald, Fennema-Notestine, Gamst, Stout, Bonner, & Hesselink, 2001; Raz, Gunning-Dixon, Head, Dupuis, & Acker, 1997) and temporal lobes (Coffey et al., 1992; Raz et al., 1997) including the parahippocampal cortex (Jack, Petersen, Xu, Waring, O'Brien, Tangalos, Smith, Ivnik, & Kokmen, 1997) and hippocampus (Coffey et al., 1992; Jack et al., 1997; Jernigan et al., 2001). However, another study reported little or no volumetric decreases in the hippocampus in healthy older adults (Good, Johnsrude, Ashburner, Henson, Friston & Frackowiak, 2001). Age-related decreases in brain weight and decreases in cortical thickness in midfrontal and superior temporal regions have been reported in a histological study; however, neuronal densities were similar to young brains (Terry, DeTeresa, & Hansen, 1987). One recent study (Smith, Rapp, McKay, Roberts, & Tuszynski, 2004) reported localized cell loss in the prefrontal cortex (area 8A) in nonhuman primates with minimal cell loss in adjacent prefrontal areas (area 46). However, other studies have reported no age-related cell loss in the frontal cortex in humans and nonhuman primates (O'Donnell, Rapp, & Hof, 1999; Peters, 2002; Peters, Rosene, Moss, Kemper, Abraham, & Tigges, 1996). Recent studies have reported an extensive loss of white matter myelinated fiber in the cerebral cortex, including the forebrain, of nonhuman primates and humans (Peters & Rosene, 2003). A study by Small, Tsai, DeLaPaz, Mayeux, and Stern (2002) showed that functional magnetic resonance imaging (fMRI) signal intensity declined in all subregions of the hippocampus (dentate gyrus, CA1, and subiculum) and the entorhinal cortex in older adults. In addition, Driscoll et al. (2006) conducted in vivo MRI in aged rats and found decreased hippocampal volume. The study also conducted histopathological analyses of the aging rat brain and reported reduced neuronal density in the hippocampus. Based on the aforementioned data, age-related changes have been well documented in the temporal and frontal lobes, regions of the brain shown to be highly involved in temporal order memory.

Although age-related impairments in temporal order memory have been well documented in older humans, few if any studies have examined temporal order memory in aged rodents. Driscoll et al., (2006) report that the observed pattern of age-related change in aged Fisher 344/Brown Norway rats closely resembles the changes found in nondemented older adults. Therefore, this strain of rats may serve as a useful animal model to study the effects of aging on cognitive function. The present study examined memory for sequentially presented objects in 24 month old Fisher 344/Brown Norway rats to determine if aging has a similar effect on temporal order memory in a rodent model of aging.

Methods

Subjects

Twenty-four Fischer 344/Brown Norway male rats (Harlan Laboratories) approximately 6 months ($n=12$) and 24 months ($n=12$) of age were used in this experiment. The strain is an inbred hybrid offspring of a cross between an inbred male Brown Norway rat and a female Fischer 344. Rats were individually housed on sawdust bedding in standard plastic cages with wire top lids covered with isolator tops and were located in a colony room. Rats had unlimited access to food and water. The colony room was maintained on a 12h:12h light/dark cycle and all testing was conducted during the light phase.

Apparatus

The testing apparatus consisted of a 50 cm $\times$ 100 cm wooden rectangular box with 60 cm high walls. All surfaces of the box were painted gray. A video camera was mounted above the apparatus to record the behavior of an animal during testing.

Stimuli

Various visually dissimilar objects 10-15 cm in height were used for the task, for example a spray nozzle for a garden hose, a toy pony, and a plastic hand soap dispenser. The objects were similar to those used in previously published experiments (Gilbert & Kesner, 2002, 2003). Objects were placed on Velcro strips 30 cm apart to prevent a rat from displacing the objects. Multiple copies of each object were used during testing to minimize specific olfactory cues associated with a particular object.

Task Procedure

Rats were tested on a task used in previous studies that has been suggested to measure temporal order memory (Hannesson et al., 2004b; Hoge & Kesner, 2007). Each rat was handled and habituated to the testing apparatus for two consecutive days prior to being tested on the temporal order memory task. The habituation process consisted of placing the rat into the box and allowing the animal to explore for 10 min. A WINTV2000 software program recorded all trials via a camera mounted above the apparatus. During all test trials, object exploration was defined as the animal's nose entering a 1 cm halo around the perimeter of the object as measured by the Object Scan software package (Clever Sys. Inc.). The Object Scan software enables the researcher to specifically track the nose of the animal to accurately measure object exploration. The task consisted of four trials: three exploratory trials and one probe test. During the first exploratory trial, the rat was placed in the center of the box between the two identical novel objects (A-A) and was allowed to explore for either 20 s of accumulated object exploration or until 5 min was reached (Ennaceur, Neave, & Aggleton, 1997). After this first exploratory trial, there was a 3 min delay in which the rat was returned to the home cage. After the delay, a second exploratory trial was conducted involving a new set of identical novel objects (B-B) and the rat once again was returned to the home cage after completion of the trial. An additional 3 min delay was then followed by a third exploratory trial involving a third set of identical novel objects (C-C). Following the third exploration trail, the rat was given an additional 3 min delay in the home cage prior to a probe test. During the probe test, one of the objects from the first exploratory trial (A) and one of the objects from the third exploratory trial (C) was placed in the box. The rat was placed back in the box and exploration was recorded for 2 min and then the rat was returned to the home cage. The amount of time the rat spent exploring each of the objects (A and C) was used as the dependent measure for the probe test. During the exploratory trials, object pairs (A-A, B-B and C-C) were assigned randomly for each rat. During the probe test, the position of Object A and Object C was randomized with regard to left or right positioning.

Results

Exploratory Trials

A $2 \times 3 \times 2$ analysis of variance (ANOVA) with group (6 months, 24 months) as a between group factor and exploratory trial (A-A, B-B, C-C) and object position during the exploratory trials (left, right) as a within group factors revealed no significant main effects of group $F(1,22) = 2.19, p=0.15$, trial $F(2,44) = 1.11, p=0.34$, or position $F(1,22) = 1.65, p=0.21$. In addition, the analysis did not detect a significant group $\times$ trial interaction $F(2,44) = 0.70, p=0.50$, a group $\times$ position interaction $F(1,22) = 0.29, p=0.59$, a trial $\times$ position interaction $F(2,44) = 0.08, p=0.93$, or a group $\times$ trial $\times$ position interaction $F(2,44) = 0.41, p=0.66$. As shown in Figure 1, the data indicate that there were no significant differences in exploration of any particular object pair or object position by 6 month and 24 month old rats during the exploratory trials.

Probe Test

A 2×2 ANOVA with group (6 months, 24 months) as a between group factor and object (A, C) as a within group factor revealed no significant main effect of group $F(1,22) = 2.80, p=0.11$. However, the analysis detected a significant main effect of object $F(1,22) = 6.16, p<.05$ and group $\times$ object interaction $F(1,22) = 4.44, p<.05$. As shown in Figure 2, a Newman Keuls post hoc comparison test of the group $\times$ object interaction revealed that young rats spent significantly more time ($p < .05$) exploring object A compared to object C during the probe test, indicating that young rats show intact memory for the temporal order of the exploratory trial objects. In contrast, old rats showed no preference for object A and spent a relatively equal amount of time exploring objects A and C.

Discussion

The main objective of the present study was to examine memory for sequentially presented objects in healthy young and aged rats. The current findings demonstrate that on the probe test when one object from the first exploratory trial (A) and one object from the third exploratory trial (C) were presented simultaneously, young rats explored object A significantly more than object C. In contrast, aged rats did not show a preference and showed equal exploration of both objects A and C. Previous studies investigating temporal order memory using the present paradigm suggest that increased exploration of the more novel object A during the probe test demonstrates intact memory for the temporal order of the object pairs from the exploratory trials (Hannesson, et al., 2004a; Hoge & Kesner, 2007; Hunsaker, Fieldsted, Rosenberg, & Kesner, 2008; Mitchell & Laiacina, 1998). Object A is suggested to be more novel than object C because it was presented further in time from the probe test (exploratory trial 1) than object C (exploratory trial 3). The results of the probe test suggest that temporal order memory may be impaired in aged rats. Although 6 month old rats appear to show increased exploration during the probe trial (Figure 1), the analysis of the data did not detect a statistically significant main effect of group, indicating that overall exploration of objects A and C combined did not differ statistically between groups. The lack of a main effect of group suggests that age-related differences during the probe test were not solely due to differences in exploration, motivation, or locomotion. This finding further supports the hypothesis that temporal order memory may be impaired in a rodent model of aging. The findings add to the growing literature demonstrating age-related learning and memory impairments in rodent models of aging (Bizon, LaSarge, Montgomery, McDermott, Setlow, & Griffith, 2009; Brushfield et al., 2008; Kraemer & Apfelbach, 2004; LaSarge, Montgomery, Tucker, Slaton, Griffith, Setlow, & Bizon, 2007; Luu, Pirogovsky, & Gilbert, 2008; Renteria, Silbaugh, Tolentino, & Gilbert, 2008; Robitsek, Fortin, Koh, Gallagher, & Eichenbaum, 2008; Roman, Alescio-Lautier, & Soumireu-Mourat, 1996; Schoenbaum et al., 2002; Shukitt-Hale, McEwen, Szprengiel, & Joseph, 2004).

The results from the exploratory trials revealed no significant age-related differences in overall exploration of the novel object pairs. These data provide further evidence that between group differences on the probe test were not due to age-related differences in overall exploration. In addition, young and aged rats showed no significant preference for any of the object pairs (A-A, B-B, or C-C). These findings suggest that any age-related differences in performance on the probe test were not due to preference for one object over another. The left vs. right side positioning of the objects during the exploratory trials also had no significant effect on exploration in the young and aged rats. Young and aged rats showed equal exploration of both left and right positioned objects, suggesting no positional bias. The findings from the exploratory trials further support the finding that the age-related differences detected during the probe test were not due to differences in exploration, motivation, or locomotion. Furthermore, previous studies using the same strain of rats in our lab have reported no significant differences between 6 month and 24 month old rats in visual object discrimination (Brushfield et. al, 2008). Therefore, the deficits observed in 24-month old rats on the probe test were not due solely to age-related impairments in visual discrimination or visual function.

Prior studies have reported impaired temporal order memory for spatial, visual object, and olfactory stimuli in rats with lesions or inactivations of the temporal or frontal lobes (Agster et al., 2002; Barker et al., 2007; Chiba et al., 1994; Decoteau & Kesner, 2000; Ergorul & Eichenbaum, 2006; Fortin et al., 2002; Gilbert et al., 2001; Hannesson et al., 2004a,b; Hoang & Kesner, 2008; Hoge & Kesner, 2007; Howland et al., 2008; Hunsaker et. al, 2008; Kesner & Novak, 1982; Kesner et al., 2002; Kesner et al., 2005; Mitchell & Laiacona, 1998; Lee et al., 2005; Manns et al., 2007). In particular, Hoge and Kesner (2007) tested rats with lesions in either the dorsal CA1 or CA3 subregions of the hippocampus on the task used in the present study. Rats with dorsal CA3 lesions did not differ from controls and spent more time exploring the object from the first exploratory trial (A) compared to the object from the third exploratory trial (C). However, rats with lesions in the CA1 subregion of the hippocampus showed significant impairments on the probe test and demonstrated increased exploration of object C relative to object A. These findings suggest that the CA1 subregion of the hippocampus may be particularly important for performance of the present task. Mitchell and Laiacona (1998) tested rats with medial frontal cortex lesions on a task similar to the present task and found that the lesions also disrupted temporal order memory. In another study, rats with crossed inactivations of perirhinal cortex and the medial prefrontal cortex showed significant impairments on the probe test of the present task compared to controls (Hannesson et al., 2004a). However, rats with unilateral inactivations of either structure alone matched the performance on controls. Therefore, the deficits observed in aged rats on the present task may be the result of age-related changes in the temporal lobes, frontal lobes, interconnections between the temporal and frontal lobes, or a combination of the aforementioned changes.

As mentioned previously, age-related impairments in temporal order memory have been well documented in older humans (Cabeza, et al., 2000; Daum, et al., 1996; Fabiani & Friedman, 1997; Golomb et al., 2008; Hartman & Warren, 2005; Kahana et al., 2002; Kessels et al., 2007; Naveh-Benjamin, 1990; Old & Naveh-Benjamin, 2008; Parkin et al., 1995; Schmitter-Edgecombe & Simpson, 2001). Episodic memory involves the ability to remember an event and locate that event in a time and place (Kesner, Gilbert, & Wallenstein, 2000; Nyberg, McIntosh, Cabeza, Habib, Houle, & Tulving, 1996; Tulving, 2002). Therefore, older adults who show deficits in temporal order processing may experience difficulty processing the temporal aspects of episodic memory. In addition, age-related deficits in temporal order memory could be associated with problems in executive function. Fuster (2001) purports that a key function of the prefrontal cortex is the temporal integration of information for the attainment of prospective behavioral goals. The prefrontal cortex is thought to mediate contingencies across time, which is a fundamental aspect of the temporal organization of events (Fuster, 2001). Thus, the frontal cortex may support processes that organize sequences of

events. Disruption in these processes may lead to impairments in cognitive domains that rely on goal directed behavior. Thus, deficits in temporal order memory in older adults may be associated with impairments in executive function, a cognitive domain that is essential to the execution of activities of daily living tasks.

Although the present results indicate that temporal order memory may be impaired in aged rats, it is possible that the findings may reflect differences in attraction to objects with different memory strengths. Young rats may show heightened exploration of an object with less memory strength (i.e. object A experienced during exploratory trial 1) compared to an object with a greater memory strength (i.e. object C experienced during exploratory trial 3). Previous studies have demonstrated that aged rats display deficits in some aspects of object recognition (Robitsek et al., 2008). Therefore, it is possible that old rats may forget the objects from the exploratory trials more rapidly, so by the probe trial, the memory strengths for object A and object C may not differ. As a result, age-related differences in attraction to objects with different memory strengths may contribute to the deficits observed on the present task. Many of the temporal order paradigms published for use in rats require extensive shaping and testing (Fortin et al., 2002; Gilbert et al., 2001; Kesner et al., 2002). Therefore, the paradigms may not be well suited for use in aged rats. Although there may be limitations to the present paradigm, the task may be attractive to researchers studying age-related changes in temporal order memory in a rodent model because it is a rapidly administered task that has been characterized in rats with lesions in brain regions affected by aging (Hannesson et al., 2004a; Hoge & Kesner, 2007; Mitchell & Laiacona, 1998).

Many previous behavioral studies involving aged rats have used 24 mo old Fisher 344 rats (F344). The present study was conducted using a Fischer 344/Brown Norway (F344/BN) hybrid strain. The F344/BN strain has been shown to live longer than Fischer 344 rats. The 50% survival age for F344/BN male rats is 34 mo, whereas the 50% survival age for F344 male rats is 24 mo (National Institute on Aging). Therefore, the rats in the present study showed significant memory impairments at 24 months of age despite having longer average longevity than other strains of rats used in prior behavioral experiments.

In conclusion, the results of the present study indicate that temporal order memory may be impaired in aged rats. In addition, it is possible that the findings may reflect differences in attraction to objects with different memory strengths. Since no significant age-related differences were detected during the exploratory trials, age-related differences on the probe trial were not due solely to group differences in exploration, motivation, locomotion, or spatial bias. An impairment in temporal order memory may be related to the general decline in episodic memory and executive function associated with aging. Age-related impairments in temporal order memory have been well documented in humans. However, the present study may be the first to document impaired temporal order memory in a rodent model of aging. The present findings suggest that aged rodents may be a suitable animal model for studying the effects of aging on temporal order memory. In addition, the present task may be very attractive to researchers examining age-related temporal order memory changes in aged rats because the task is rapidly administered. Thus, the results may have important implications for the selection of memory paradigms for future research studies on aging. Driscoll et al. (2006) report that the observed pattern of age-related change in aged Fisher 344/Brown Norway rats closely resembles the changes found in nondemented older adults. Therefore, this strain of rats may serve as a useful animal model to study the effects of aging on cognitive function.

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References

- Agster KL, Fortin NJ, Eichenbaum H. The hippocampus and disambiguation of overlapping sequences. *Journal of Neuroscience* 2002;22:5760–5768. [PubMed: 12097529]
- Barker GR, Bird F, Alexander V, Warburton EC. Recognition memory for objects, place, and temporal order: A disconnection analysis of the role of the medial prefrontal cortex and perirhinal cortex. *The Journal of Neuroscience* 2007;27:2948–2957. [PubMed: 17360918]
- Bizon JL, LaSarge CL, Montgomery KS, McDermott AN, Setlow B, Griffith WH. Spatial reference and working memory across the lifespan of male Fischer 344 rats. *Neurobiology of Aging* 2009;30:646–655. [PubMed: 17889407]
- Brushfield AM, Luu TT, Callahan BD, Gilbert PE. A comparison of discrimination and reversal learning for olfactory and visual stimuli in aged rats. *Behavioral Neuroscience* 2008;122:54–62. [PubMed: 18298249]
- Cabeza R, Anderson ND, Houle S, Mangels JA, Nyberg L. Age-related differences in neural activity during item and temporal-order memory retrieval: A positron emission tomography study. *Journal of Cognitive Neuroscience* 2000;12:197–206. [PubMed: 10769316]
- Cabeza R, Mangels J, Nyberg L, Habib R, Houle S, McIntosh AR. Brain regions differentially involved in remembering what and when: A PET study. *Neuron* 1997;19:864–870.
- Chiba AA, Kesner RP, Gibson CJ. Memory for temporal order of new and familiar spatial location sequences: Role of the medial prefrontal cortex. *Learning and Memory* 1997;4:311–317. [PubMed: 10706368]
- Chiba AA, Kesner RP, Reynolds AM. Memory for spatial location as a function of temporal lag in rats: Role of hippocampus and medial prefrontal cortex. *Behavioral and Neural Biology* 1994;61:123–131. [PubMed: 8204078]
- Coffey CE, Wilkinson WE, Parashos IA, Soady SAR, Sullivan RJ, Patterson LJ, Figiel GS, Webb MC, Spritzer CE, Djang WT. Quantitative cerebral anatomy of the aging human brain: A cross-sectional study using magnetic resonance imaging. *Neuropsychology* 1992;42:527–36.
- Daum I, Graber S, Schugens MM, Mayes AR. Memory dysfunction of the frontal type in normal ageing. *Neuroreport* 1996;7:2625–2628. [PubMed: 8981435]
- Daum I, Mayes AR. Memory and executive function impairments after frontal or posterior cortex lesions. *Behavioural Neurology* 2000;12:161–173. [PubMed: 11568428]
- DeCoteau WE, Kesner RP. A double dissociation between the rat hippocampus and medial caudoputamen in processing two forms of knowledge. *Behavioral Neuroscience* 2000;114:1096–1108. [PubMed: 11142642]
- Downes JJ, Mayes AR, MacDonald C, Hunkin NM. Temporal order memory in patients with Korsakoff's syndrome and medial temporal amnesia. *Neuropsychologia* 2002;40:853–861. [PubMed: 11900736]
- Driscoll I, Howard SR, Stone JC, Monfils MH, Tomanek B, Brooks WM. The aging hippocampus: A multi-level analysis in the rat. *Neuroscience* 2006;139:1173–1185. [PubMed: 16564634]
- Ekstrom AD, Bookheimer SY. Spatial and temporal episodic memory retrieval recruit dissociable functional networks in the human brain. *Learning and Memory* 2007;14:645–654. [PubMed: 17893237]
- Ennaceur A, Neave N, Aggleton JP. Spontaneous object recognition and object location memory in rats: The effects of lesions in the cingulate cortices, the medial prefrontal cortex, the cingulum bundle and the fornix. *Experimental Brain Research* 1997;113:509–519.
- Ergorul C, Eichenbaum H. Essential role of the hippocampal formation in rapid learning of higher-order sequential associations. *Journal of Neuroscience* 2006;26:4111–7. [PubMed: 16611828]
- Fabiani M, Friedman D. Dissociations between memory for temporal order and recognition memory in aging. *Neuropsychologia* 1997;35:129–141. [PubMed: 9025117]
- Fortin NJ, Agster KL, Eichenbaum H. Critical role of the hippocampus in memory for sequences of events. *Nature Neuroscience* 2002;5:458–462.
- Fuster JM. Executive frontal functions. *Experimental Brain Research* 2000;133:66–70.
- Fuster JM. The prefrontal cortex—an update: Time is of the essence. *Neuron* 2001;30:319–333. [PubMed: 11394996]

- Gallagher M, Rapp PR. The use of animal models to study the effects of aging on cognition. *Annual Review of Psychology* 1997;48:339–370.
- Gilbert PE, Kesner RP. Recognition memory for complex visual discriminations is influenced by stimulus interference in rodents with perirhinal cortex damage. *Learning and Memory* 2003;10:525–30. [PubMed: 14657264]
- Gilbert PE, Kesner RP. Role of the rodent hippocampus in paired-associate learning involving associations between a stimulus and a spatial location. *Behavioral Neuroscience* 2002;116:63–71. [PubMed: 11895184]
- Gilbert PE, Kesner RP, Lee I. Dissociating hippocampal subregions: A double dissociation between dentate gyrus and CA1. *Hippocampus* 2001;11:626–636. [PubMed: 11811656]
- Golomb JD, Peelle JE, Addis KM, Kahana MJ, Wingfield A. Effects of adult aging on utilization of temporal and semantic associations during free and serial recall. *Memory & Cognition* 2008;36:947–956.
- Good CD, Johnsrude IS, Ashburner J, Henson RN, Friston KJ, Frackowiak RS. A voxel-based morphometric study of ageing in 465 normal adult human brains. *Neuroimage* 2001;14:21–36. [PubMed: 11525331]
- Hannesson DK, Howland JG, Phillips AG. Interaction between perirhinal and medial prefrontal cortex is required for temporal order but not recognition memory for objects in rats. *Journal of Neuroscience* 2004a;24:4596–4604. [PubMed: 15140931]
- Hannesson DK, Vacca G, Howland JG, Phillips AG. Medial prefrontal cortex is involved in spatial temporal order memory but not spatial recognition memory in tests relying on spontaneous exploration in rats. *Behavioral Brain Research* 2004b;153:273–285.
- Hartman M, Warren LH. Explaining age differences in temporal working memory. *Psychology and Aging* 2005;20:645–656. [PubMed: 16420139]
- Hoang LT, Kesner RP. Dorsal hippocampus, CA3, and CA1 lesions disrupt temporal sequence completion. *Behavioral Neuroscience* 2008;122:9–15. [PubMed: 18298244]
- Hoge J, Kesner RP. Role of CA3 and CA1 subregions of the dorsal hippocampus on temporal processing of objects. *Neurobiology of Learning and Memory* 2007;88:225–231. [PubMed: 17560815]
- Hopkins RO, Kesner RP, Goldstein M. Memory for novel and familiar spatial and linguistic temporal distance information in hypoxic subjects. *Journal of the International Neuropsychological Society* 1995a;1:454–468. [PubMed: 9375231]
- Hopkins RO, Kesner RP, Goldstein M. Item and order recognition memory in subjects with hypoxic brain injury. *Brain and Cognition* 1995b;27:180–201. [PubMed: 7772332]
- Howland JG, Harrison RA, Hannesson DK, Phillips AG. Ventral hippocampal involvement in temporal order, but not recognition, memory for spatial information. *Hippocampus* 2008;18:251–257. [PubMed: 18064705]
- Hunsaker MR, Fieldsted PM, Rosenberg JS, Kesner RP. Dissociating the roles of dorsal and ventral CA1 for the temporal processing of spatial locations, visual objects, and odors. *Behavioral Neuroscience* 2008;122:643–650. [PubMed: 18513134]
- Inoue M, Mikami A. Prefrontal activity during serial probe reproduction task: encoding, mnemonic, and retrieval processes. *Journal of Physiology* 2006;95:1008–1041.
- Jack CR Jr, Petersen RC, Xu YC, Waring SC, O'Brien PC, Tangalos EG, Smith GE, Ivnik RJ, Kokmen E. Medial temporal atrophy on MRI in normal aging and very mild Alzheimer's disease. *Neurology* 1997;49:786–94. [PubMed: 9305341]
- Jernigan TL, Archibald SL, Fennema-Notestine C, Gamst AC, Stout JC, Bonner J, Hesselink JR. Effects of age on tissues and regions of the cerebrum and cerebellum. *Neurobiology of Aging* 2001;22:581–94. [PubMed: 11445259]
- Kahana MJ, Howard MW, Zaromb F, Wingfield A. Age dissociates recency and lag recency effects in free recall. *Journal of Experimental Psychology, Learning, Memory, and Cognition* 2002;28:530–540.
- Kesner RP, Gilbert PE, Barua LA. The role of the hippocampus in memory for the temporal order of a sequence of odors. *Behavioral Neuroscience* 2002;116:286–290. [PubMed: 11996313]
- Kesner RP, Gilbert PE, Wallenstein GV. Testing neural network models of memory with behavioral experiments. *Current Opinion in Neurobiology* 2000;10:260–265. [PubMed: 10753789]

- Kesner RP, Hopkins RO, Fineman B. Item and order dissociation in humans with prefrontal cortex damage. *Neuropsychologia* 1994;32:881–891. [PubMed: 7969864]
- Kesner RP, Hunsaker MR, Gilbert PE. The role of CA1 in the acquisition of an object-trace-odor paired associate task. *Behavioral Neuroscience* 2005;119:781–786. [PubMed: 15998199]
- Kesner RP, Novak JM. Serial position curve in rats: Role of the dorsal hippocampus. *Science* 1982;218:173–175. [PubMed: 7123228]
- Kessels RP, Hobbel D, Postma A. Aging, context memory and binding: A comparison of “what, where and when” in young and older adults. *International Journal of Neuroscience* 2007;117:795–810. [PubMed: 17454244]
- Knutson KM, Wood JN, Grafman J. Brain activation in processing temporal sequence: An fMRI study. *NeuroImage* 2004;23:1299–1307. [PubMed: 15589094]
- Kraemer S, Apfelbach R. Olfactory sensitivity, learning and cognition in young adult and aged male wistar rats. *Physiological Behavior* 2004;81:435–442.
- LaSarge CL, Montgomery KS, Tucker C, Slaton GS, Griffith WH, Setlow B, Bizon JL. Deficits across multiple cognitive domains in a subset of aged Fischer 344 rats. *Neurobiology of Aging* 2007;28:928–936. [PubMed: 16806587]
- Lee I, Jerman TS, Kesner RP. Disruption of delayed memory for a sequence of spatial locations following CA1-or CA3-lesions of the dorsal hippocampus. *Neurobiology of Learning and Memory* 2005;84:138–147. [PubMed: 16054848]
- Lehn H, Steffenach HA, van Strien NM, Veltman DJ, Witter MP, Hayberg AK. A specific role of the human hippocampus in recall of temporal sequences. *Journal of Neuroscience* 2009;29:3475–3484. [PubMed: 19295153]
- Lewinsohn PM, Zieler RE, Libet J, Eyeberg S, Nielson G. Short-term memory: a comparison between frontal and nonfrontal right- and left-hemisphere brain-damaged patients. *Journal of Comparative and Physiological Psychology* 1972;81:248–255. [PubMed: 5084440]
- Luu TT, Pirogovsky E, Gilbert PE. Age-related changes in contextual associative learning. *Neurobiology of Learning and Memory* 89:81–85. in press. [PubMed: 17967551]
- Manns JR, Howard MW, Eichenbaum H. Gradual changes in hippocampal activity support remembering the order of events. *Neuron* 2007;56:530–540. [PubMed: 17988635]
- Mayes AR, Holdstock JS, Isaac CL, Montaldi D, Grigor J, Gummer A, Cariga P, Downes JJ, Tsivilis D, Gaffan D, Gong Q, Norman KA. Associative recognition in a patient with selective hippocampal lesions and relatively normal item recognition. *Hippocampus* 2004;14:763–784. [PubMed: 15318334]
- Milner B. Interhemispheric differences in the localization of psychological processes in man. *Brain Medical Bulletin* 1971;27:272–277.
- Milner B, Petrides M, Smith ML. Frontal lobes and the temporal organization of memory. *Human Neurobiology* 1985;4:137–142. [PubMed: 4066424]
- Mitchell JB, Laiacona J. The medial prefrontal cortex and temporal memory: Tests using spontaneous exploratory behaviour in the rat. *Behavioral Brain Research* 1998;97:107–113.
- Naveh-Benjamin M. Coding of temporal order information: An automatic process? *Journal of Experimental Psychology: Learning, Memory, and Cognition* 1990;16:117–126.
- Ninokura Y, Mushiaki H, Tanji J. Integration of temporal order and object information in the monkey lateral prefrontal cortex. *Journal of Neurophysiology* 2004;91:555–60. [PubMed: 12968014]
- Ninokura Y, Mushiaki H, Tanji J. Representation of the temporal order of visual objects in the primate lateral prefrontal cortex. *Journal of Neurophysiology* 2003;89:2868–2873. [PubMed: 12740417]
- Nyberg L, McIntosh AR, Cabeza R, Habib R, Houle S, Tulving E. General and specific brain regions involved in encoding and retrieval of events: What, where, and when. *Proceedings of the National Academy of Sciences of the United States of America* 1996;93:11280–11285. [PubMed: 8855347]
- O'Donnell KA, Rapp PR, Hof PR. Preservation of prefrontal cortical volume in behaviorally characterized aged macaque monkeys. *Experimental Neurology* 1999;160:300–310. [PubMed: 10630214]
- Old SR, Naveh-Benjamin M. Differential effects of age on item and associative measures of memory: A meta-analysis. *Psychology and Aging* 2008;23:104–118. [PubMed: 18361660]

- Oztekin I, McElree B, Staresina BP, Davachi L. Working memory retrieval contributions of the left prefrontal cortex, the left posterior parietal cortex and the hippocampus. *Journal of Cognitive Neuroscience* 2009;21:581–593. [PubMed: 18471055]
- Parkin AJ, Walter BM, Hunkin NM. Relationships between normal aging, frontal lobe function, and memory for temporal and spatial information. *Neuropsychology* 1995;9:304–312.
- Peters A. Structural changes in the normally aging cerebral cortex of primates. *Progress in Brain Research* 2002;136:455–465. [PubMed: 12143402]
- Peters A, Rosene DL. In aging, is it gray or white? *Journal of Comparative Neurology* 2003;462:139–43. [PubMed: 12794738]
- Peters A, Rosene DL, Moss MB, Kemper TL, Abraham CR, Tigges J. Neurobiological bases of age-related cognitive decline in the rhesus monkey. *Journal of Neuropathology and Experimental Neurology* 1996;55:861–874. [PubMed: 8759775]
- Petrides M, Milner B. Deficits on subject-ordered tasks after frontal- and temporal-lobe lesions in man. *Neuropsychologia* 1982;20:249–262. [PubMed: 7121793]
- Raz N, Gunning-Dixon FM, Head D, Dupuis JH, Acker JD. Neuroanatomical correlates of cognitive aging: evidence from structural magnetic resonance imaging. *Neuropsychology* 1997;12:95–114. [PubMed: 9460738]
- Renteria AF, Silbaugh BC, Tolentino JC, Gilbert PE. Age-related changes in conditioned flavor preference in rats. *Behavioral Brain Research* 2008;188:56–61.
- Robitsek RJ, Fortin NJ, Koh MT, Gallagher M, Eichenbaum H. Cognitive aging: a common decline of episodic recollection and spatial memory in rats. *Journal of Neuroscience* 2008;28:8945–8954. [PubMed: 18768688]
- Roman FS, Alescio-Lautier B, Soumireu-Mourat B. Age-related learning and memory deficits in odor-reward association in rats. *Neurobiology of Aging* 1996;17:31–40. [PubMed: 8786800]
- Rowe JB, Passingham RE. Working memory for location and time: activity in prefrontal area 46 relates to selection rather than maintenance in memory. *Neuroimage* 2001;14:77–86. [PubMed: 11525340]
- Schmitter-Edgecombe M, Simpson AL. Effects of age and intentionality on content memory and temporal memory for performed activities. *Aging, Neuropsychology, and Cognition* 2001;8:81–97.
- Schoenbaum G, Nugent S, Saddoris MP, Gallagher M. Teaching old rats new tricks: Age related impairments in olfactory reversal learning. *Neurobiology of Aging* 2002;23:555–564. [PubMed: 12009505]
- Sekuler R, McLaughlin C, Kahana MJ, Wingfield A, Yotsumoto Y. Short-term visual recognition and temporal order memory are both well-preserved in aging. *Psychology and Aging* 2006;21:632–637. [PubMed: 16953725]
- Shimamura AP. Memory and the prefrontal cortex. *Annals of the New York Academy of Sciences* 1995;769:151–159. [PubMed: 8595022]
- Shimamura AP, Janowsky JS, Squire LR. Memory for the temporal order of events in patients with frontal lobe lesions and amnesic patients. *Neuropsychologia* 1990;28:803–813. [PubMed: 2247207]
- Shukitt-Hale B, McEwen JJ, Szprengiel A, Joseph JA. Effect of age on the radial arm water maze—a test of spatial learning and memory. *Neurobiology of Aging* 2004;25:223–229. [PubMed: 14749140]
- Small SA, Tsai WY, DeLaPaz R, Mayeux R, Stern Y. Imaging hippocampal function across the human life span: is memory decline normal or not? *Annals of Neurology* 2002;51:290–295. [PubMed: 11891823]
- Smith DE, Rapp PR, McKay HM, Roberts JA, Tuszyński MH. Memory impairment in aged primates is associated with focal death of cortical neurons and atrophy of subcortical neurons. *Journal of Neuroscience* 2004;24:4373–4381. [PubMed: 15128851]
- Solomon PR, Beal MF, Pendlebury WW. Age-related disruption of classical conditioning: A model systems approach to memory disorders. *Neurobiology of Aging* 1988;9:535–546. [PubMed: 3062464]
- Szatkowska I, Szymanska O, Grabowska A. The role of the human ventromedial prefrontal cortex in memory for contextual information. *Neuroscience Letters* 2004;364:71–75. [PubMed: 15196680]
- Terry RD, DeTeresa R, Hansen LA. Neocortical cell counts in normal human adult aging. *Annals of Neurology* 1987;21:530–539. [PubMed: 3606042]

- Tulving E. Episodic memory: From mind to brain. *Annual Reviews in Psychology* 2002;53:1–25.
- Woodruff-Pak DS, Thompson RF. Classical conditioning of the eyelid response in rabbits as a model system for the study of brain mechanisms of learning and memory in aging. *Experimental Aging Research* 1985;11:109–122. [PubMed: 4092718]

Figure 1.

Mean ($\pm$ standard error) object exploration time (sec) for 6 month old and 24 month old rats of objects pairs (A, B, C) positioned on the left and the right side of the apparatus during the three exploratory trials.

Figure 2. Mean ($\pm$ standard error) object exploration time (sec) for 6 month old and 24 month old rats during the probe test when one of the objects from the first exploratory trial (A) and one of the objects from the third exploratory trial (C) was presented simultaneously.