

Age-Related Deficits on the Radial Maze and in Fear Conditioning: Hippocampal Processing and Consolidation

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ABSTRACT: Young adult, middle-aged, and old male F-344 rats were assessed for their hippocampal ability. This was accomplished by examining the animals on two different paradigms, each incorporating a simultaneous measure of hippocampal-dependent and -independent processing. The animals were fear conditioned and then tested for retention of the conditioning context and tone. This was followed by an 8-arm radial maze task which combined spatial working and cued reference memory elements. The two paradigms are compared in terms of task demands, potential confounds, and validity for aging studies. The results indicate that the performance of the animals on the two tasks is correlated. Age-related deficits limited to the hippocampal aspects of the above tasks were found, with no deficits found in the analogous but hippocampus-independent aspects of these tasks.

The function of the hippocampus in incorporating new memories is time-related. Therefore, the possibility of age-related changes in consolidation was examined. It has previously been shown on the fear conditioning paradigm that the hippocampus is involved in retention of the aversive context for approximately 28 days. In the present study, an attempt was made to test the animals for retention of the conditioning context both early into the period of consolidation (10 days) and after consolidation should have been completed (52 days). The results indicate that, initially, the old animals show comparable retention to young rats. When examined later, young animals showed a stronger retention of the conditioning context than they had previously. The aged rats, however, did not seem to benefit from this additional period of time and in fact showed a decrease in retention of the conditioning context. The data are interpreted in terms of consolidation, alternative explanations of the data are presented, and suggestions are given for future research. Finally, the implications of such age-related changes in hippocampal consolidation on learning and memory are discussed. *Hippocampus* 1998;8:402-415.

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KEY WORDS: aging; spatial learning; working memory; rats; spaced conditioning; massed conditioning

INTRODUCTION

Aged rats perform as well as young adult rats in solving simple cued discrimination tasks (Barnes et al., 1980), but perform poorly on tasks that

require the integration of spatial information (Barnes et al., 1980; Pelley et al., 1987; Gallagher and Burwell, 1989). Such findings have led to a focus on the hippocampus as the source of these deficits. The underlying rationale is the similarity between the nature of behavioral deficits found during aging and those seen with hippocampal lesions. A potential pitfall of this correlational approach is that observed behavioral deficits which mimic those found in lesioned animals may be due to non-hippocampal age-related changes. The aging process is characterized by many types of changes, only some of which are cognitive. For example, aging in rats is characterized by alterations in visual ability (Shinowara et al., 1982; O'Steen et al., 1995), thermoregulation (Lindner and Gribkoff, 1991; Bruner and Vargas, 1994), motor ability (Petit and Markus, 1987), immune response (Miller, 1996), and neuroendocrine function (Frohman, 1994), all of which are potential confounds when using behavioral tasks to assess learning and memory function. In order to identify age-related behavioral deficits that are specific to hippocampal function, it is imperative to use behavioral measures that can dissociate hippocampal and non-hippocampal processing.

The approach used in the present study is to investigate the effects of natural aging on learning and memory, by testing animals on tasks that *simultaneously* examine hippocampal and non-hippocampal abilities. Young adult, middle-aged, and old rats were examined on two behavioral paradigms: radial maze learning and fear conditioning. These tasks were chosen because they encompass both a hippocampal-dependent and a hippocampal-independent component. Fear conditioning utilizes the fact that when a rat is placed into an aversive situation (e.g., a brightly lit open field or an inescapable shock chamber) it exhibits a "fear response," characterized among other things by defecation and freezing (Bolles, 1970; Blanchard et al., 1976; Davis, 1992). *Conditioned* fear responses are elicited by exposing the animal to cues previously paired with an aversive experience. For example, the animal is placed in a novel chamber and after a few minutes a tone is presented,

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followed by a mild foot shock. Later, if the animal is presented with only the tone or is placed back into the chamber, it will show the fear response (i.e., freeze). These two types of conditioning, to the explicit stimulus (tone) and to the chamber/context, have been shown to depend on different brain structures. Recognition of the background context is related to hippocampal function, while recognition of an explicit stimulus remains intact even in animals with hippocampal lesions (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Phillips and LeDoux, 1994). Thus, within the contextual fear conditioning paradigm, a dissociation can be shown between hippocampal-dependent and -independent processing. Likewise, one can demonstrate such a dissociation on a radial arm maze. Specifically, rats with lesions of the hippocampus show impairments on spatial working memory tasks, but resemble control animals on cued reference memory tasks (see, Jarrard, 1993). The radial maze procedure used in the present study was designed to simultaneously assess ability on a cued reference memory task and a spatial working memory task.

Hippocampal involvement in learning and memory shows a time-related gradient. Kim and Fanselow (1992) showed that hippocampal lesions 1 or 7 days after fear conditioning in young rats prevent subsequent retention of the conditioning context. If, however, the hippocampus is lesioned 28 days after conditioning, the animals show normal retention of the conditioning context. This has been proposed to reflect a process in which the hippocampus is involved in the establishment, or consolidation, of memories in the neocortex (Squire, 1992; Eichenbaum et al., 1994; McClelland et al., 1995; Buzsáki, 1996). In light of the findings that hippocampal involvement in memory formation is transient, the approach used in the present study was to examine the animals for contextual retention during this period of consolidation (10 days post-conditioning) as well as after this process was presumably complete (52 days post-conditioning). The use of these retention intervals provided a means for assessing possible age-related changes in hippocampal consolidation.

MATERIALS AND METHODS

Subjects

Young adult (4 months), middle-aged (10 months), and old (23 months) male F-344 rats (Harlan Sprague-Dawley, Houston, TX) were housed in pairs and sustained on lab chow and water ad libitum. The rats were weighed and handled on a daily basis for 2–3 weeks, after which the animals were housed individually. The animal room was maintained on a 12 h light/dark cycle (lights on at 7:00 AM). The animals were run through the experimental battery in two waves: the first set consisted of only middle-aged and old rats, and the second set was run with the addition of a young group. The numbers and ages of the animals are shown in Table 1.

TABLE 1. *Number and Ages of the Rats at the Beginning of the Experiment: Total (Set 1; Set 2)*

Group	Age (months)	Interference	Control
Young	4	5 (0; 5)	5 (0; 5)
Middle-aged	10	7 (5; 2)	7 (5; 2)
Old	23	8 (5; 3)	7 (4; 3)

Fear Conditioning

Adaptation

In order to familiarize the animals with the experimental procedure, the animals were given three adaptation trials over a period of 9 days (Fig. 1). Each adaptation trial consisted of placing the rat for a period of 3–5 min in a novel chamber within a novel room. These trials all took place during the light part of the cycle, and the chambers were cleaned with a 50% alcohol solution between animals.

Conditioning

Four days after the last adaptation trial, the animals underwent three sessions of tone-foot-shock pairings in a shock chamber (28 × 21 × 21 cm; Med Associates, East Fairfield, VT) in a novel room. Each session consisted of a 100 s baseline period, followed by 30 s of tone (70 dB). Immediately following termination of the tone, a 2 s 0.4 mA current was delivered to the animal from a shock generator/scrambler (Lafayette Instruments, Lafayette, IN). Upon completion of the session, the rat was returned to the animal housing area. This procedure was repeated twice with a 90–120 min interval between conditioning sessions, and all conditioning sessions were conducted during the dark part of the cycle. The conditioning room itself was illuminated, white noise was present in the background, and the chamber was cleaned with a 50% alcohol solution between animals. The rat's movement was recorded at 3 Hz by a microwave-based motion detector system (Radio Shack, Fort Worth, TX) mounted above the conditioning chamber. The motion detection sensitivity was adjusted to pick up small movements but not the animal's heartbeat or respiration. In addition, the animal was monitored via a closed circuit video system for qualitative changes in behavior.

Interference

Following conditioning a second experimenter divided the animals into an interference group and a control group. Both groups were handled and weighed daily. The interference group was placed on three occasions into the three adaptation chambers, however the sequence was changed from that used during adaptation (Fig. 1). These placements were conducted during the 10 day interval between the conditioning and the retention tests. The control group remained in the animal room during this interval.

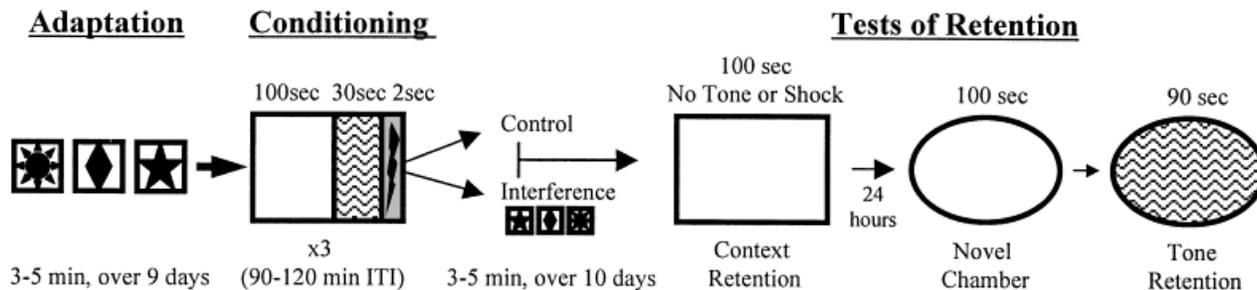


FIGURE 1. Conditioning procedure. All animals were given three separate adaptation trials, consisting of 3–5 min in a novel chamber within a novel room, over the span of 9 days. All animals underwent three conditioning sessions in a distinctive shock chamber in a novel room, with a 90–120 min intertrial interval between sessions. Each session consisted of a 100 s baseline period, followed by 30 s of tone. Immediately following termination of the tone, a 2 s 0.4 mA shock was delivered to the animal's feet. The rats were then divided into interference and control groups. The interference group was placed on three occasions back into the three adaptation chambers, however in a different sequence from that used during

adaptation. The control group remained in the animal room. Ten days after conditioning all animals were placed back into the original shock chamber for a period of 100 s, however, no tone or shock was administered. The following day the animals were placed into a novel chamber in a novel room. The animals were monitored for a baseline period of 100 s, followed by sounding of the conditioned tone for 90 s. A motion detection system recorded the motor activity of the animals while in each chamber, and the inverse of the amount of movement (i.e., freezing) was used as a measure of retention for the original conditioning context and tone.

Short-term retention of context

Ten days after conditioning, context retention was tested by repeating the procedure used during conditioning and placing the animals back into the original conditioning chamber for a period of 100 s, however, no tone or shock was administered (the experimenter was blind as to which animals received interference throughout the tests for retention).

Short-term retention of tone

The following day the animals were placed into a novel chamber in a novel room. To ensure novelty, this trial was conducted during the lights-on period, the chamber was oval rather than rectangular, composed of clear Plexiglas not metallic, cleaned with soap and water instead of alcohol, and there was no white noise in the background. The animal's activity was monitored for a period of 100 s, and this was followed by the sounding of the conditioned tone for 90 s.

Long-term retention of context and tone

Fifty-two days after conditioning the animals were again tested for retention of the original conditioned context and tone. The procedures for the long-term retention tests were the same as for the short-term retention. It should be noted that during the interval between the short- and long-term tests of retention the animals were examined on the radial arm maze.

Shock Sensitivity

The animals' shock sensitivity was tested to ensure no age-related differences in sensitivity to the aversive stimulus. Shock sensitivity testing took place in a different room and apparatus than the conditioning, interference, or retention trials. The animals were placed with their hindlimbs and body in a narrow

conductive metallic container, with their forelimbs on a second conductive surface. A weak electrical current was passed through the two surfaces as an experimenter, blind to the current level being used, monitored the animal. The current was gradually increased until the rat removed his forelimbs. This was done five times for each rat with the median used as an index of the animal's shock sensitivity. Shock sensitivity was tested a few days after the short-term retention tests for Set 1 and a few days after the long-term retention tests for Set 2. The results indicate no effect of when the shock sensitivity testing took place, on the measures of shock sensitivity, or the fear response to the conditioned context or tone during the tests of long-term retention [$F(1, 19) = 0.20$, $P > 0.10$; $F(1, 15) = 0.45$, $P > 0.10$; and $F(1, 15) = 0.22$, $P > 0.10$, respectively].

8-Arm Radial Maze

Maze adaptation

Following the completion of the short-term fear conditioning retention tests, the animals were food deprived to about 80% of their ad libitum body weight and trained for 9 days to run on an 8-arm radial maze (black Plexiglas, 60 × 10 cm arms extending from an octagonal central platform). The maze was in the center of a dimly lit room (4.5 × 4.5 m) with many objects along the walls. The animals were placed at the maze center with all arms accessible and baited with chocolate sprinkles. The rats were removed from the maze after visiting six arms or 15 min, whichever came first. Arms were rebaited only after the animal left the arm (due to a technical problem arms were not rebaited on the sixth adaptation session), and the maze was cleaned with a 50% alcohol solution between animals. On the 9th day the animals were screened for their ability to visit six arms in under 15 min. Only animals reaching this criterion were trained on the memory

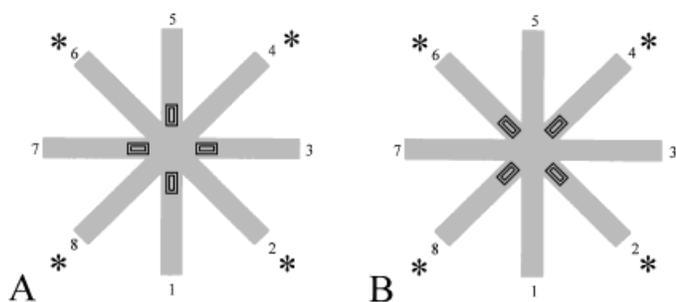


FIGURE 2. Maze configuration. **A:** The configuration of the 8-arm radial maze throughout the 14 training trials. All maze arms were identical, except for small textured inserts at the beginning of the odd-numbered arms. Arms with inserts were never baited, while the other arms (asterisks) were baited only once per training session. Note the symmetrical arrangement of the maze; thus even if animals learned the cued reference memory task (i.e., not to enter arms with inserts), getting to all four correct arms constituted a spatial working memory task. **B:** The configuration of the 8-arm radial maze during the strategy probe trial. Note that the baited arms are now cued. Animals attending to the cues will avoid these arms, while those animals attending to space will be unaffected.

tasks, consequently animals with overt motoric or motivational deficits were excluded from the experiment.

Memory tasks

Following adaptation the animals were trained on two memory tasks. Four symmetrical arms were baited, while the other four arms were never baited and were cued with a black textured insert (Fig. 2A). The rats were given one training session per day for 14 days, each day placed in the center of the maze facing a different arm in a quasirandom order. A rat was left on the maze until he had eaten on all four baited arms, or for a maximum of 15 min. When an animal ate on an arm, the chocolate was not replaced. Due to the configuration of the maze this experiment encompassed two types of tasks. The cued arms constituted a cued reference memory task since they were never baited. The uncued arms constituted a spatial working memory task since there were no local cues to inform the animal whether a given uncued arm had been visited. Thus three types of errors were recorded: a *working memory error* when an animal returned to a baited arm already visited, a *reference memory error* when an animal visited an arm never baited, and a *working reference error* when an animal returned to an unbaited arm already visited. An arm was considered “visited” if the animal’s forepaws passed beyond the center of the arm. The criterion for having learned the cued reference memory task was 2 out of 3 days without committing any reference memory errors, and the criterion for the spatial working memory task was 2 out of 3 days without any working memory errors.

“Strategy probe” trial

On day 15, the textured cue arms were rotated 45° and baited with chocolate (Fig. 2B). The local reference cues that before

signaled arms with no reward were now on the baited arms. This situation provided information on the strategy used by the animal. Animals predominantly using a spatial strategy would continue to go down correct arms regardless of the cue position. Conversely, animals tending to use a local cue strategy would avoid the cued arms regardless of their spatial orientation. At the end of the trial, the animals were put back on the full feeding schedule.

Data Analysis

In order to accurately analyze the fear conditioning results, data on the animals’ movement were taken only after the first 10 s in each condition. This was done to remove possible confounds related to placing the animals in the chamber or the animals’ orienting response. As mentioned above, the animals were run through the experimental battery in two waves. All results were first analyzed to see if there was an effect of set (i.e., old and middle-aged groups of Sets 1 and 2 were compared). If no significant effect of set was found, the data from the two sets were combined for further analysis.

RESULTS

Over the course of the experiment, several of the old subjects died; this is reflected in the changes in the degrees of freedom for each behavioral test. In addition, if an animal was sick or died within 10 days of a behavioral test, his data were excluded from the analysis of that test. The data were not excluded from one aged rat who died from choking on food, verified by necroptic tracheotomy.

Shock Sensitivity

Analysis of the mean shock sensitivity revealed no significant difference in sensitivity with age [$F(2, 34) = 0.85, P > 0.10$]. As can be seen in Figure 3, the shock sensitivity levels are well below the current level used during conditioning.

Short-Term Tests of Retention

As can be seen in Figure 4, there were no age differences in context retention [Set 1: $F(1, 15) = 3.38, P > 0.09$; Set 2: $F(2, 14) = 1.67, P > 0.10$]. Animals in the first set receiving interference exhibited significantly less freezing [$F(1, 15) = 5.45, P < 0.05$]; this was not found in the second set of animals [$F(2, 14) = 0.01, P > 0.10$]. No age by interference interactions were observed [$F(1, 15) = 0.02, P > 0.10$, and $F(2, 14) = 0.47, P > 0.10$, Sets 1 and 2, respectively].

Twenty-four hours later, the animals were placed in a novel chamber. There were no set effects in the response to the novel chamber or to the tone [$F(1, 21) = 1.87, P > 0.10$;

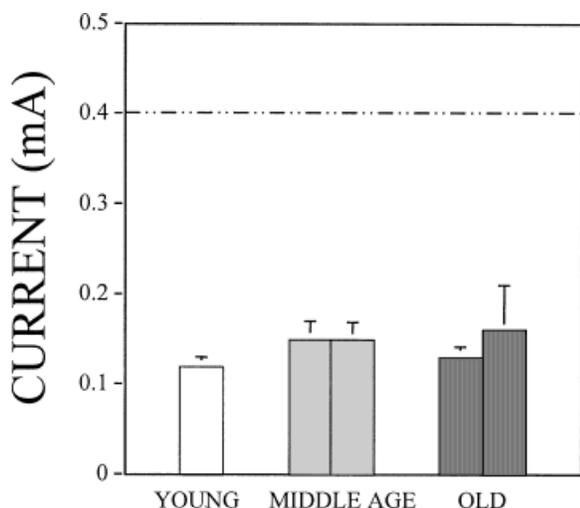


FIGURE 3. Shock sensitivity. The average amount of current needed to elicit a forepaw withdrawal response. The animals were placed on two conductive surfaces. Electrical current was gradually increased until an experimenter, blind to the current level being used, observed the rat respond. For a given age group, data from Set 1 are presented on the left and data from Set 2 on the right; there were no young animals in Set 1. No age-related differences in sensitivity to the aversive stimulus were observed. The dashed line indicates the stimulus level used for conditioning.

[$F(1, 21) = 0.17, P > 0.10$, respectively]. Consequently, the data from the two sets were combined. As can be seen in Figure 5A, during the 90 s prior to the sounding of the conditioned tone all age groups exhibited similar amounts of freezing in the new environment [$F(2, 33) = 2.55, P > 0.09$]. Animals receiving interference exhibited significantly less freezing than control rats [$F(1, 33) = 19.46, P < 0.001$], and no age by interference interactions were observed [$F(2, 33) = 0.36, P > 0.10$]. At the end of the 100 s baseline period the conditioned tone was

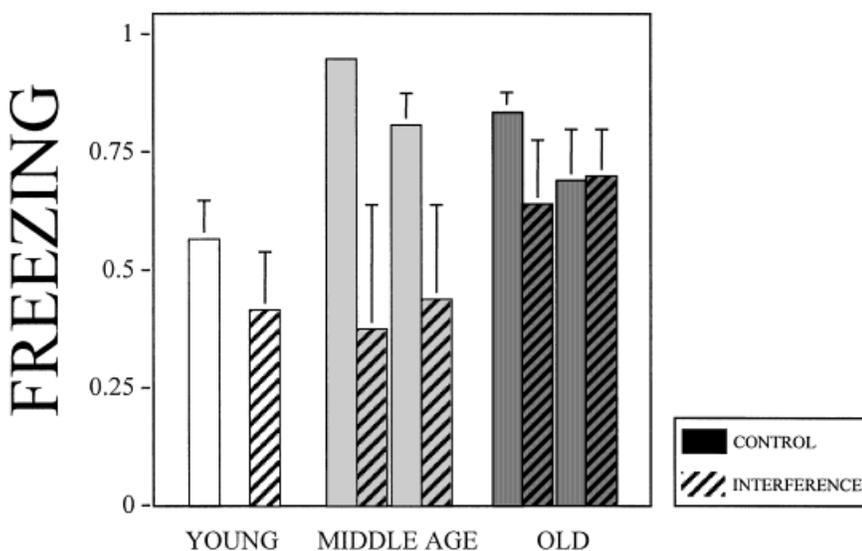


FIGURE 4. Short-term context retention. Proportion of time freezing when placed back into the original shock chamber. For a given age group, data from Set 1 are presented on the left and data from Set 2 on the right; there were no young animals in Set 1. There were no age effects in context retention for either Set 1 or Set 2. Those animals experiencing interference (diagonal bars) displayed less freezing compared to controls in Set 1 ($P < 0.05$); this was not significant in Set 2. There were also no age by interference interactions observed in either set.

presented and the animals' response examined. While all animals displayed a strong fear response to the conditioned tone (Fig. 5B), there were no effects of age [$F(2, 33) = 1.73, P > 0.10$], interference [$F(1, 33) = 0.00, P > 0.10$], or age by interference interactions [$F(2, 33) = 0.86, P > 0.10$] in the level of freezing.

Long-Term Tests of Retention

Fifty-two days after conditioning, and following the 8-arm radial maze tests (see below), the animals were again examined for retention of spatial context by placing them back into the original conditioning environment with no tone or shock presented. There were no differences between the animals in the two sets. In addition, since all animals had experienced considerable "interference" during the 2 month interval (maze training), and because there were no significant differences between groups, the interference and control data were combined. As can be seen in Figure 6, old animals displayed far less freezing to the conditioned context than the young or middle-aged animals [$F(2, 30) = 17.05, P < 0.001$]. A post hoc analysis revealed that old animals differed significantly from both the young and middle-aged groups (Scheffe $P < 0.001$, for each comparison).

Twenty-four hours later, the animals were placed back into the "novel" chamber (Fig. 7A). During the 90 s prior to the sounding of the conditioned tone the old animals in Set 1 froze less than the middle-aged animals [$F(1, 13) = 20.91, P < 0.001$]. This effect, however, was not found in Set 2 [$F(2, 15) = 0.02, P > 0.10$]. At the end of the 100 s the conditioned tone was presented and the animals' response examined. While all animals displayed a strong fear response to the conditioned tone (Fig. 7B), there was a significant effect of age on freezing [$F(2, 30) = 4.30, P < 0.05$], with old animals showing a stronger fear response to the tone than young animals (Scheffe $P < 0.05$).

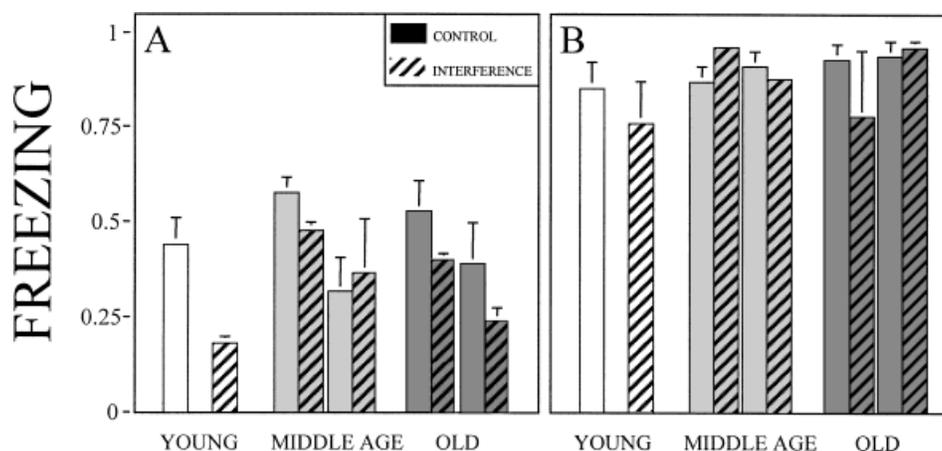


FIGURE 5. A: Short-term novel exposure. Proportion of time freezing in a novel chamber prior to sounding of the conditioned tone. For a given age group, data from Set 1 are presented on the left and data from Set 2 on the right; there were no young animals in Set 1. Those animals experiencing interference (diagonal bars) during the interval between conditioning and retention tests displayed less

freezing compared to controls ($P < 0.001$). No age-related differences or age by interference interactions were observed. B: Short-term tone retention. Proportion of time freezing during tone presentation in the novel chamber. Note that all animals displayed a stronger freezing response once the conditioned tone was presented, with no differential effects of age or interference.

8-Arm Radial Maze

The results of the cued reference memory task (Fig. 8) were similar for all ages. Although the middle-aged and old rats of Set 2 displayed significantly more reference memory errors than those of Set 1 [$F(1, 20) = 8.48$, $P < 0.01$], there were no significant age differences observed in the mean total errors for either Set 1 or Set 2 [$F(1, 13) = 2.07$, $P > 0.10$, and $F(2, 16) = 0.61$, $P > 0.10$, respectively]. Furthermore, examining the number of trials to

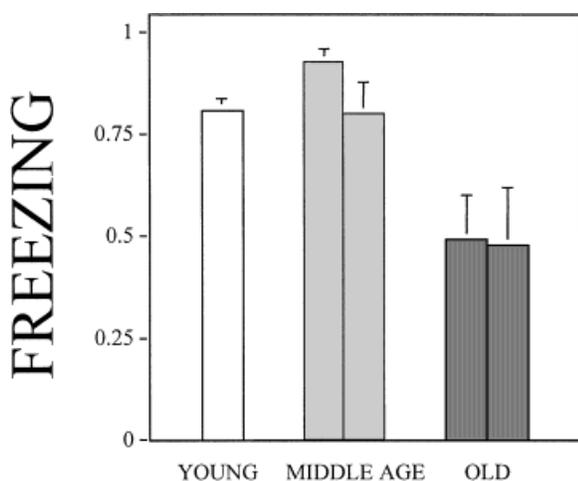


FIGURE 6. Long-term context retention. Proportion of time freezing when returned to the original shock chamber 52 days after conditioning. The old animals displayed significantly less freezing than both the young and middle-aged groups ($P < 0.001$, for each comparison). For a given age group, data from Set 1 are presented on the left and data from Set 2 on the right; there were no young animals in Set 1.

criterion revealed no significant differences between age groups [young: 10.90 ± 0.97 , middle-aged: 11.08 ± 1.14 , and old: 9.64 ± 1.07 , mean \pm S.E.M., respectively; $F(2, 31) = 0.524$, $P > 0.10$].

On the spatial working memory task, an age-related deficit was observed (see Fig. 9). With regard to mean total errors, there were no differences between the animals in the two sets. There was a significant effect of age on mean total error [$F(2, 31) = 10.80$, $P < 0.001$], and a post hoc analysis revealed that the old rats made significantly more working memory errors than both the middle-aged (Scheffe $P < 0.01$) and the young (Scheffe $P < 0.001$) rats. Analysis of the number of trials to criterion revealed an age effect for both Set 1 [middle-aged: 6.22 ± 0.72 and old: 9.17 ± 1.22 ; $F(1, 13) = 4.93$, $P < 0.05$] and Set 2 [young: 5.40 ± 0.52 , middle-aged: 9.50 ± 1.55 , and old: 11.80 ± 1.20 ; $F(2, 16) = 14.48$, $P < 0.001$]. Post hoc analysis for Set 2 data showed that the old rats took significantly longer to reach criterion than both the middle-aged (Scheffe $P < 0.05$) and the young (Scheffe $P < 0.001$) rats.

As mentioned in Materials and Methods, returning a subsequent time to a cued, unbaited arm was termed a working reference memory error. Analysis of these errors revealed no differences between age groups [young: 4.50 ± 0.54 , middle-aged: 4.38 ± 1.01 , and old: 5.73 ± 1.24 ; $F(2, 33) = 0.548$, $P > 0.10$].

In addition to examining spatial working memory and cued reference memory, the animals' strategy for solving the radial maze was investigated using the "probe trial" (local cues in conflict with spatial cues; see Materials and Methods). A significant effect of age was observed with regard to the first arm chosen [$F(2, 31) = 5.33$, $P < 0.01$]. Post hoc tests revealed that the old rats followed the local cues more than the young did, with a significantly greater

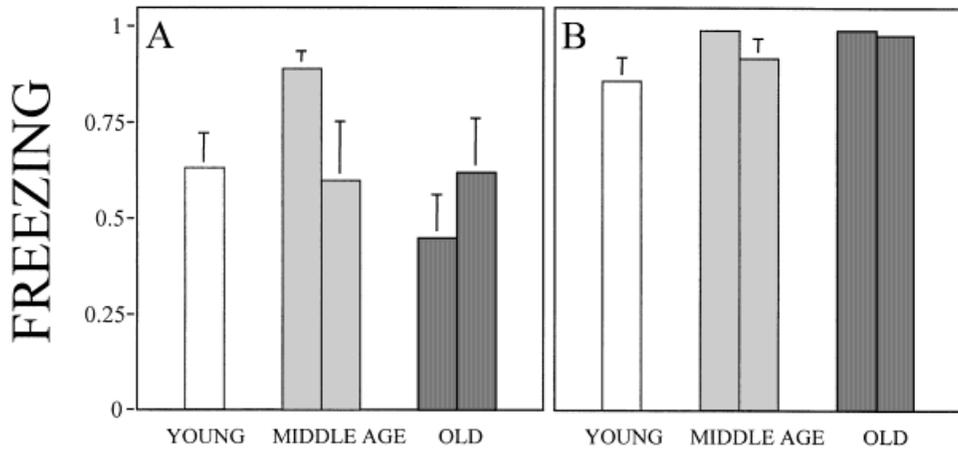


FIGURE 7. A: Long-term novel exposure. Proportion of time freezing in the novel chamber prior to sounding of the conditioned tone. The old animals in Set 1 exhibited significantly less freezing than the middle-aged animals ($P < 0.001$). No age differences in freezing behavior were observed in Set 2. B: Long-term tone

retention. Proportion of time freezing during tone presentation in the novel chamber. Note that while all animals exhibited a strong fear response, the young animals froze less than the old rats ($P < 0.05$). For a given age group, data from Set 1 are presented on the left and data from Set 2 on the right; there were no young animals in Set 1.

percentage making an error on the first choice (Scheffe $P < 0.05$). The middle-aged animals seemed to be intermediate, and were not significantly different than either the young or old animals (Fig. 10).

Correlations of Task Performance

The total number of spatial working memory errors, cued reference memory errors, and the amount of freezing during the

long-term retention tests were converted to Z-scores and correlated. As can be seen in Figure 11, a significant correlation was found between performance on the spatial working memory task and contextual fear conditioning ($r = 0.40$, $P < 0.05$), and between the cued reference memory task and tone conditioning ($r = 0.47$, $P < 0.01$). No other significant correlations were found ($P > 0.10$, for all).

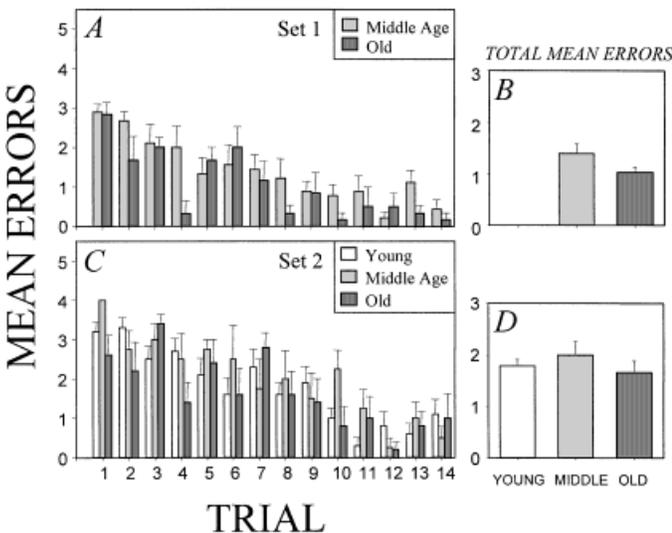


FIGURE 8. Acquisition of the 8-arm radial maze cued reference memory task. Performance of the cued reference memory task was equivalent across age groups. Average amount of cued reference memory errors over 14 trials, 1 trial per day (A), and average total reference memory errors (B) for Set 1. Average amount of cued reference memory errors over 14 trials (C) and average total reference memory errors (D) for Set 2.

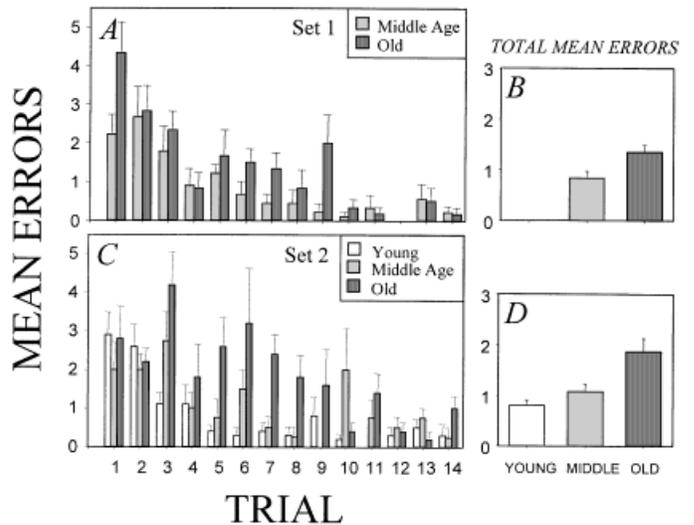


FIGURE 9. Acquisition of the 8-arm radial maze spatial working memory task. Aged rats display a deficit in performance of the spatial working memory maze task. Average amount of working memory errors over 14 trials, 1 trial per day, on the spatial radial maze task (A), and average total working memory errors (B) for Set 1. Average amount of working memory errors over 14 trials, 1 trial per day, on the spatial radial maze task (C), and average total working memory errors (D) for Set 2. Post hoc analyses revealed that the old animals differed from both the middle-aged ($P < 0.01$) and young ($P < 0.001$) animals.

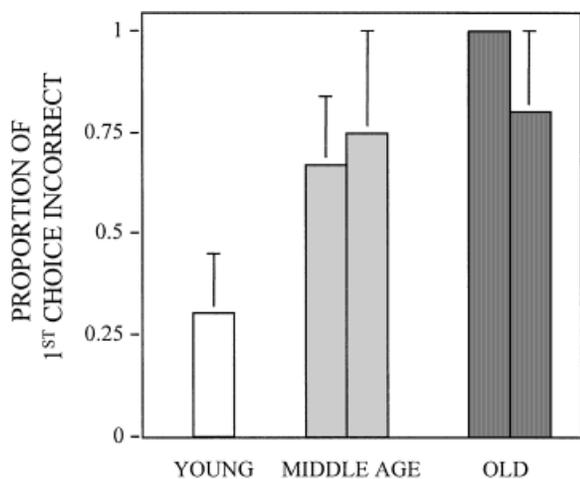


FIGURE 10. Strategy probe trial. The animals' strategy for solving the radial maze was examined by rotating the maze inserts (see Fig. 2B). Almost all of the old rats avoided the local cues, making significantly more errors than the young rats on the first choice ($P < 0.05$). This would suggest that when given a choice the young rats prefer a spatial strategy to solve the maze, while the old rats rely more on the reference cues to guide them. The middle-aged animals appear to be intermediate, and not significantly different from either the young or old animals. For a given age group, data from Set 1 are presented on the left and data from Set 2 on the right; there were no young animals in Set 1.

DISCUSSION

Aging Selectively Affects Processing of Spatial and Contextual Information

Contextual fear conditioning

The results of the short-term retention tests indicate that conditioning was equivalent across age groups. Despite the fact that all animals were initially conditioned to the same degree, an age-related deficit in context retention was manifest after approximately 2 months. This deficit was specific to the hippocampal-dependent portion of the fear conditioning task. No age-related deficits were found in response to the conditioned tone, a process known to be hippocampus-independent (Kim and Fanselow, 1992; Phillips and LeDoux, 1992).

These findings are in agreement with a number of other studies showing no short-term deficit in contextual fear conditioning in aged animals (Brown et al., 1997; Houston et al., 1997). Stoehr and Wenk (1995), however, found that aged rats showed a reduced contextual fear response compared to young rats when examined only 24 h after conditioning. The different results may be due to variations in the conditioning procedure; for example, Stoehr and Wenk (1995) used a massed conditioning paradigm, whereas in the current study and others (Brown et al., 1997; Houston et al., 1997) a distributed conditioning procedure was employed. It has long been known that there are differences in massed vs. distributed learning (Ebbinghaus, 1885; Underwood,

1961), and more recently it was shown that this distinction is relevant also for fear conditioning (Fanselow et al., 1993). Taken together, these data may indicate age-related changes in the effects of massed and distributed fear conditioning.

The current results indicate that even though a distributed conditioning procedure was used, and the aged rats were conditioned to the same degree as the young, they still show less long-term retention of the hippocampal-dependent contextual conditioning. This effect seems to be specific to hippocampal processing, since no such age effect was found for the tone.

Effect of interference

The animals receiving interference exhibited significantly less freezing than controls when placed in the novel environment. Interestingly, the differential response to being placed into the novel chamber disappeared as soon as the conditioning tone was presented (Fig. 5). This suggests that the interference effects were modality-specific. Presumably, the control rats had generalized their fear to any chamber they were placed into, while the interference rats that were exposed to three "safe" environments after conditioning showed conditioned discrimination (D'Amato, 1970). These results indicate that despite the attempt to adapt the

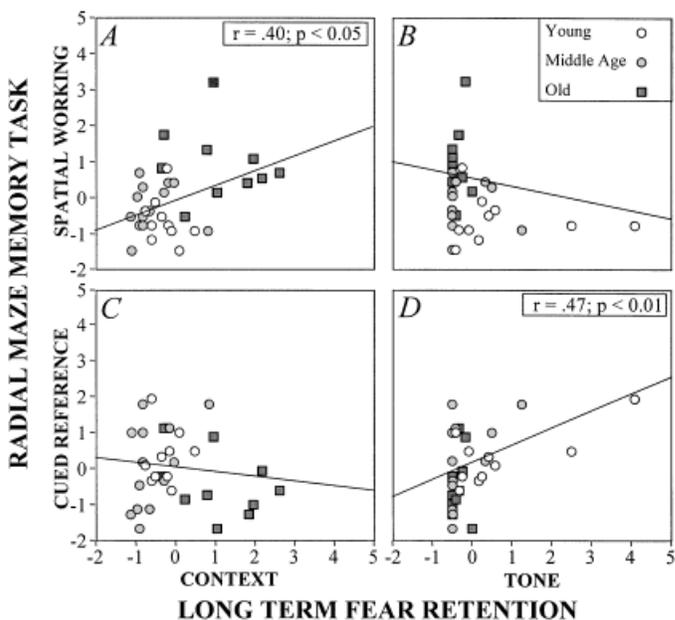


FIGURE 11. Correlations between tasks. A comparison of the amount of freezing during fear conditioning retention trials with the mean number of errors on the radial maze. A: Animals showing a stronger fear response during context retention (i.e., less movement) also made less errors on the spatial working memory task (Pearson $r = 0.40$, $P < 0.05$). B: No relationship was seen between performance on the spatial working memory task and the long-term tone retention test ($P > 0.10$). C: No relationship was seen between performance on the cued reference memory task and the long-term context retention test ($P > 0.10$). D: Animals showing a stronger fear response during tone retention (i.e., less movement) also made less errors on the cued reference memory task (Pearson $r = 0.47$, $P < 0.01$).

animals to the general procedure (i.e., being carried out of the animal housing area, placed into an enclosed container, left alone in a room, etc.), the animals showed a generalization of the fear conditioning to many different elements of the procedure. At present we are conducting a number of experiments to determine which factors may be important for "context."

Although there was an overall interference effect in the novel environment, this was unaffected by age. Previous reports have shown no age differences in the effects of interference as long as the animal is not trained on a task very similar to the one previously learned (Winocur, 1984, 1988). In the present study, the animals were reexposed to the adaptation environments, which were chosen because they were very different from the conditioning chamber. Thus, under these interference conditions, no specific age-related deficit would be expected and none was found.

Effect of set

As noted in Materials and Methods, the animals were run through the experimental battery in two waves (Sets 1 and 2). In a few of the experimental conditions, significant differences were found between corresponding age groups of different sets. This may be due to the smaller sample sizes used in Set 2. Overall, however, both sets of animals showed similar trends, and the main effects of hippocampus-related age deficits were found in both sets.

The Fear Conditioning Paradigm and Aging

When adapting paradigms normally used to assess hippocampal function in young animals for aging studies there is a danger that non-hippocampal aging effects will confound the results. Thus age-related changes in motor ability, thermoregulation, response to food deprivation, etc., can have an impact on 8-arm radial maze and/or water maze performance. This could also potentially be the case for contextual fear conditioning. The fear conditioning paradigm used in the present study was based on a similar paradigm previously used in young rats (Kim and Fanselow, 1992). However, a number of modifications were incorporated into the procedure to make it more suitable for an aging study.

Reduction of assessment intervals

With young animals contextual freezing was assessed over 8 min (Kim and Fanselow, 1992) and novel context and tone over 11 min. Willig and colleagues (1987) showed that while initially (2.5 min) there is no age-related difference in exploration of a novel environment, at 5 min and onward aged rats show reduced exploration. Pilot experiments in our laboratory gave similar results; while unconditioned animals did not display freezing behavior, there was a tendency for all animals to reduce their activity levels over time, with the old animals showing a greater decrease in activity. The issue of age-related reductions in exploration is important for fear conditioning paradigms since the

variable used to assess the fear response (i.e., freezing) is inversely related to the animals' level of activity. To circumvent this potential confound all time intervals used in the present study were kept at 190 s or less. To further ensure that the present findings were not confounded by an age-related reduction in activity levels, the response to the novel environment was examined in greater detail. As noted in Materials and Methods, the rats spent 100 s in the novel chamber before the conditioned tone was presented. If there was an age-related decline in activity levels it should have been found toward the end of this period. An examination of the amount of movement during the last 30 s in the novel environment showed that the old animals were not significantly different from the young, therefore it seems likely that the marked increase in freezing found during the presentation of the conditioning tone (freezing about 90% of the time; see Figs. 5B, 7B) stems from a conditioned fear response and not simply from a gradual reduction in exploration.

Modification of the conditioning parameters

In Kim and Fanselow (1992), 15 massed context-tone-shock pairings were used, with a current level of 1.0 mA for 2 s. This produces very strong freezing in intact young rats. However, very strong conditioning can lead to a ceiling effect with all animals showing "equal" conditioning. In the present study, a weaker conditioning protocol was used that resulted in intermediate levels of freezing of approximately 50–75%. This provides for a more sensitive assessment of age-related changes in retention.

Shock sensitivity

The fear conditioning task is based on pairing stimuli with an aversive shock. Therefore, any age-related changes in the aversiveness of the shock could be a potential confound. To ensure that there were no age-related differences in sensitivity to the aversive stimulus, shock sensitivity was examined. There was no age-related difference in shock sensitivity, and it should be noted that the level of sensitivity found (about 0.14 mA) was far lower than the 0.4 mA used for conditioning (see Fig. 3). Consequently, any differences in conditioning or retention cannot be attributed to gross age-related changes in shock sensitivity.

Sensory impairment

Another possible confound in fear conditioning, and many other behavioral paradigms, could be a decline in sensory perception with age (i.e., visual, olfactory, auditory, or tactile). It is possible that the aged rats did not show as much contextual fear because they could not perceive the chamber as well as the younger animals. This did not seem to be the case, since 10 days after conditioning all animals displayed a similar fear response when placed back into the conditioning chamber.

Interference

Old animals showed a deficit in long-term contextual fear conditioning in relation to young and middle-aged rats. During

the interval between short- and long-term retention tests the animals were trained daily on the radial maze. It is possible that interference from the maze learning had a greater effect on the old rats, and this is the source of the deficit in long-term context retention. This does not seem to be likely since an explicit examination of the effects of interference showed no age-related deficits (see above). In addition, Winocur (1988) showed that aged animals do not show an increased susceptibility to interference when the source of interference is quite different than the original learning. In the present study, the fear conditioning task differed greatly from the maze task, and therefore should not have affected the old and young rats differently.

Consequently, the deficits observed in contextual fear conditioning using the present procedure seem to be related specifically to cognitive aspects of aging, rather than to non-hippocampal age-related changes in exploration, activity levels, or sensory ability.

Spatial Working Memory on the Radial Maze

Old rats committed significantly more spatial working memory errors than young rats while simultaneously displaying equivalence on the cued reference memory task. Previous research has shown that animals without a hippocampus can learn a cued reference memory task, but are impaired on spatial working memory tasks (Jarrard, 1993). Thus, the old animals display deficits similar to those shown by animals with hippocampal damage. These results support and extend on previous reports that aged rats show deficits on spatial memory tasks (e.g., Geinisman et al., 1995; Gallagher and Rapp, 1997; Barnes, 1998).

These findings cannot be attributed to major differences in task difficulty since the overall number of errors on both tasks was similar. Therefore, it seems likely that the aged rats' deficits are linked to impaired spatial working information processing. This conclusion is further supported by the results of the strategy probe trial indicating that younger animals tend to rely more on a spatial strategy to solve the maze task, while the old animals rely more on local cues. Similar results have been reported by others (Barnes et al., 1980; Winocur, 1988).

There are several possible confounds that could lead to deficits on the 8-arm radial maze tasks. General age-related changes in response to food deprivation, locomotive ability, or motivation could all lead to poor maze performance. These factors did not seem to play an important role in the current study since all animals were able to perform the cued reference memory task equally well. Thus the deficits observed in the spatial working memory task seem to be related specifically to hippocampal processing, rather than to other non-hippocampal age-related factors.

Correlation Between Tasks

While both fear conditioning and the 8-arm radial maze can be used to dissociate hippocampal- and non-hippocampal-related processing, they differ greatly due to the fact that one is aversive and the second is appetative. Furthermore, the radial maze is a

locomotor instrumental task requiring active exploration of the environment. Fear conditioning, however, is primarily a classical conditioning paradigm, with the dependent measure being a species-specific defensive response (Bolles, 1970). In the present study, correlations were found in the performance on these two tasks across age groups (see Results). Animals that did well on the spatial working memory task also showed a stronger long-term contextual fear response. Similarly, animals that performed well on the cued reference memory aspects of the maze also showed enhanced long-term conditioning to the tone. Both spatial working memory and contextual fear conditioning have been shown to depend on intact hippocampal function, while cued reference memory and tone conditioning are independent of the hippocampus. Thus, a correlation was found only between those components of the tasks presumed to measure the same underlying type of information processing.

Despite the fact that *as a group* the old animals were deficient on both types of hippocampal tasks, for an individual rat, performance on the fear conditioning paradigm was not predictive of maze ability. Similar results have been reported when comparing other spatial tasks such as the circular platform (Barnes and McNaughton, 1985), water-maze (Morris, 1984), and radial arm maze (Olton and Samuelson, 1976). Despite the fact that age-related deficits are found on all the above "hippocampal" mazes, when the performance of an individual rat on more than one maze has been examined, correlations have not been seen (Gallagher and Burwell, 1989; Markowska et al., 1989; Stewart et al., 1989; Zyzak et al., 1995; Dudchenko et al., 1997; Martin et al., 1997).

One possible reason for the lack of correlation at the level of the individual is that optimal performance on these "hippocampus-dependent tasks" relies also on many other "hippocampus independent" variables (Hodges, 1996). Aging is characterized by a multitude of neurobiological changes. For example, aging in rats is characterized by alterations in visual ability (Shinowara et al., 1982; O'Steen et al., 1995), thermoregulation (Lindner and Gribkoff, 1991; Bruner and Vargas, 1994; Rick et al., 1996), motor ability (Petit and Markus, 1987; Rick et al., 1996; see also Woodruff-Pak, 1988), as well as immune response (Miller, 1996), neuroendocrine function (Frohman, 1994), and response to food deprivation (Ando and Ohashi, 1991). Consequently, the lack of predictive power across "hippocampal tasks" could be due to the fact that the performance on different mazes depends on different complements of non-hippocampal factors. Thus individual differences in any one of these "non-hippocampal" variables could lead to differential behavior on different "hippocampal" tasks.

A second interpretation of the lack of correlation is that different hippocampal-dependent tasks rely on different aspects of hippocampal function. For example, synaptic changes in the hippocampus (Woolley et al., 1990; Woolley and McEwen, 1992) may cause a deficit in hippocampal aversive tasks, while producing no change or even an improvement in performance on hippocampal appetative tasks (for discussion see Desmond and Levy, 1997; Markus and Zecevic, 1997).

Life Span Changes in Memory Function

The current study examined both middle-aged and old rats. This allows for the distinction between those changes which are limited to old age from general life span changes in hippocampal function. If the age-related changes in hippocampal processing progress as a monotonic function, then the middle-aged rats should perform somewhere between the young and old animals. If, however, the age-related memory deficit is specific to old age, the performance on hippocampal-dependent tasks should change as a step function. Previous research has shown both types of changes (Wallace et al., 1980; Ando and Ohashi, 1991; Fischer et al., 1992; see also Kadar et al., 1990).

On both the 8-arm radial maze and fear conditioning hippocampal tasks, the middle-aged animals performed as well as the young ones. One could interpret these findings to mean that the changes in hippocampal function occur only in old age. However, the data from the probe trial show that the strategy used by most of the middle-aged rats resembled that of the old rats. Most young rats preferred a spatial strategy (approximately 70%), while most of the middle-aged and old rats chose to follow the local cues (approximately 70% and 90%, respectively). In other words, although the middle-aged performed as well as the young rats on both types of hippocampal tasks, there seems to be a subtle change in the manner in which they interact with their environment.

Aging and Hippocampal Consolidation

Many aging studies have examined long-term retention, however, the definition of "long term" has varied across studies to include minutes, hours, days, or months (Bruning et al., 1975; Winocur, 1988; Rozenzweig et al., 1993; Fisk et al., 1994). In light of the findings that hippocampal involvement in memory formation is transient, a different approach would be to classify retention intervals by their relative dependence on the hippocampal system. In other words, examine retention during the period of hippocampal involvement compared to "post-hippocampal retention," the period when the hippocampus is no longer required for recall of a memory.

The importance of the hippocampus for establishing memories in the neocortex has long been of interest (Scoville and Milner, 1957; Squire, 1979; Eichenbaum et al., 1994), and there is physiological evidence supporting such an interaction between the hippocampus and neocortex (McClelland et al., 1995; Buzsáki, 1996; Qin et al., 1996; Skaggs and McNaughton, 1996). To date only a limited number of studies have examined the time course of consolidation, however, one of them used a fear conditioning paradigm similar to ours. Kim and Fanselow (1992) showed that the hippocampus is involved in the consolidation of contextual fear conditioning for approximately 28 days. In the present study, animals were examined for contextual conditioning after 10 days, presumably approximately halfway through this consolidation process. When examined later at 52 days, a period after which presumably full consolidation had occurred, it was found that young animals displayed increased contextual freezing relative to the short-term retention test, while the old animals exhibited a

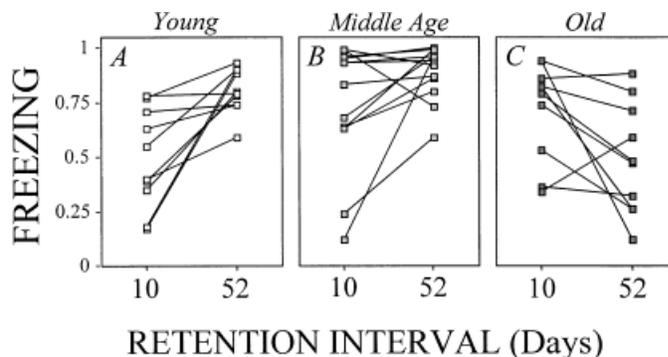


FIGURE 12. The contextual fear response 10 and 52 days after conditioning. **A:** Data from the young rats. Note the increase in the fear response at the longer retention interval (10 days: 0.49 ± 0.07 , 52 days: 0.81 ± 0.03 , respectively; paired *t*-test, $P < 0.01$). **B:** Data from the middle-aged group. This group showed mixed results, with some animals exhibiting increases and others decreases in their response (10 days: 0.73 ± 0.08 , 52 days: 0.89 ± 0.03 ; paired *t*-test, $P = 0.056$). **C:** Data from the aged rats showing a greater short-term fear response in comparison to the long-term response (10 days: 0.71 ± 0.07 , 52 days: 0.49 ± 0.08 ; paired *t*-test, $P < 0.05$).

reduction in the contextual freezing response. As can be seen in Figure 12, all young rats showed an equal or greater fear response when tested after the period of consolidation, while almost all the aged rats showed a decline.

A paired *t*-test comparison of the mean freezing response revealed that these changes were significant (young: $P < 0.01$; old: $P < 0.05$). To better examine this issue, the relative amount of short-term to long-term freezing was calculated using the following formula:

$$R_{\text{freeze}} = \frac{\text{Short-Term Freezing}}{\text{Short-Term} + \text{Long-Term Freezing}}$$

Thus an R_{freeze} of 0.5 indicates equal freezing during the short- and long-term tests of retention, an R_{freeze} of 0.67 indicates twice as much freezing during the short-term than the long-term retention, while a R_{freeze} of 0.33 indicates twice as much freezing during the long-term than the short-term retention test. Using this index it was found that the old animals ($R_{\text{freeze}} = 0.60 \pm 0.05$) significantly differed from both the middle-aged ($R_{\text{freeze}} = 0.43 \pm 0.3$; Scheffe $P < 0.05$) and young ($R_{\text{freeze}} = 0.36 \pm 0.04$; Scheffe $P < 0.001$) groups. One possible explanation of these data is that there is an age-related decrease in the duration and/or efficiency of hippocampal consolidation which impedes proper memory formation. Similar results have been reported by others (Houston et al., 1997).

In the present study, the same animals were tested for both short- and long-term retention allowing for a within animal analysis. However, this type of repeated testing inevitably introduces the possibility that the testing for short-term retention *in itself* was the source for the changes found in long-term retention. In other words, simply experiencing the conditioning context and tone during short-term retention may have acted as an extinction trial. If this were the case, then the differences found during long-term retention would be due to faster extinction in the aged

rats. The literature on extinction during aging has produced mixed results. Some studies show faster extinction rates in old rats (Solyom and Miller, 1965; Stephens et al., 1985), others find no difference (Birren, 1962; Kay and Sime, 1962; Sarter and Markowitsch, 1983; Port et al., 1996), and still others find that aged animals take longer to extinguish (Botwinick et al., 1962; Goodrick, 1968; Bartus et al., 1979; Sarter and Markowitsch, 1983). On tasks similar to the present fear conditioning paradigm, no differences have been found in extinction (Schneider-Rivas et al., 1995). If the short-term retention test had acted as an extinction trial, it would have been expected to cause a reduction in the fear response (possibly at different rates) in all age groups. Yet this was not the case, since the young rats displayed an enhanced response at the second test of retention.

Another possible interpretation of these data is that both old and young animals learned to the same degree, however, aged rats displayed a faster rate of forgetting. The present experiment cannot distinguish between the possibility of age-related changes in memory consolidation, storage, or retrieval. This issue must be further examined in the future by performing hippocampal lesions on old and young animals at different time intervals after new learning.

It is important to note that the age-related deficits found at the long-term test of retention are context-specific and were not found for the non-hippocampal conditioning (the tone). Thus whatever the process taking place (i.e., consolidation, extinction, or forgetting), it is the hippocampus-specific aspects that are affected by age.

As noted above, one of the interpretations of the present findings is that aged rats show a decreased *duration* of hippocampal consolidation. An examination of the age-related neurobiological changes in the hippocampus seems to support such a hypothesis. Geinisman (1977) showed a decrease in the number of perforant path fibers to the hippocampus during aging. Since these axons are the primary source of cortical input to the hippocampus (Amaral and Witter, 1989), a reduction in this pathway would inevitably decrease the richness and/or variety of cortical information passed on to the hippocampus. This age-related decline in the quality of the input could lead to a higher probability of new information "pushing out" or replacing previous hippocampal representations even before consolidation has been completed. In fact, there is some neurophysiological evidence indicating that synaptic changes in the hippocampus are less persistent in aged rats. For example, Barnes and McNaughton (1985) showed that long-term potentiation decays faster in the dentate gyrus of aged rats. Similarly, Norris et al. (1996) demonstrated that long-term depression is easier to induce in aged rats. Taken together, these neurobiological changes during aging would lead to a faster decay of the hippocampal representation. As the representation decays, the hippocampal contribution to the consolidation of memories in the neocortex would decline.

The current behavioral results, together with previous neurobiological findings, may indicate that the age-related deficits in learning and memory are a consequence of a decline in hippocampal consolidation.

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