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Conditional Learning in Aged Rats: Evidence of Hippocampal and Prefrontal Cortex Impairment

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WINOCUR, G. *Conditional learning in aged rats: Evidence of hippocampal and prefrontal cortex impairment.* NEUROBIOL AGING **13**(1) 131–135, 1992.—Groups of normal old rats and young adult rats were administered a test of conditional discrimination learning in which different visual stimuli were associated with responses to different levers. Initially, rats were tested in a zero-delay condition in which they selected their responses in the presence of the conditional stimuli. They were later tested at 5- and 15-s delays between stimulus presentation and the appearance of the levers. Old rats were impaired in learning the basic conditional discrimination, a test thought to be sensitive to frontal lobe dysfunction. Age differences increased with the length of the interval, revealing a time-dependent memory loss that was attributed to impaired hippocampal function.

Aging Learning and memory Hippocampus Prefrontal cortex

THERE is growing evidence that learning and memory changes with normal aging can be related to functional decline in specific brain regions (3,19). In this regard, the hippocampus, a structure that is known to be important for memory function, has attracted considerable attention. The hippocampus is extremely sensitive to the aging process and appears to be one of the first brain regions to show significant structural (9) and physiological (6) changes with advancing age.

At the behavioural level, there are striking similarities in the performance of normal aged subjects and young adults with restricted damage to the hippocampus on tests of learning and memory (30). This observation has been made in several species, including humans, but the question has been most extensively investigated in the rat. Studies have demonstrated parallel deficits on numerous tests including, for example, spatial memory (5), complex maze learning (16), reversal learning (8), delayed nonmatching to sample (22), and passive avoidance conditioning (31). In contrast, old animals often perform normally on tests that are not sensitive to the effects of hippocampal damage, such as simultaneous discrimination learning (8), one-way active avoidance (13), and operant conditioning tasks in which reinforcement is governed by a continuous reinforcement schedule (11).

A second brain region that has been identified as being especially vulnerable to the effects of aging is the prefrontal cortex. Historically, this area has been identified with executive functions that control the organization of information and behavioural planning (27). The elderly frequently exhibit signs of prefrontal impairment that include an exaggerated tendency to perseverate responses, difficulties in naming and in abstracting information to form concepts (2). In clinical investigations, involving brain imaging techniques, age differences on neuropsychological tests of frontal lobe function have been correlated with structural change in the prefrontal area (3,26).

The evidence linking age-related cognitive changes to brain dysfunction prompted a series of experiments in which normal old rats and young adult rats with lesions to the hippocampus or prefrontal cortex were compared on various behavioural tasks. This research focussed on different aspects of learning and memory, including an analysis of episodic and nonepisodic memory. The results point to a clear dissociation between the effects of hippocampal and prefrontal lesions. For example, hippocampal and prefrontal groups were found to be impaired on tests of complex maze learning (31) and delayed alternation (30,32), but their respective deficits were clearly different. In both cases, the impairment of rats with hippocampal lesions was related to a selective failure in recalling specific or episodic information. In contrast, rats with prefrontal damage showed normal memory for specific events but were impaired in nonepisodic learning and memory related to the acquisition of skill or rule-based behaviour. Aged groups were consistently impaired on measures of episodic and nonepisodic memory, reflecting impaired function in both brain regions (29,33).

The present study continued this line of investigation by comparing groups of old and young rats on a test of conditional learning that incorporated independent measures of episodic and nonepisodic memory. In conditional learning, subjects are required to associate different responses with different discriminative stimuli. The present test was conducted in an operant chamber that was outfitted with a panel of lights and two retractable levers. Rats were trained to press one lever in response to a particular visual stimulus, and a second lever in response to another stimulus. Initially, the discriminative stimuli and the levers were presented simultaneously to assess basic conditional learning ability. Following this condition, episodic memory was tested by introducing a delay between the presentation of the stimuli and the appearance of the levers. The delay condition required rats to recall the discriminative stimulus for each trial as part of the process of response selection.

In previous research (32), rats with lesions to the prefrontal cortex were severely impaired in learning the basic conditional discrimination, but their performance was not affected by in-

creases in the delay between stimulus presentation and the opportunity to respond. In contrast, rats with hippocampal lesions normally learned the conditional discrimination, but their performance declined significantly at longer delays. The major objectives of the present study were to assess the performance of old rats on the conditional learning test and to compare their performance with that of the lesioned groups. On the basis of previous work, old rats were expected to show signs of hippocampal and prefrontal dysfunction. Accordingly, it was predicted that old rats, like rats with prefrontal damage, would be impaired in learning the conditional discrimination in the zero-delay condition and, like rats with hippocampal lesions, their performance would decline further at long stimulus-response intervals.

METHOD

Subjects

Twenty-eight male, Long-Evans rats, obtained from the Trent University Breeding Centre, served as subjects for this experiment. At the beginning of the experiment, the old rats (N = 13) were 26 months old and the young adult rats (N = 15) were 6 months old. All the rats were experimentally naive. Upon being weaned, they were placed in group cages, housing 4 to 6 rats, where they remained until approximately 1 month before the experiment. At that point, they were transferred to individual wire-mesh cages where they remained until the end of testing. Water was available at all times but food was provided in accordance with experimental conditions. The rats were examined regularly by a veterinarian to ensure that they were healthy and able to participate in the study.

Apparatus

All testing was conducted in 5 identical operant chambers, each outfitted with 2 retractable levers, a centrally located visual display panel, and a food-well. The display panel consisted of a bank of 2 rows of 3–3.5 candle-power miniature lamps (#313), each 1 cm in diameter and spaced 3 cm apart. The food-well was situated centrally, 5 cm below the lights and between the levers. A pellet dispenser delivered 45 mg Noyes food pellets to the food-well. Three walls and the ceiling of each box were made of Plexiglas. The fourth wall, containing the levers, etc. was made of metal, and the floor consisted of steel rods spaced 1.5 cm apart.

Procedure

Three weeks before the beginning of training, rats were handled regularly and gradually reduced to about 80% normal body weight. Throughout the experiment, rats were maintained at a constant weight by being fed 20–25 g of lab chow each day.

The experiment began with a 3-stage training program:

Stage 1. Rats received daily sessions in which they were trained to press both levers according to a continuous reinforcement (CRF) schedule. On the first day, rats received some hand-shaping but, in later sessions, they were allowed to press the levers at their own rates. In each session, a rat could obtain a maximum of 160 food pellets, distributed equally across left and right lever-presses. When one lever was pressed 80 times, that lever remained in the chamber but subsequent presses of that lever no longer delivered pellets. Each session in Stage 1 ended when 160 pellets were delivered, or after a 30-min time limit. The criterion for completing Stage 1 was 3 consecutive sessions in which the maximum number of pellets was obtained.

Stage 2. The day after criterion was reached on Stage 1, rats advanced to Stage 2 where they were trained to become familiar with levers retracting after reinforcement. Each daily session began with both levers present. Pressing either lever produced a food pellet, followed by the retraction of both levers for 10 s. The levers then reappeared and remained in the chamber until a response was performed. The cycle was then repeated. Each lever could be pressed 80 times for reinforcement. When 80 pellets had been obtained with one lever, that lever no longer reappeared and the session continued with only the other lever appearing and retracting. This procedure served to discourage the build-up of position preferences. The criterion for completing Stage 2 was obtaining 160 reinforcements in 3 consecutive 30-min sessions.

Stage 3. The day after criterion was reached on Stage 2. Stage 3 was instituted to train rats to respond differentially to the presence and absence of lights on a given trial. Daily sessions consisted of 100 light and 100 no-light trials, with the order of presentation randomly determined. A light trial began with the illumination of the 4 middle lights and the simultaneous appearance of both levers. When the rat pressed a lever, a pellet was dispensed, the lights turned off, and the levers retracted. A 10-s interval followed each light trial. In the light trials, rats were permitted a maximum of 50 responses to each lever. When that number was reached, the lever was retracted and did not reappear in the session. In the no-light trials, both levers appeared for 10 s or until the rat pressed one of the levers. No pellets were provided in the no-light trials. If the rat did not press a lever during the 10 s, both levers retracted for 10 s. If the rat did press a lever in a no-light trial, a 30-s intertrial interval followed.

A session was terminated when 200 trials (100 light and 100 no-light) were completed. The criterion for completion of Stage 3 was 3 consecutive sessions in which the ratio of lever presses in the light trials to lever presses in the no-light trials was 2:1 or better.

Testing. The conditional discrimination learning (CDL) test was introduced the day after criterion was reached on Stage 3. Initially, subjects were tested in a zero-delay condition in which each session began with the illumination of the top, bottom, or both lights at either extreme of the light panel. The lights served as discriminative stimuli and their onset coincided with the appearance of both levers. The levers remained in the chamber until a response was performed. A stimulus on the left side of the panel was the signal for pressing the left lever, while a stimulus on the right side was the signal for a right-lever response. A correct response produced a food pellet, followed by the stimulus offset, retraction of both levers, and a 10-s intertrial interval. An incorrect response led to stimulus offset, the withdrawal of the levers, no reinforcement, and a 30-s intertrial interval. Each session consisted of 80 trials in which the left lever was correct and 80 trials in which the right lever was correct, the order being randomly determined. One 160-trial session was administered each day for 30 consecutive days.

The day after the last session in the zero-delay condition, a delay condition was instituted. The procedure was the same for this condition except for a delay period between the offset of the discriminative stimulus and the appearance of the levers. Subjects received 15 daily sessions with a 5-s delay and then 15 sessions with a 15-s delay.

RESULTS

Records were kept of the groups' progress through each stage of the training program. Occasionally, old rats appeared to advance more slowly, but statistical comparisons did not reveal significant age differences in reaching any of the training criteria.

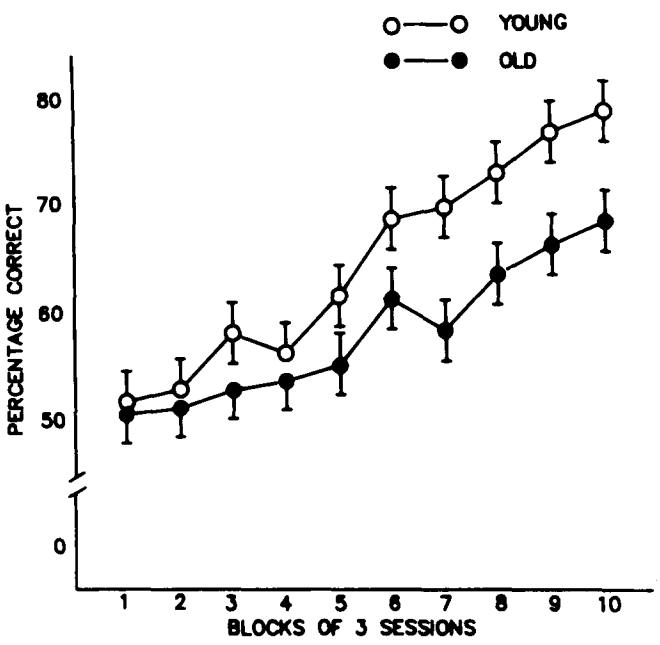

FIG. 1. Performance of old and young groups in the zero-delay condition, averaged over 10 blocks of 3 sessions. Values shown are group means ± the standard error of the mean (S.E.M.).

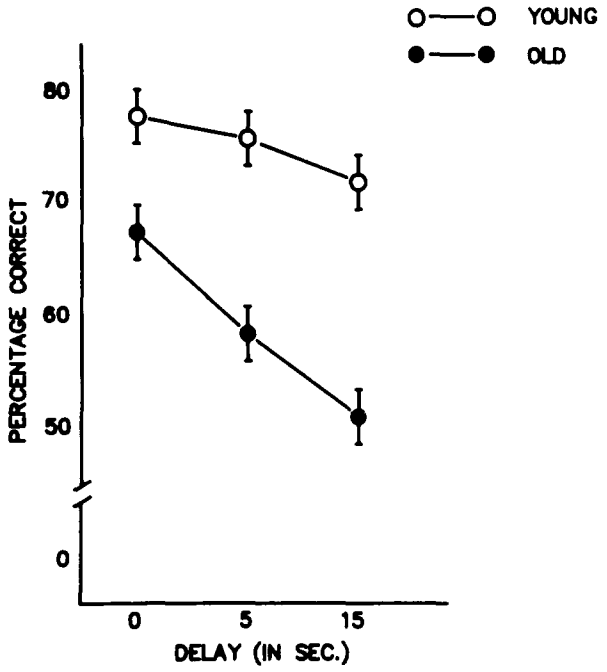

FIG. 2. Performance of old and young groups in all 3 conditions. The scores represent the mean over the last block of 3 test sessions in each condition. Values shown are group means ± S.E.M.

The results for the zero-delay condition of CDL testing are presented in Fig. 1. The data are expressed in terms of the percentage correct responses over the 30 test sessions, averaged over 10 blocks of 3 sessions. It is clear that the old group was much slower in learning the conditional discrimination and that, even by the end of testing, the performance of the aged rats was far below that of the young controls. These differences were confirmed by analysis of variance (ANOVA) that revealed a significant Age by Block interaction, $F(9,234) = 11.06$, $p<0.001$, as well as significant main effects of Age, $F(1,26) = 8.49$, $p<0.025$ and Block, $F(9,234) = 11.57$, $p<0.001$.

When the 5-s delay was introduced between stimulus presentation and the appearance of the levers, both groups, as expected, displayed an initial drop in performance. The young rats quickly recovered and reestablished their previous accuracy rate of 75–80%. The old rats recovered more slowly and did not reach the level of performance they had attained in the zero-delay condition. Virtually the same pattern was observed in the 15-s delay condition. There was a slight decline in the young group's performance in this condition, but the decline in the old group was much greater. In fact, the accuracy rate of the old group over the course of testing in the 15-s delay condition did not rise significantly above chance levels.

The results for the delay conditions are presented in Fig. 2, in terms of the percentage correct scores, averaged over the last block of 3 test sessions. The figure also presents the groups' corresponding scores for the zero delay condition. ANOVA, performed on these data, revealed significant main effects of Age, $F(1,26) = 9.97$, $p<0.01$ and Delay, $F(2,52) = 6.95$, $p<0.005$. The Age × Delay interaction was also statistically significant, $F(2,52) = 3.18$, $p<0.05$, indicating that increases in the delay interval differentially affected the performance of the age groups.

DISCUSSION

In this experiment, old and young adult rats were compared on a test of conditional discrimination learning in which they were required to press one lever in response to a particular stimulus and another lever in response to a second stimulus. Initially, the discriminative stimuli and the levers were presented simultaneously to assess each group's ability to form conditional associations and select appropriate responses. The groups were then tested in successive delay conditions where the stimuli preceded the appearance of the levers by 5 and 15 seconds. The delays added the requirement that rats remember the stimulus that was specific to each trial. Relative to the young controls, old rats were impaired in all conditions but the magnitude of the age difference increased with the length of the stimulus-response interval.

The results indicate significant age differences in learning a conditional discrimination rule and in remembering specific information over extended delays. In studies like this it is important to consider the possibility that age differences in performance reflect differences in noncognitive functions such as, for example, motivation or sensorimotor abilities. However, this is not likely in the present case. All the old rats completed the rigorous training schedule and, at no time, were there age differences in reaching the criterion for advancing from one training stage to the next. The high level of performance by the aged group was particularly evident in Stage 3 where rats were administered 200 trials each day and were required to discriminate between light and no-light trials and respond appropriately.

The behaviour of the aged groups may be compared with the results of a recent experiment (32) in which groups of young adult rats with lesions to the hippocampus or prefrontal cortex were administered the same task under identical conditions. That study demonstrated a clear dissociation between the effects of the two lesions. Prefrontal lesions severely impaired conditional learning but the prefrontal group's performance was not affected by the introduction of a delay between the presentation of the stimuli and the opportunity to respond. In contrast, rats with hippocampal lesions learned the conditional discrimination as

well as controls but were unable to perform the task in the delay conditions where it was necessary to remember the trial-specific stimuli. In the present study, the old rats' impairment in learning the conditional discrimination and recalling discriminative stimuli were evidence of both prefrontal and hippocampal dysfunction.

The hippocampus is one of the first brain regions to show significant changes as part of the aging process and there is growing evidence that learning and memory decline in old age can be related to progressive deterioration in this structure. Studies, involving animals and humans, have demonstrated that aged subjects are frequently impaired on tests that are known to be sensitive to effects of hippocampal damage. In studies where normal aged rats and younger rats with hippocampal lesions were compared directly [e.g., (5, 16, 30)], the results consistently indicated similar deficits in the two populations. The present work extended these observations with respect to episodic or specific memory in a test of conditional learning. Further, the results show that age-related impairment in episodic memory can be distinguished from learning deficits that arise from dysfunction in other brain areas.

The finding that episodic memory loss in the aged group was delay-dependent is consistent with previous reports of rapid forgetting in old rats [(14, 23, 30, 31, 37); but see (1)] and is similar to that seen after selective hippocampal damage (17, 30, 35). Since short-term memory processes are considered to be normal in both cases, the retention deficit at long intervals may be attributed to a failure during that part of the learning process in which enduring representations are formed of acquired information. This type of interpretation has been used to explain the unique form of amnesia that occurs in animals and humans with hippocampal damage (15,25) and would seem to apply equally well to the anterograde memory deficit in old age.

The poor performance of old rats in the zero-delay condition of the present research is reminiscent of the deficit in rats with lesions to the prefrontal cortex on the same task (32). It also shows that age-related impairment in learning and memory extends beyond measures of specific recall. Nonepisodic memory performance has been studied in aging populations but the results are inconclusive. In humans, investigations of implicit memory or memory without awareness, for the most part, suggest that this type of memory is relatively protected from the aging process (18). There are, however, some notable exceptions. Old people are reliably impaired on certain rule learning tasks, such as the Tower of London (24), and in acquiring general skills, such as reading geometrically transformed script (21). There is also evidence that elderly humans fail to show implicit memory in semantic priming tests that use a word-stem completion paradigm (12).

An interesting feature that appears to distinguish those nonepisodic tests that yield age differences in humans from those that do not is the extent to which the frontal lobes are important for successful performance. Those tests in which old people typically fail to show implicit memory appear to be sensitive to the effects of frontal lobe damage. It has been shown, for example, that patients with frontal lobe lesions are impaired on the Tower of London test (24), learning to read geometrically transformed words (4) and in word-stem completion tests (20). The elderly are less likely to show deficits on tests of implicit memory that do not pose difficulty to frontal lobe patients (18).

Animal research has indicated a similar pattern regarding frontal lobe involvement in age-related nonepisodic memory decline. The present results show that old rats, like rats with prefrontal damage, have difficulty learning a discrimination habit that is governed by a conditional rule. In previous work, parallel deficits were observed in old rats and rats with prefrontal lesions on a test of alternation rule learning (29,32) and in acquiring a general skill related to maze learning (33). Further evidence comes from a recently completed study (34) in which hippocampal, prefrontal and aged groups of rats were compared on a size discrimination learning problem. A different pair of stimuli were presented on each trial and subjects had to respond to the larger or smaller of the two. There was no difference between any of the groups in learning the size-discrimination rule, or in reestablishing the original level of performance when retested several weeks later. Clearly, old rats had no difficult learning and remembering rule-based behaviour in a task that did not depend on the integrity of the prefrontal cortex. Taken together, the evidence from animal and human research seems to warrant the hypothesis that variable performance by aged subjects on tests of nonepisodic memory depends on the nature of the tests and the extent to which they depend on prefrontal function.

Finally, while the present results offer further evidence of the importance of hippocampal and prefrontal contributions to learning and memory decline with age, these are certainly not the only brain areas that affect cognitive performance in senescence. Indeed, other structures, such as locus ceruleus (36) and basal ganglia (10), have been implicated in important ways. The present work affirms the neuropsychological approach as a valuable research strategy for studying age-related cognitive changes in animal models. Combined with the use of sensitive testing instruments, this approach promises, in addition to an enhanced understanding of lost and spared function in old age, a greater appreciation of the impact of changes in brain function on learning and memory.

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