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The study of behavioral dysfunctions

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Age-related changes in learning and memory in rats, assessed in the Morris water escape task

Every living organism is affected by changes as consequence of the normal aging process. These changes affect the basal metabolism, the regulation of blood and tissue chemistry, the regulation of body temperature, the absorption and digestion of food, the resistance to injury and disease, and the speed of healing processes and recovery from diseases (Kisker, 1972). The aging organism becomes more vulnerable to adverse environmental influences.

In addition to these changes, sensorimotor changes also occur with advancing age. There is a pronounced deterioration of vision, hearing, smell, and, albeit to a lesser degree, taste with aging. Proprioception, however, appears to be unaffected by age (Adams, Victor & Ropper, 1997). On the (sensori)motor level, activity decreases, reaction time increases, and fine-tuned motion becomes compromised. Body posture, stance, and gait change as the individual ages. The typical 'senile' gait, for example, is characterized by a "(...) *slightly stooped posture, varying degree of slowness and stiffness of walking, shortening of the stride, slight widening of the base, and a tendency to turn en bloc (...)*". (Adams, Victor & Ropper, 1997, p. 122).

The volume of the brain decreases, the gyri shrink, and the sulci, which separate the gyri, become wider. Cognitive functions appear to decline steadily with advancing age, although this decline is normally slow and mild. The most pronounced age-associated cognitive changes are observed in the learning of new material, in problem solving, and in particular, in general memory. Aged people show signs of benign forgetfulness, also called age-associated memory impairment (AAMI). Diagnostic criteria for AAMI are the absence of dementia, a performance in standard tests of memory functions that is at least one standard deviation below the mean, and an age of at least 50 years (Adams, Victor & Ropper, 1997; Giannakopoulos et al., 1997).

Because AAMI is generally considered a symptom of normal aging, the transition to more severe forms which are symptomatic for dementias, a group of disorders that is characterized by progressive loss of cognitive abilities, is often not recognized in time. Moreover, it is difficult to know whether a symptom is part of the normal age-related changes or whether it is the first manifestation of a dementia. As a consequence, therapeutic interventions which might be able to delay the progression of the disease are not as effective as they could be, or are ineffective, because they were not started early enough. One needs to have an understanding of the normal aging process in order to be able to identify pathological changes in the aged. However, these processes are still poorly understood. Animal models might be helpful for gaining further insight into normal aging.

The proportion of aged people in the population is increasing steadily (Martin, 1991; Holden, 1996; Butler, 1997), and the age distribution of the population is no longer pyramid-shaped but is slowly becoming mushroom-shaped (Holden, 1996). The increasing proportion of people aged 65 and older is a big challenge to society and its institutions, to health care policymakers, to the health care system,

and to the private sector (Butler, 1997). They are all responsible for establishing conditions which ensure a good quality of life for the aged, now and in the future.

What is aging?

All attempts to define the aging process are hampered by a lack of knowledge (Masoro, 1991). Aging might be considered as an interdisciplinary life span problem (Ordy, 1975). Ingram (1983), for example, proposed the definition: "*Aging is the manifestation of time-related biological processes that result in decreased viability and increased vulnerability of the organism and thus enhance the probability of death.*" (p. 225). Hazzard (1991) defined aging as "*time-dependent decline in function which affects all tissues and organ systems*" (p. 645). Increasing insight into the role of genes in the process of aging has brought forward the evolutionary theory of aging which states that "*organisms in age-structured populations senesce because of weakening of the force of natural selection as they grow older, which allows certain gene action to reach deleterious levels of expression.*" (Martín & Mian, 1997, p. 18). Alzheimer's disease, for example, normally occurs when people have exceeded their reproductive age, and the occurrence of Alzheimer-specific pathology has no direct effect on the reproductive success of the afflicted.

Changes in selected biomarkers (e.g. Ingram & Reynolds, 1986), or in chronological age and survival curves (Ordy, 1975; Barnes, 1990) could serve as measures of aging. Distributions based on age of death can easily be obtained, whereas measures of gradual changes due to the aging process are much more difficult to obtain. Thus although death as end-point has been used as a criterion for the definition of aging, this has been criticized as being a poor measure of biological aging (Collier & Coleman, 1991; Ingram, 1996). Instead, the 'biological or functional age' might be used (Ingram & Reynolds, 1986; Barnes, 1990). There is increasing evidence that different functions age at different speeds (Campbell, Krauter & Wallace, 1980; see also Chapter 2.3).

The aging rodent

Although aging research in animals has been performed for many decades (e.g. Stone, 1929a,b), it has only recently gained extensive attention, and in fact could be considered a relatively young discipline of increasing importance to biology, medicine, and experimental psychology. Changes occurring with age might be due to extrinsic factors, e.g. environmental or ecological hazards, or due to factors intrinsic to the organism (Finch, 1991). Investigations of the effects of external factors on life expectancy are the province of toxicology (Tucker, 1993) and of experimental animal science. The latter has tended to focus on the effects of housing conditions (e.g. Clough, 1991), handling (Meaney et al., 1991), bacteriological state (Sebesteny, 1991), virological state (van der Logt, 1991), and diet (e.g. Coates, 1991; Roth, Ingram & Lane, 1995) on aging rodents. Normally, extrinsic factors are highly controlled and standardized in experimental aging research in order to eliminate them as sources of confounding effects. The intrinsic factors are under strong genetic control (Finch, 1991): this is not only true for the life expectancy of different species, including humans (Mann, 1997; Yashin, Iachine & Harris, 1999), but also for different genetic strains and lines within specific species (e.g. for rats: Burek, 1978; Mos & Hollander, 1987; Deerberg, 1991; for mice: Russell, 1972; Ordy, 1975; Jucker & Ingram, 1997).

Further evidence for the notion that the survival characteristics of populations are under genetic control has been provided by Takeda and coworkers (1981). They have developed sublines of mice which show biological characteristics of accelerated aging (SAM-P: senescent-accelerated prone mouse) compared with a control line which is considered to show normal aging characteristics (SAM-R:

senescent-accelerated resistant mouse). Two of these SAM-P lines, SAM-P8 and SAM-P10, age very rapidly and have a median life span of approximately 12 months (Jucker & Ingram, 1997). These SAM-P mouse lines show an earlier onset of the age-related deterioration in learning and memory that is correlated with the accelerated aging. However, after evaluating the age-related neuropathological changes in the SAM-P lines, Jucker and Ingram (1997) concluded that these lines do not provide models for normal, though accelerated aging. Instead, they might be useful to study developmental dysfunctions of particular biological systems and their effects on the aging process.

Appropriate lines of mice or rats can be selected from the enormous genetic pool provided by the numerous strains of rats and mice available (Altman & Katz, 1979; Festing, 1980); however, the availability of old animals is limited. The National Institute of Aging, for example, maintains colonies of the inbred Fischer 344 and Brown Norway strains, and of the Fischer 344*Brown Norway hybrids for gerontological studies, and provides animals from these colonies to investigators from other institutes and laboratories (Masoro, 1991; Sprott, 1991). However, aged rats from other strains, such as Sprague-Dawley rats (e.g. Brandeis et al., 1991) and Long-Evans rats (e.g. Gallagher & Burwell, 1989), are also frequently used in experimental aging research. In Europe, the inbred Brown Norway (e.g. Burek, 1978; Mos & Hollander, 1987; van der Staay, van Nies & Raaijmakers, 1990) and WAG strains (e.g. de Koning-Verest, Knook & Wolthuis, 1980; Mos & Hollander, 1987), and different Wistar-derived outbred strains, such as the Janvier Wistar (e.g. Gozlan et al., 1990; Huguet & Tarrade, 1992; Klappdor et al., 1997b) and Harlan Winkelmann Wistar strains (HsdWin:Wu previous name: WISW:Wu; e.g. Schuurman et al., 1987; van der Staay & de Jonge, 1993), have been used in experimental gerontology.

We used four of the above-mentioned strains in the experiments described in this present chapter: the outbred Janvier and the Harlan Winkelmann Wistar strains, the inbred Fischer 344 strain, and the Fischer 344 * Brown Norway hybrids.

Effects of age on (spatial) learning and memory

Because longitudinal studies are quite time-consuming, even in rodents with their relatively short life-span of 2 to 4 years, the most frequently applied experimental design in aging research consists of cross-sectional comparisons between age groups. Most commonly, the behavior of a group of young adult rats is compared with that of a group of old rats. These cross-sectional age comparison studies have consistently revealed that cognitive performance is impaired at advanced age. The experiments described in this chapter focus on learning and memory in tasks which possess spatial components. Spatial discrimination tasks range from straight alleys and two-compartment boxes to mazes of varying complexity, among them the holeboard, the radial maze, and the Morris maze.

Ordy and colleagues (1978) assessed avoidance of a food-cup at the end of a straight alley, the approach of which had been punished by footshock. The running time of the oldest group was faster and the distance run greater than that of the middle-aged and adult groups, when retention was tested 2 and 6 hours after shock, indicating poor retention of the aversive experience. In an inhibitory avoidance task, which also has a spatial component, namely distinction between an illuminated and a dark area, Schuurman and colleagues (1986) tested 2-, 13-, 19-, and 25-month-old Wistar rats. They found an age-related decrease in inhibitory avoidance, with the 25-month-old rats showing virtually no avoidance at all.

Birren (1962) tested Sprague-Dawley rats, approximately 3-, 15-, and 24-months of age, in a two-choice water maze task and found that the aged rats had retarded acquisition of the task. The

acquisition of a position response, and of a series of reversals in a Y-maze was assessed by Botwinick, Brinley, and Robbin (1962) in young, middle-aged, and aged rats. Clear deficits of the oldest group were only apparent in the reversals, which faded away when further reversals were given. When the test was made more difficult, by designing a maze pattern with four serial choice points, the oldest group was slower to learn the original problem, an impairment that was also seen in the first reversal. Again, this difference rapidly faded away with further reversals (Botwinick, Brinley & Robbin, 1963). In a 14-unit T-maze (Stone maze) in which the rats were rewarded with food, aged rats made more errors on their way to the goal than their young counterparts (Ingram, 1985). Similar results were found in a shock-motivated 14-unit T-maze, where aged Fischer 344 rats (Kametani et al., 1989; Spangler et al., 1994), aged Brown Norway rats, and aged Fischer 344*Brown Norway rats were found to perform worse than young conspecifics (Spangler et al., 1994).

Age-related impairments in spatial memory have been reported in rats tested in the circular maze, where holes are located equidistantly around the periphery of a circular platform. One of these holes provides access to an escape tunnel. Aged rats made consistently more errors before finding the escape tunnel than young rats did (e.g. Barnes & McNaughton, 1985; Barnes, Eppich & Rao, 1989; Gallagher & Burwell, 1989; Algeri et al., 1991).

The effects of aging on the spatial memory performance of rats have also been studied in a holeboard task, which allows the simultaneous assessment of spatial working memory (WM) and spatial reference memory (RM). Olton, Becker, and Handelmann (1979, p. 314) defined WM, based on a concept of Honig (1978) as type of memory needed to solve a WM procedure. *"In a working memory procedure, stimulus information is useful for one trial of an experiment, but not for subsequent trials (...). The animal must remember not only which stimuli have been presented, but also when they were presented (...)."* In fact, WM is defined by the procedure applied to test it. In contrast, WM as defined by Baddeley and colleagues (Baddeley & Hitch, 1974; Baddeley & Lieberman, 1980) refers to an aspect of the memory system that stores information and processes it. This appears to be the task of the short term memory. RM refers to information that is not only useful within a single trial, but that is useful across a series of trials, usually the entire experiment (Olton, Becker, and Handelmann, 1979, 1980). Typically, information about how a task must be solved, and where the baits can be found in, for example, a radial arm maze, or a holeboard, is stored in RM.

Testing Brown-Norway rats of five ages (4, 13, 19, 25, and 30 months) in a holeboard task where 4 of 16 holes were baited with a food reward, we observed a clear age-related decline of spatial WM and RM performance (van der Staay, van Nies & Raaijmakers, 1990). The decline was most profound between 19 and 25 months of age. The speed of visiting holes and the development of a preferred pattern of hole visits did not influence spatial discrimination performance.

Means and Kennard (1991) tested 3-, 12-, and 22-month-old Fischer 344 rats in a two-choice win-stay water escape task. This task is run in a circular water tank with a T-shaped barrier that separates the start partition opposite to the horizontal stem of the T, and two choice partitions, one of which is provided with an escape platform. From a fixed starting position, a rat was forced to choose the segment containing the escape platform (the information run). In the choice run both segments were accessible, but only the partition provided with an escape platform during the information run contained the platform, i.e. a win-stay strategy is required. The partition which contained the escape platform was determined semi-randomly. Aged rats were impaired in acquiring this version of the task. When inter-trial intervals of variable length are inserted between the information run and the choice run, the time-

dependent decay of WM performance can be measured in this task. Again, impairments were seen in the aged rats, whereas the performance of the young and middle-aged rats did not differ.

Age-related impairments in spatial discrimination learning have been reported in rats tested in different versions of the radial arm maze. Aged rats have been found to perform worse than their younger counterparts in both the WM version of this task, in which all arms are baited with food reward, (e.g. Steward, Mitchell & Kalant, 1989; Luine, Bowling & Hearn, 1990; Kadar et al., 1990; Huidobro et al., 1993), and the version in which only a subset of arms is baited to allow the simultaneous assessment of WM and RM (Pitsikas & Algeri, 1992; Arendash, Sanberg & Sengstock, 1995).

By contrast, no age differences were found in non-spatial versions of the radial maze task, modified to measure either WM or RM (Barnes et al., 1987). Similar differences between spatial and non-spatial versions were found for the 8-arm radial water maze: the clearest impairments of both WM and RM were seen in the spatial version, where 25-month-old rats made more errors than their 3- and 11-month-old conspecifics (Pitsikas & Algeri, 1992). These clear differences between non-spatial and spatial versions of tests support the notion that the processing of spatial information is compromised in aged rats (Barnes, 1990). Life-long calorie restriction protected against the decline in spatial orientation performance, emphasizing that external factors can strongly affect the aging process (Foster & Lal, 1991; Roth, Ingram & Lane, 1995).

The Morris water escape task is the most frequently used task to assess spatial orientation in rats. Age-associated impairments in spatial discrimination performance in the Morris task are well documented (e.g. Gage, Dunnett & Björklund, 1984; Decker, Pelleymounter & Gallagher, 1988; Steward, Mitchell & Kalant, 1989; Meaney et al., 1991; Sirviö et al., 1991; Fischer et al., 1991; Nilsson & Gage, 1993; Yamazaki et al., 1995; Abrous et al., 1997; Fong, Neff & Hadjiconstantinou, 1997).

Description of the experiments performed

Because of its prominent role in the investigation of age-related cognitive impairments, we chose the Morris water escape task as the behavioral paradigm in the experiments described in this chapter. We assessed different aspects of the age-associated changes in Morris water escape behavior, and discuss the implications of our findings for experimental designs in aging research.

- We investigated the effects of age on the performance of rats of different strains in the Morris water escape task by comparing the performance of old rats with that of younger counterparts in a cross-sectional design (Chapter 2.1). Cross-strain comparisons might provide information about the generality of behavioral changes observed (Barnes, 1990).
- Cross-sectional experiments with only two ages have as drawback the possibly erroneous conclusions drawn about the shape of the underlying aging function. Aging appears to follow a non-monotonic function in most cases (Markowska et al., 1989; Barnes, 1990; Baxter & Gallagher, 1996), but can be differentiated on the basis of individual processes (van der Staay, Blokland & Raaijmakers, 1990). Therefore, we studied the effects of aging on performance of the water escape task in a cross-sectional experiment with rats of four different ages, followed by an experiment involving two age groups (Chapter 2.2, first and second experiments).
- Longitudinal studies might provide an alternative to cross-sectional designs to investigate the progressive age-related decline in spatial learning and memory. In two experiments, we repeatedly tested aging rats in the Morris water escape task. The animals acquired the task at the age of approximately 24 months. They were re-tested about 3 and 6 months later. The last test was performed at an age of approximately 30 months. At this age less than 50% of the population had

survived (Chapter 2.3). The 50% mortality point might be taken as a criterion to judge animals as old (Barnes, 1990).

- Successful spatial navigation in the standard Morris task relies on an intact RM, but the Morris task can be modified in such a way that WM can also be assessed (Whishaw, 1985, 1987). We investigated the effects of age on the performance of young and old rats in this modified version of the Morris task in the third experiment of Chapter 2.2.
- Finally, when performing Morris water escape experiments with aged outbred Harlan Winkelmann Wistar rats, we got the impression that behavioral shifts in the strain occurred over time, i.e. that the replicability of results was poor. Old rats of this strain apparently lost their ability to successfully negotiate the circular water tank. We investigated this further in Chapter 2.4.

2.1

Effects of age on Morris water escape behavior of outbred Wistar, hybrid Fischer 344*Brown Norway, and inbred Fischer 344 rats

Abstract

The aim of the present study was to assess whether strains of rats which are available for gerontological research show the expected age-associated decline in spatial orientation performance in the Morris maze. The standard Morris water escape task, where a rat is required to find an invisible, submerged escape platform in a water tank, is a test system that has consistently been found to be sensitive to age-associated changes in spatial orientation performance. The spatial discrimination of adult and aged outbred Janvier Wistar (WISRJ) rats, young and old inbred Fischer 344 (F344) rats, and hybrid Fischer 344*Brown Norway (FBNF1) rats was compared in three separate experiments. In an additional experiment we assessed the possible influence of transport stress on the performance of aged F344 rats in the Morris water maze task.

We conclude that gerontological or gerontopharmacological studies of *spatial discrimination learning in the Morris task* should not be performed with F344 rats. FBNF1 rats and WISRJ rats appear to be a better choice. We cannot comment on the usefulness of these rat strains for non-behavioral studies or for behavioral studies of aspects other than spatial discrimination learning.

Introduction

According to Barnes (1979), spatial discrimination tasks could serve as valuable experimental paradigms to study the cognitive and neurobiological changes which accompany aging. To be considered as a valid tool in aging research, spatial discrimination tasks should be sensitive to these naturally occurring events. The standard Morris water escape task is a test system that has extensively been used to investigate age-associated changes in the spatial orientation performance of rats.

The results of age comparison studies have, however, proved to be variable ranging from no age-related impairments (Lindner & Schallert, 1988) and transient acquisition deficits in aged rats (Rapp, Rosenberg & Gallagher, 1987), to severe and permanent impairments in the ability of aged rats to find and escape onto a submerged platform (Aitken & Meaney, 1989; Lindner & Schallert, 1988; van der Staay & de Jonge, 1993; Bickford et al., 1997).

A factor that may have influenced this variation in the occurrence and severity of age-associated deficits is the rat strain used. Lindner and Schallert (1988), for example, found no age-related impairment in performance of the Morris water escape task in an age comparison study with 3-, 24-, and 28-month-old Long-Evans rats. By contrast, they found a clear age-dependent decline in water escape performance when using Fischer 344 rats. The latter finding is in line with the majority of cross-

sectional studies on water escape behavior in rats (e.g. Aitken & Meaney, 1989; Gage, Dunnett & Björklund, 1984; Rapp, Rosenberg & Gallagher, 1987; van der Staay & de Jonge, 1993; Fong, Neff & Hadjiconstantinou, 1997).

Many factors appear to determine a rat's performance in the Morris water escape task, for example, housing conditions, the test environment, schedule and time of testing, and the equipment used (Andrews, 1996). Therefore, testing young(er) and old(er) rats of different strains in the same test environment might provide an answer to the question whether age-related impairments in Morris water escape behavior are a general phenomenon (Barnes, 1990), or whether they reflect the effects of conditions which are unique for a particular laboratory (Andrews, 1996).

The aim of the present study was to assess whether strains of rats which are readily available for gerontological research show the expected age-associated decline in spatial orientation performance in the Morris maze when tested under the experimental conditions in our laboratory. To this end, we carried out three experiments in which we compared the performance of adult and aged outbred Janvier Wistar (WISRJ) rats, young and aged Fischer 344*Brown Norway (FBNF1) hybrid rats, and inbred Fischer 344 (F344) rats. The WISRJ strain is often used in aging research in Europe (e.g. Gozlan et al., 1990; Huguet & Tarrade, 1992; Roux et al., 1994, 1995; Klapdor et al., 1997b), whereas the F344 strain and the FBNF1 hybrids have been selected by the National Institute of Aging, Bethesda, USA, as being especially suited for aging studies (Masoro, 1991; Sprott, 1991). The FBNF1 hybrids show a normal distribution of age-related pathologies which occur in the later phase of life (Sprott, 1991). Interestingly, these rats do not suffer from an age-related decline in the acquisition of the Stone 14-unit T-maze up to 31 months of age, whereas all other rodent strains tested so far show this age-associated impairment (Ingram et al., 1994).

Experiment 1: acquisition of the standard water escape task by 12- and 24-month-old outbred Janvier Wistar rats.

Material and Methods

Animals: adult and aged male Janvier Wistar rats (WISRJ) were supplied by the Centre d'Élevage Roger Janvier (le Genest Saint Isle, France). They were allowed to habituate to the animal facilities of our laboratory (CNS-Research, Bayer, Cologne, Germany) for at least 1 week, before behavioral testing started. The results in the present experiment are for the control rats used in a drug-finding study. The 12-month-old (n=10) and 24-month-old rats (n=9) were given orally a 1% tylose suspension 30 minutes before each of the daily acquisition sessions. Tylose was used as the solvent of the drug tested and has earlier been found not to have an effect on learning behavior in the Morris task (data not shown).

Apparatus: the Morris water escape performance was assessed in a water tank which consisted of a circular black tub with a slightly sloping wall (Material: polyethylene; inner dimensions: diameter at top 153 cm, diameter at bottom 143 cm, depth 63 cm), filled with 43.5 cm of clear tap water at a temperature of approximately 22°C (see Fig. 1). The escape platform consisted of a black polyethylene cylinder (diameter 10.8 cm), submerged 1.5 cm below the surface of the water. In this version of the test the water was not made opaque because the black escape platform was virtually invisible in the black tank. The water tank was situated in a room illuminated by white fluorescent tubes. Abundant

extra-maze cues were provided by the furniture in the room, including desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that was playing softly. All testing was done between 9:00 and 15:00. A video camera, mounted in the center above the circular pool, provided a picture of the pool on a TV-monitor. The movements of the rat were registered automatically by a video-tracking system (EthoVision®, Noldus Information Technology, Wageningen, The Netherlands) and stored in an MS-DOS compatible microcomputer.

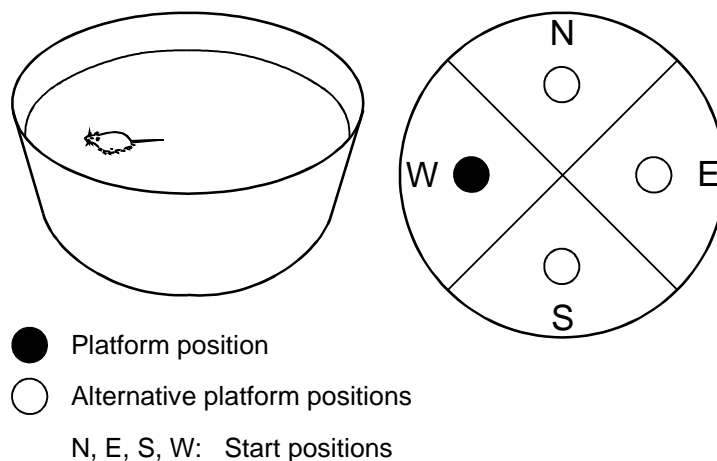


Figure 1. The Morris water escape task. The invisible, submerged escape platform was always in the same quadrant. A trial was terminated as soon as the rat had swum and climbed onto the escape platform, as depicted in this illustration (left panel). The platform position in quadrant West, the alternative platform positions, and the four alternative start positions (N, E, S, W) at the rim of the pool are schematically shown in the right panel.

Procedures (acquisition): the animals received four trials during five daily acquisition sessions. A trial was started by placing a rat into the pool, facing the wall of the tank. Each of four starting positions (north, east, south, and west) was used once in a series of four trials; their order was randomized. The escape platform was always in the same quadrant. A trial was terminated as soon as the rat had climbed onto the escape platform or when 90 seconds had elapsed, whichever event occurred first. A rat was allowed to stay on the platform for 30 seconds. Then it was taken from the platform and the next trial was started. If a rat did not find the platform within 90 seconds it was put on the platform by the experimenter and was allowed to stay there for 30 seconds. After completion of the fourth trial (on the fifth day after completion of the probe trial, see below), the rat was gently dried with crêpe paper and returned to its home cage. The animal was kept warm under an infrared bulb (Original Hanau Solilux, 150 W) fixed about 60 cm above the floor of the cage.

Probe trial: after the fourth trial of the fifth daily session, an additional trial was given as a probe trial: the platform was removed, and the time the rat spent in the four quadrants was measured for 60 seconds. In the probe trial, all rats started from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

Statistical analysis

Acquisition: four different measures were taken to evaluate the performance of the rats during acquisition training: escape latency, traveled distance, distance to platform, and swimming speed.

- Escape latency is the time (s) taken to find and escape onto the submerged platform (Morris, 1984).

- Traveled distance (cm) is the total distance swum to find and escape onto the submerged platform (Morris, 1984).
- Distance to platform (cm) (Gallagher, Burwell & Burchinal, 1993) was calculated as the mean distance to the platform across all samples drawn by the video-tracking system between the start of a trial and the moment the rat climbed onto the platform.
- Swimming speed was calculated as traveled distance (cm) divided by escape latency (s).

The measures were averaged per rat within each session. Age differences in the acquisition of the water escape task were assessed with an analysis of variance (ANOVA; Winer, 1971) with repeated measures over sessions. In addition, age differences in particular sessions were analyzed by ANOVA.

Probe trial: the following measures were evaluated for the probe trial: time (s) in quadrants, traveled distance (cm) in quadrants, time (s) in annulus, and traveled distance (cm) in annulus. The swimming time and distance swum per quadrant were assessed with a repeated measures ANOVA over quadrants (time in the quadrant north, east, south, and west are considered as levels of the repeated measures factor), complemented by ANOVAs on the swimming times *per* quadrant. Time in the annulus and traveled distance in the annulus were analyzed by an ANOVA with the factor Age.

Results

Acquisition

Escape latency (Fig. 2, upper left panel): averaged over the sessions, the aged Janvier rats had longer escape latencies than the middle-aged rats (General mean: $F_{1,17} = 21.13$, $p < 0.01$). The escape latencies decreased across the five daily acquisition sessions (Sessions: $F_{4,68} = 10.15$, $p < 0.01$) to a similar extent (Sessions by Age interaction: $F_{4,68} < 1.0$, n.s.). However, when individual sessions were considered, age differences were apparent from the third day of training onward ($F_{s_{1,17}}$, and associated p-values for sessions 1 to 5, in that order: 3.01, n.s.; 3.02, n.s.; 6.38, $p < 0.05$; 22.01, $p < 0.01$; 11.69, $p < 0.01$).

Traveled distance to reach the escape platform (Fig. 2, upper right panel): the old rats swam, on average, further before they found the escape platform than the middle-aged rats did (General mean: $F_{1,17} = 15.78$, $p < 0.01$). The distance swum decreased over the five training sessions (Sessions: $F_{4,68} = 10.00$, $p < 0.01$) to a similar extent (Sessions by Age interaction: $F_{4,68} = 1.77$, n.s.). The age differences were evident from the third session onward, when individual days of training were considered ($F_{s_{1,17}}$, and associated p-values, for sessions 1 to 5, in that order: 0.58, n.s.; 3.26, n.s.; 6.99, $p < 0.05$; 32.62, $p < 0.01$; 8.13, $p < 0.05$).

Distance to platform (Fig. 2, lower left panel): the mean distance to the escape platform was smaller for the middle-aged than for the aged rats, averaged over the five acquisition sessions (General mean: $F_{1,17} = 16.49$, $p < 0.01$). The distance to the platform decreased across training sessions (Sessions: $F_{4,68} = 10.72$, $p < 0.01$) to a similar extent in both age groups (Sessions by Age interaction: $F_{4,68} = 1.73$, n.s.). The age differences were evident from the second session onward, when individual days of training were considered ($F_{s_{1,17}}$, and associated p-values for sessions 1 to 5, in that order: 0.25, n.s.; 5.63, $p < 0.05$; 6.33, $p < 0.05$; 16.15, $p < 0.01$; 8.89, $p < 0.01$).

Swimming speed (Fig. 2, lower right panel): on average, the two age groups had a similar swimming speed (General mean: $F_{1,17} = 2.34$, n.s.). The speeds changed slightly over sessions (Sessions: $F_{4,68} =$

3.17, $p < 0.05$), but similarly in both the middle-aged and the aged rats (Sessions by Age interaction: $F_{4,68} < 1.0$, n.s.).

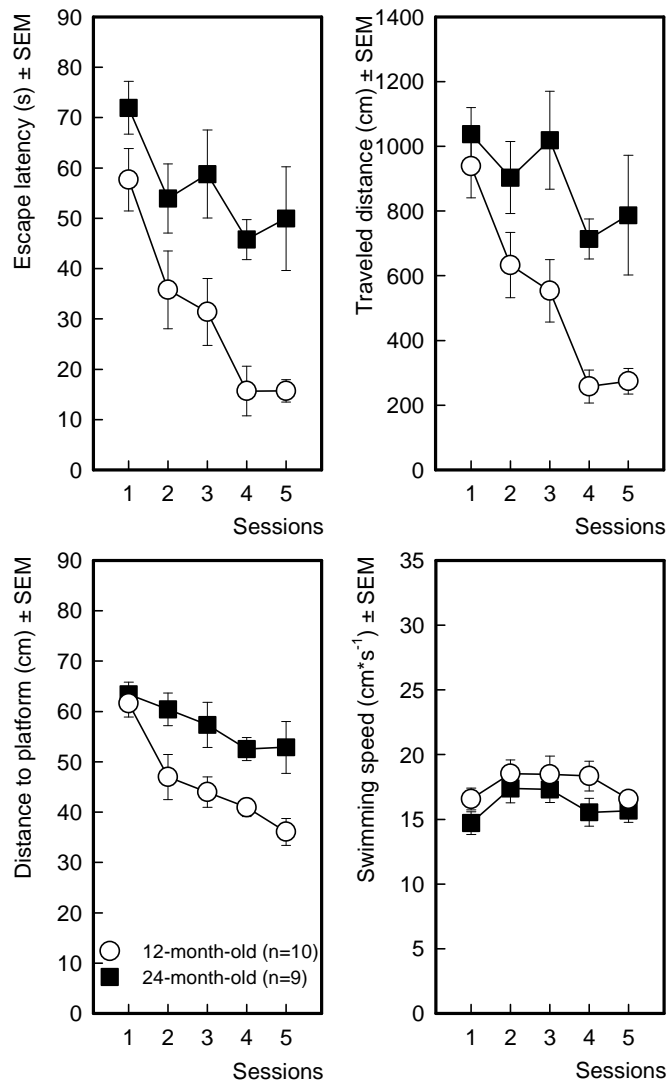


Figure 2. Morris water escape task: acquisition of a water escape task in a circular pool by middle-aged and old Janvier Wistar rats. The means and standard errors of the means (SEM) of the latency (s) (upper left panel) and the traveled distance (cm) (upper right panel) to escape onto a submerged platform, the distance to platform (cm) (lower left panel), and the swimming speed ($\text{cm}\cdot\text{s}^{-1}$) (lower right panel) are depicted for the five acquisition sessions.

Probe trial

Time in quadrants (Fig. 3, upper left panel): the time spent in the four quadrants was different (Quadrants: $F_{3,51} = 12.62$, $p < 0.01$), indicating that there was a bias for the training quadrant. This bias was present in the middle-aged, not in the aged rats (Quadrants by Age interaction: $F_{3,51} = 3.01$, $p < 0.05$).

Traveled distance in quadrants (Fig. 3, lower left panel): on average, middle-aged and aged rat swam a similar distance during the 60-seconds probe trial (General mean: $F_{1,17} < 1.0$, n.s.). Although the distances swum in the quadrants were different (Quadrants: $F_{3,51} = 12.61$, $p < 0.01$), a bias for the previous training quadrant was obvious for the middle-aged rats only (Quadrants by Age interaction: $F_{3,51} = 2.74$, $0.10 > p > 0.05$).

Time in the annulus (Fig. 3, upper right panel): there was no difference between the two age-groups for the time spent in the annulus ($F_{1,17} = 3.01$, n.s.).

Traveled distance in the annulus (Fig. 3, lower right panel): the middle-aged and the aged Janvier rats swum a similar distance in the annulus ($F_{1,17} = 2.69$, n.s.).

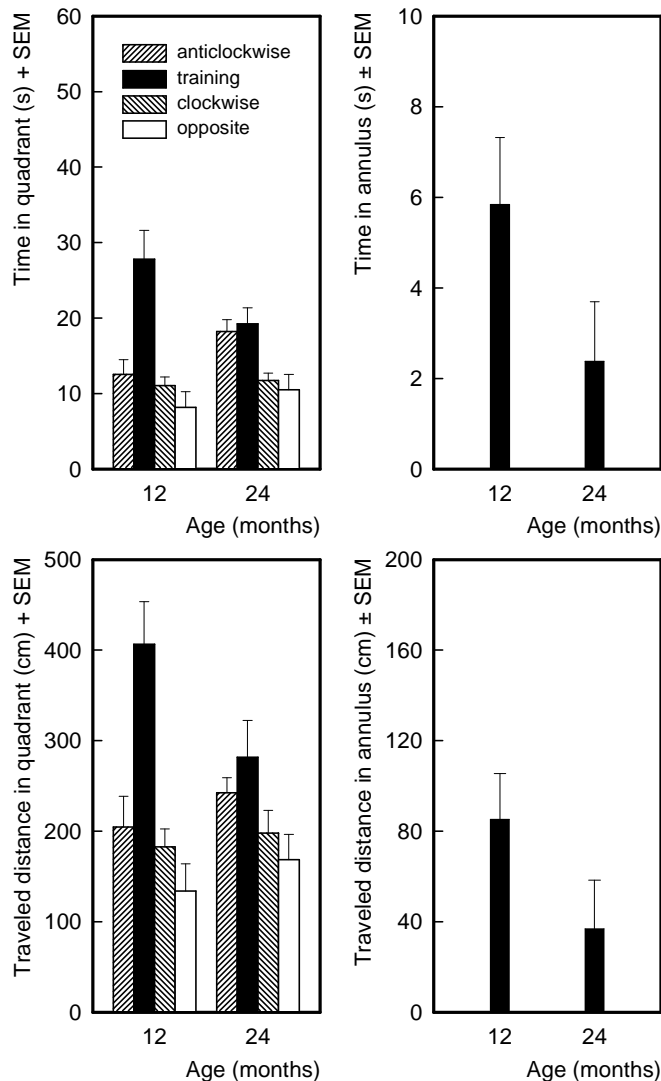


Figure 3. Performance of ten middle-aged and nine old Janvier Wistar rats in the probe trial. The means and standard errors of the means (SEM) of the times (s) spent in the four quadrants (upper left panel), the traveled distance in quadrants (cm) (lower left panel), the time (s) in the annulus (upper right panel), and traveled distance in the annulus (cm) (lower right panel) are shown.

Experiment 2: acquisition of the standard water escape task by 3- and 24-month-old hybrid Fischer 344*Brown Norway rats

Material and Methods

Animals: male Fischer 344*Brown Norway (FBNF1) hybrids were supplied by Harlan Sprague Dawley (Indianapolis, USA). All rats had been shipped to our institute (Cologne, Germany) at least 1 month before behavioral testing started. The animals were housed in groups of three to five in standard Makrolon® type IV cages on sawdust bedding in an air-conditioned room (temperature: about 20°C,

humidity: 60%). All rats were kept under an artificial 12/12-hour light-dark cycle (lights on from 7:00 to 19:00). The data in the present experiment were for eight 4-month-old rats and eight 26-month-old rats used as controls in a study in which the behavioral effects of a putative cognition-enhancing compound were tested. The control rats were given orally a Na-citrate buffer 30 minutes before each of the daily acquisition sessions. Na-citrate buffer was used as the solvent of the drug tested and was found not to have an effect on learning behavior in independent control experiments.

Apparatus: the same apparatus as in the first experiment was used.

Procedure: the procedure to assess the acquisition was identical to that used in experiment 1. The probe trial was performed as in experiment 1, except that the trial was terminated after 30 seconds.

Statistical analysis: the data of the acquisition and the probe trial were analyzed as in experiment 1.

Results

Acquisition

Escape latency (Fig. 4, upper left panel): averaged over the sessions, the aged FBNF1 hybrids had longer escape latencies than the young rats (General mean: $F_{1,14} = 29.40$, $p < 0.01$). In the first training session, the escape latency of the young rats was only marginally shorter than that of the aged rats ($F_{1,14} = 3.27$, $0.1 > p > 0.05$). The escape latencies decreased across the five daily acquisition sessions (Sessions: $F_{4,56} = 10.49$, $p < 0.01$), but faster for the young rats (Sessions by Age interaction: $F_{4,56} = 3.57$, $p < 0.05$).

Traveled distance to reach the escape platform (Fig. 4, upper right panel): the old FBNF1 rats swam, on average, further before they found the escape platform than the young rats did (General mean: $F_{1,14} = 38.44$, $p < 0.01$). There were no differences between the two age groups on the first training session ($F_{1,14} < 1.0$, n.s.). In the course of training, the rats reduced the swum distance to reach the platform (Sessions: $F_{4,56} = 12.25$, $p < 0.01$). The young rats, however, learned faster than the aged rats (Sessions by Age interaction: $F_{4,56} = 6.37$, $p < 0.01$).

Distance to platform (Fig. 4, lower left panel): the distance to the escape platform, averaged over the five acquisition sessions, was shorter for the young than for the aged rats (General mean: $F_{1,14} = 64.59$, $p < 0.01$). This difference between age groups was evident from the first day of training onward ($F_{s,1,14}$ and associated p-values, for sessions 1 to 5, in that order: 6.11, $p < 0.05$; 32.79, $p < 0.01$; 57.30, $p < 0.01$; 61.9, $p < 0.01$; 111.26, $p < 0.01$). Young and aged rats reduced the distance swum to reach to platform across training sessions (Sessions: $F_{4,56} = 28.87$, $p < 0.01$). This reduction was more pronounced for the young rats (Sessions by Age interaction: $F_{4,56} = 6.48$, $p < 0.01$).

Swimming speed (Fig. 4, lower right panel): on average, the old rats swam slower than the young rats (General mean: $F_{1,14} = 22.95$, $p < 0.01$). Swimming speed was stable across sessions (Sessions: $F_{4,56} = 2.08$, n.s.; Sessions by Age interaction: $F_{4,56} < 1.0$, n.s.).

Probe trial

Time in quadrants (Fig. 5, upper left panel): the time spent in the four quadrants was different (Quadrants: $F_{3,42} = 46.99$, $p < 0.01$), indicating that there was a bias for the training quadrant, and this bias was stronger in the young rats than in the aged rats (Quadrants by Age interaction: $F_{3,42} = 10.09$, $p < 0.01$). This was confirmed by an analysis of the time spent in the training (west) quadrant ($F_{1,14} = 14.97$, $p < 0.01$).

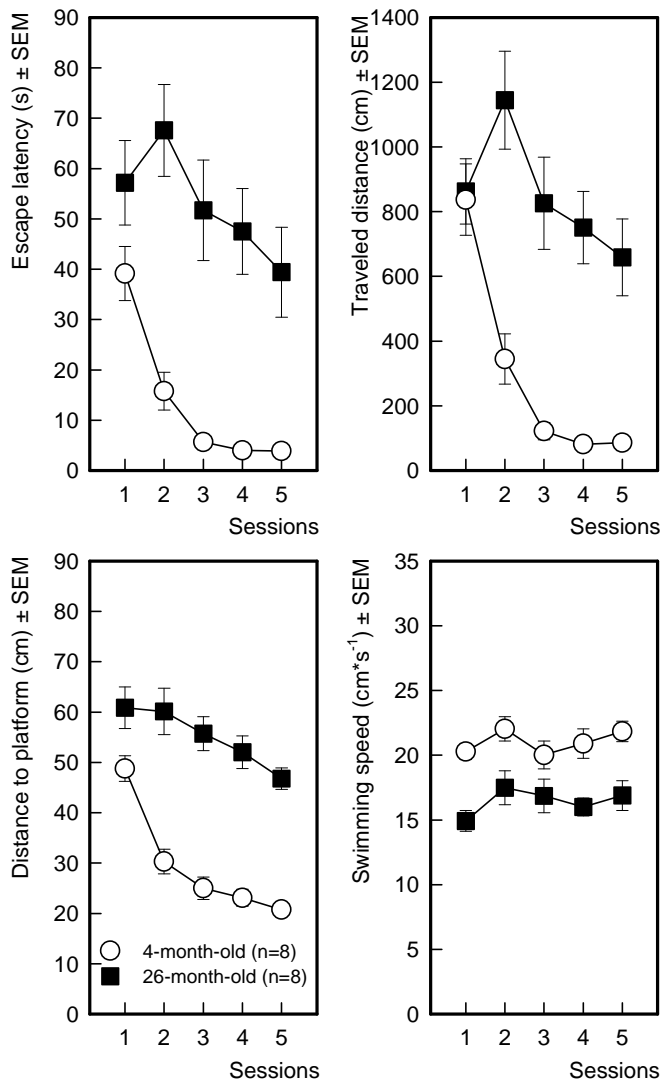


Figure 4. Morris water escape task: acquisition of a water escape task in a circular pool by young and old Fischer 344*Brown Norway hybrids. The means and standard errors of the means (SEM) of the latency (s) (upper left panel) and the traveled distance (cm) (upper right panel) to escape onto a submerged platform, the distance to platform (cm) (lower left panel), and the swimming speed (cm*s⁻¹) (lower right panel) are depicted for the five acquisition sessions.

Traveled distance in quadrants (Fig. 5, lower left panel): on average, the young rats swam further during the 30-second probe trial than the old rats did (General mean: $F_{1,14} = 12.93$, $p < 0.01$). The distances swum in the quadrants were different (Quadrants: $F_{3,42} = 52.97$, $p < 0.01$), but a bias for the previous training quadrant was obvious for the young rats only (Quadrants by Age interaction: $F_{3,42} = 20.72$, $p < 0.01$). This was confirmed by an analysis of the distance swum in the training (west) quadrant ($F_{1,14} = 55.92$, $p < 0.01$).

Time in the annulus (Fig. 5 upper right panel): the young spent more time in the annulus than the aged rats did ($F_{1,14} = 23.31$, $p < 0.01$).

Traveled distance in the annulus (Fig. 5, lower right panel): the young rats swam further in the annulus than the aged rats did ($F_{1,14} = 93.41$, $p < 0.01$).

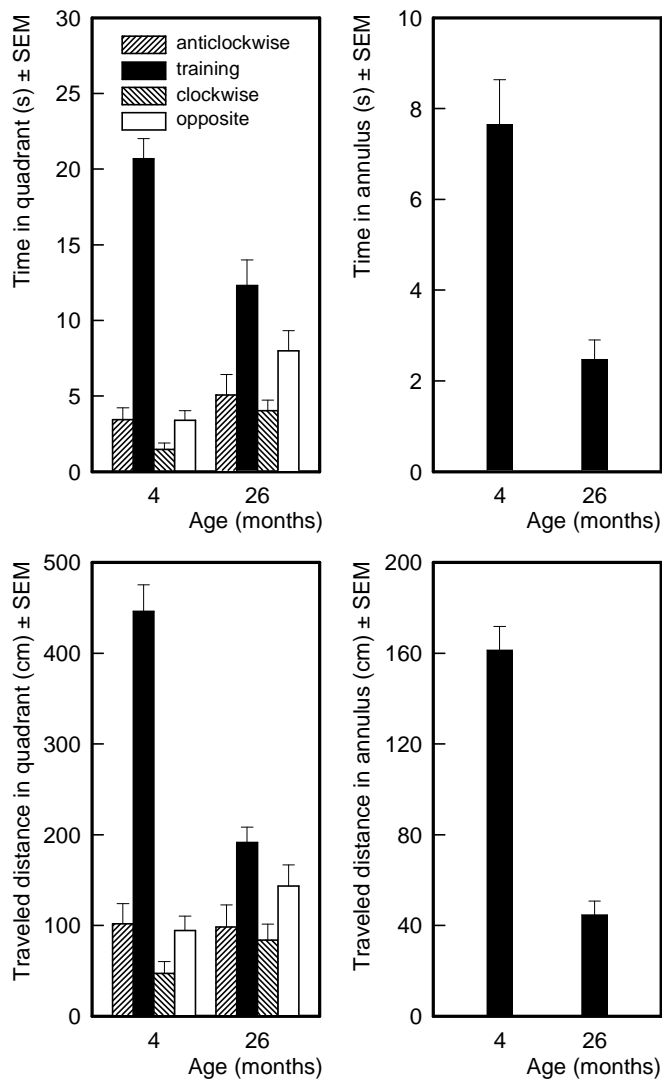


Figure 5. Behavior of eight young and eight old Fischer 344*Brown Norway hybrids in the probe trial. The means and standard errors of the means (SEM) of the times (s) spent in the four quadrants (upper left panel), the traveled distance in quadrants (cm) (lower left panel), the time (s) in the annulus (upper right panel), and traveled distance in the annulus (cm) (lower right panel) are shown.

Experiment 3: acquisition of the standard water escape task by young and aged Fischer 344 rats

Material and Methods

Animals: male Fischer 344 (F344) rats were supplied by Harlan Sprague Dawley (Indianapolis, USA). All rats were shipped to our animal facilities at least 1 month before behavioral testing started. The data in the present experiment were from the control groups of animals used in a study in which the behavioral effect of a putative cognition-enhancing compound was assessed. Ten 6-month-old and ten 26-month-old rats were used. These control rats were given orally a Na-citrate buffer 30 minutes before each of the daily acquisition sessions.

Apparatus: the same equipment was used as in the previous experiments.

Procedure: the rats were tested in the Morris water escape task as described in experiment 2.

Statistical analysis: the data for the acquisition sessions and the probe trial were analyzed as in experiment 1.

Results

Acquisition

Escape latency (Fig. 6, upper left panel): averaged over the sessions, the aged F344 rats had longer escape latencies than the young rats (General mean: $F_{1,18} = 41.85$, $p < 0.01$). This age difference was found from the first training session on ($F_{1,18} = 6.45$, $p < 0.05$). The escape latencies changed across the five daily acquisition sessions (Sessions: $F_{4,72} = 4.39$, $p < 0.01$), but only for the young rats (Sessions by Age interaction: $F_{4,72} = 4.88$, $p < 0.01$).

Traveled distance to reach the escape platform (Fig. 6, upper right panel): the old rats swam, on average, further before they found the escape platform than the young rats did (General mean: $F_{1,18} = 9.48$, $p < 0.01$). On the first day of training, the old and young rats swam the same distance to reach the platform ($F_{1,18} < 1.0$, n.s.). There was no general effect of sessions (Sessions: $F_{4,72} < 1.0$, n.s.), because in the course of training the aged rats swam longer distances before they reached the platform, whereas in the young F344 rats, these distances decreased over sessions (Sessions by Age interaction: $F_{4,72} = 7.19$, $p < 0.01$).

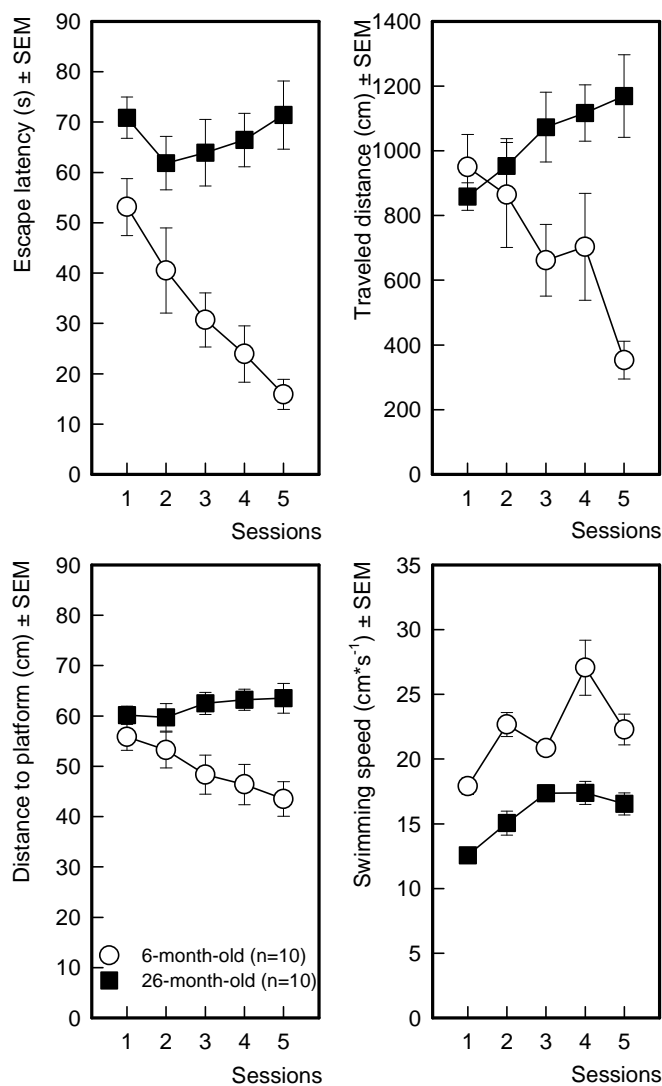


Figure 6. Morris water escape task: acquisition of a water escape task in a circular pool by young and old Fischer 344 rats. The means and standard errors of the means (SEM) of the latency (s) (upper left panel) and the traveled distance (cm) (upper right panel) to escape onto a submerged platform, the distance to platform (cm) (lower left panel), and the swimming speed (cm*s⁻¹) (lower right panel) are depicted for the five acquisition sessions.

Distance to platform (Fig. 6, lower left panel): the distance to the escape platform, averaged over the five acquisition sessions, was shorter for the young rats than for the aged rats (General mean: $F_{1,18} = 15.14$, $p < 0.01$). This difference between age groups was evident from the third day of training onward ($F_{s_{1,18}}$ and p -values, for sessions 1 to 5, in that order: 1.75, n.s.; 2.08, n.s.; 10.03, $p < 0.01$; 13.76, $p < 0.01$; 19.53, $p < 0.01$). There was no general effect of sessions (Sessions: $F_{4,72} = 1.13$, n.s.), because the mean distance to the platform increased slightly in aged rats in the course of training, whereas it decreased in the young F344 rats (Sessions by Age interaction: $F_{4,72} = 4.39$, $p < 0.01$).

Swimming speed (Fig. 6, lower right panel): on average, the old rats swam slower than the young rats (General mean: $F_{1,18} = 55.84$, $p < 0.01$), but swimming speed increased over sessions (Sessions: $F_{4,72} = 14.35$, $p < 0.01$). This increase was continuous for the young rats, whereas it was somewhat irregular across sessions for the aged rats (Sessions by Age interaction: $F_{4,72} = 3.24$, $p < 0.05$).

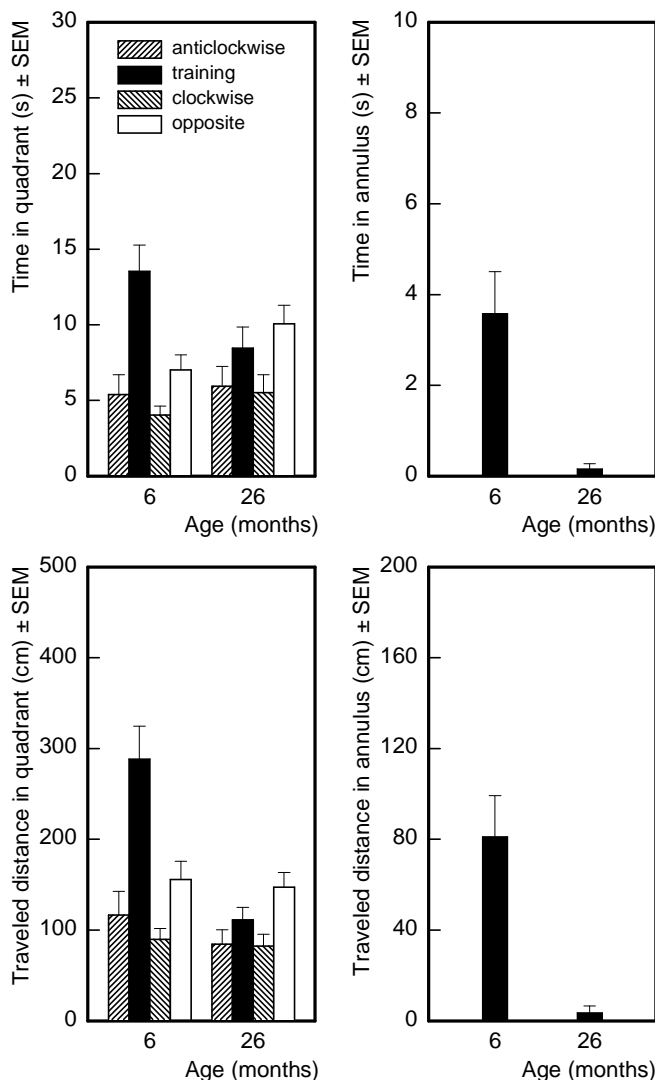


Figure 7. Performance of ten young and ten old Fischer 344 rats in the probe trial. The means and standard errors of the means (SEM) of the times (s) spent in the four quadrants (upper left panel), the traveled distance in quadrants (cm) (lower left panel), the time (s) in the annulus (upper right panel), and traveled distance in the annulus (cm) (lower right panel) are shown.

Probe trial

Time in quadrants (Fig. 7, upper left panel): the time spent in the four quadrants was different (Quadrants: $F_{3,54} = 7.63$, $p < 0.01$), indicating that there was a bias for the training quadrant. This bias was present in the young rats but not in the aged rats (Quadrants by Age interaction: $F_{3,54} = 2.97$, $p < 0.05$). In particular, the young F344 rats spent more time in the training (west) quadrant than the aged rats did ($F_{1,18} = 5.19$, $p < 0.05$).

Traveled distance in quadrants (Fig. 7, lower left panel): on average, the young rats swam further during the 30-second probe trial than the old rats did (General mean: $F_{1,18} = 89.01$, $p < 0.01$). The distances swum in the quadrants were different (Quadrants: $F_{3,54} = 9.88$, $p < 0.01$), but a bias for the previous training quadrant was obvious for the young rats only (Quadrants by Age interaction: $F_{3,54} = 6.07$, $p < 0.01$). This was confirmed by an analysis of the distance swum in the training (west) quadrant ($F_{1,18} = 20.84$, $p < 0.01$).

Time in the annulus (Fig. 7, upper right panel): the young rats spent more time in the annulus than the aged rats did ($F_{1,18} = 13.36$, $p < 0.01$). The aged rats hardly ever swam in the annulus.

Traveled distance in the annulus (Fig. 7, lower right panel): the young F344 rats swam further in the annulus than the aged rats did ($F_{1,18} = 17.74$, $p < 0.01$).

Discussion

The expected age-associated deficits in spatial orientation performance in the Morris water escape task were found in the outbred WISRJ rat (exp. 1), the FBNF1 hybrids (exp. 2), and the inbred F344 rats (exp. 3). These data corroborate the findings of others who observed that aged rats, compared with younger animals, exhibit an impairment in acquiring a standard Morris water escape task, and at best show only a weak bias for the previous training quadrant during the probe trial (e.g. Pellemounter, Smith & Gallagher, 1987; Rapp, Rosenberg & Gallagher, 1987; Decker, Pellemounter & Gallagher, 1988; Steward, Mitchell & Kalant, 1989; Brandeis et al., 1990; Rapp & Gallagher, 1996).

The aged FBNF1 hybrids showed a clear deficit in the Morris task, whereas no such age-related impairment was found previously in the Stone 14-unit T-maze (Ingram et al., 1994). Although both the Morris water maze and the Stone maze used by Ingram and colleagues are aversively motivated, a possible explanation for the failure to find age-associated impairments in the Stone maze is that the aversive stimulus is an electric footshock. We have observed previously, when comparing adult rats of different strains, that electric footshock is a very ineffective motivator for FBNF1 rats in inhibitory and active avoidance tasks (van der Staay & Blokland, 1996a).

The distance swum to reach the platform increased in the aged F344 rats across the five daily training sessions. A similar observation was reported by Lindner and Gribkoff (1991, experiments 3, and 4), who trained 23-month-old F344 rats, and by Mabry and colleagues (1996), who tested 22-month-old F344 rats in the standard Morris task in 5 successive training sessions. When Lindner and Gribkoff (1991) continued training for another 5 days, they found that the distance swum slightly decreased. However, the distance swum during the tenth session was not different from that swum during the very first acquisition session, confirming that 23-month-old F344 rats are already unable to acquire the Morris task.

The measure *distance to platform* (Gallagher, Burwell & Burchinal, 1993) shows that the aged rats in general swam at a greater distance from the platform than the young rats did. In fact, the mean distance to the platform ranged between about 60 to 40 cm; at the surface of the water the radius of the water tank was about 75 cm. The swimming of the aged rats can best be described as being dominated by a wall hugging strategy (Puumala et al., 1996). This behavior makes it less likely that rats will find the platform, unless they adopt an active exploration strategy and leave the rim of the tank faster and more frequently (Yau et al., 1994). The latter behavior was shown by the younger rats. The definition of an annulus that consists of a concentric area equivalent to the breadth of the escape platform and which is located equidistant from the rim of the pool (Denenberg et al., 1990) might help to identify a particular search strategy, namely swimming at a fixed distance from the rim. This strategy will automatically guide the animal to the escape platform.

Cross-sectional studies comparing two age groups

Cross-sectional experiments with rats of only two ages do not provide information about the shape of the underlying aging function. Age-associated cognitive impairments, motor coordination deficits, and sensory dysfunctions appear to occur quite independently at different ages (Gage, Dunnett & Björklund, 1984). Moreover, in most cases aging appears to follow a non-monotonic function (Markowska et al., 1989; Barnes, 1990; Baxter & Gallagher, 1996; Ingram, 1996). Cross-sectional designs with two age groups can answer one question only: do old(er) rats suffer from behavioral impairments when compared with young(er) conspecifics? This question can be answered affirmatively for the three strains tested. However, direct comparisons between the three strains should be made with care. It is not clear whether the old rats of the three strains are at the same stage of aging. There was a ceiling effect in the performance of the old F344 rats: they did not learn the water escape behavior and consequently, a further decline in (general) cognitive abilities in this strain will not be detected in the Morris task. By contrast, the aged rats of the other two strains tested were still able to learn. It is conceivable that a further decline might occur with increasing age. To obtain relevant information regarding this question and regarding the question at what age age-related deficits first occur, longitudinal studies, or cross sectional studies with multiple age points, are necessary (e.g. Ingram, 1996). A series of articles showing the preservation of the spatial memory of rats in the Morris task over many months, however, make the Morris water escape task less suited for longitudinal studies (e.g. Pitsikas, Biagini & Algeri, 1991; Gyger, Kolly & Guigoz, 1992; van der Staay & Blokand, 1996b, and Chapter 2.3).

Although the rats of the three strains were *tested* under highly standardized conditions, there were many differences between the three experiments. For example, the strains were reared under different conditions for a significant period of their lives. The FBNF1 and the F344 had been housed under identical conditions, because both genotypes came from the SPF barrier facilities of Harlan Sprague Dawley (Indianapolis, USA), where the animals are maintained under conditions strictly defined by the National Institute of Aging (Masoro, 1991; Sprott, 1991). Housing conditions for the WISRJ rats at the breeder's facilities were clearly different from those of the F344 and FBNF1 rats. After shipment to the animal facilities of our laboratory, housing conditions for all animals were identical.

Ideally, housing conditions should be standardized at the breeders. In aging research, this aspect of the animals' history often constitutes a 'black box' which exerts important, although unrecognized and unrecognizable, effects on behavior later in life. The duration and distance of transportation (addressed below), duration of the adaptation period in the laboratory before testing, and many other factors might contribute to the behavior assessed. Moreover, the differences seen between the three strains might,

at least partially, represent inherent differences between the strains in the ability to acquire the Morris water escape task, which already existed when the rats were young (van der Staay & Blokland, 1996a).

Swimming speed as confounding factor in age-comparison studies

There was an age-related decrease in the swimming speed of the aged FBNF1 hybrids and the F344 rats. The aged rats needed more time to reach the escape platform, they swam a longer distance before they escaped onto the platform, and they swam slower. This finding makes the measure *time to escape onto the escape platform* less suited to assess age-related impairments in learning and memory. Our data contrast with those of Brandeis and colleagues (Brandeis et al., 1990), who reported that swimming speed did not change with age, except for a transient decrease in swimming speed during the first training sessions. The measure *escape latency* in the experiments with FBNF1 and F344 rats might have been biased by the age-related decline in swimming speed. However, in all three experiments, the distance swum to reach the platform, which can be considered as an unbiased measure for spatial discrimination performance in the Morris task, was longer in the aged rats than in the younger rats. This finding supports the notion that the aged rats had a deficit in their spatial orientation performance.

The adult and old Wistar rats did not differ with respect to their swimming speed, i.e. the impairment in the acquisition of the water escape task seen in the 24-month-old WISRJ rats, when compared with the 12-month-old rats, was not paralleled by an age-related decrease in the swimming speed. Thus, the age-related decreases in cognitive functions and in motor skills appear to progress independently (e.g. Gage, Dunnett & Björklund, 1984; Blokland & Raaijmakers, 1993a; Forster et al., 1996; van der Staay & Blokland, 1996b).

The role of transport stress as a confounding factor

In experiment 3, the aged F344 rats did not show any improvement in their ability to locate the platform across sessions. These rats had been supplied by Harlan Sprague Dawley, Indianapolis, USA, and had been transported to our laboratory (CNS-Research, Bayer, Cologne, Germany) as aged animals. We wondered whether the poor performance of these rats might have been due to the long and presumably stressful journey. It has been suggested that stress affects the performance of mice (Francis et al., 1995) and rats (Mabry et al., 1996; Hölscher, 1999) in the Morris water escape task. Therefore, we performed an experiment in which four 22- and four 24-month-old F344 rats were transported from Harlan Sprague Dawley (Indianapolis, USA) to Harlan CPB (Zeist, Netherlands) about 3 months before shipment to our laboratory. Six 22-, and five 24-month-old Fischer 344 rats from the same aging colony, from the same barrier, and from the same cohorts at Harlan Sprague Dawley in Indianapolis were transported directly to our laboratory. The transportation was coordinated in such a way that all rats arrived at our laboratory at the same time.

In the Morris water escape task, neither the 22- nor the 24-month-old rats showed any improvement over sessions, as had previously been found with 26-month-old rats (exp. 3). These data also fully corroborate observations by Lindner and Gribkoff (1991) and Marby and colleagues (1996). There was no evidence for the notion that the long, potentially stressful shipment from Harlan, Indianapolis, USA, to Cologne was the cause of the poor performance of the aged F344 rats. However, putative effects of transport-induced stress might not have been apparent because of ceiling effects. As the rats transported from the Netherlands did not perform better than those transported from the USA, we conclude that the poor performance is directly related to the age of the animals.

Therefore, it is not advisable to use aged (22 months and older) Fischer 344 rats in the Morris water task. If F344 rats are used in age comparison studies, then the rats should be younger than 22 months. Rats of this strain aged 16 or 17 months already appear to show clear age-associated deficits in learning and memory (e.g. Steward, Mitchell & Kalant, 1989; Frick et al., 1995). These deficits are not so severe that the animals are no longer capable of learning.

To summarize, aging studies *on spatial discrimination learning* in the Morris water escape task should not be performed with F344 rats aged 22 months or older. FBNF1 hybrids and WISRJ rats appear to be better suited. Inbred strains and F₁ hybrids between inbred strains possess a number of advantages that make them valuable for aging research (Russell, 1972; Festing, 1991). They are genetically exactly defined, which increases the reproducibility of results due to the reproducibility of individuals. Moreover, the use of genetically defined strains offers the advantage of predictability. As their genotype is specified exactly, knowledge about the genotype accumulates with every experiment. Results from different studies in which the same genotypes are used can be compared more readily.

By contrast, the WISRJ is an outbred strain, which is 'genetically undefined' (Festing, 1991). Owing to 'genetic drift' in outbred populations, the replicability of results should not be taken for granted (Falconer & Mackay, 1996, pp. 48-64). Earlier, we had found that another rat strain supplied by Harlan Winkelmann, the outbred Wistar HsdWin:Wu (then called WISW:Wu), is not suited for studies which focus on spatial orientation learning in the Morris task (van der Staay, 1997, and Chapter 2.4). This strain has undergone considerable change, and the aged Wistar rats of this particular strain are no longer able to acquire the water escape response in the standard Morris task. Their learning curves are similar to those for the aged F344 rats in experiment 3 of the present study.

2.2

Effects of age on the acquisition of a standard Morris task and of a repeated acquisition task in Wistar rats^{*}

Abstract

The standard Morris water escape task in a circular pool predominantly measures spatial reference memory (RM), but a version of the task described by Whishaw (1985, 1987) makes it possible to measure spatial working memory (WM) as well. In this paradigm, each of the four start positions in the pool is used randomly in each series of the four trial pairs of a daily training session, i.e. rats are randomly started from one of the four starting positions in the first trial of a pair and from the same start position on the next trial of the pair. The escape platform is positioned in a different position in each daily session. The decrease in escape latency and in the distance swum to reach the escape platform from the first to the second trial within a trial pair is considered a measure of spatial WM.

We performed three experiments. In the first two experiments, we assessed the effects of aging on the acquisition of the standard Morris water escape task. Based on the results of these experiments, we compared the performance of 3-month-old rats with that of 24-month-old animals in the repeated acquisition paradigm to assess the effects of age on WM. The young rats acquired the task within the first sessions. In contrast, the 24-month-old animals did not acquire the task, even after 12 daily training sessions. It is not clear, however, whether the poor performance of the old rats on the repeated acquisition task reflects impaired WM or whether they did not acquire the procedural aspects of the task.

Introduction

The standard water escape task (Morris, 1984), in which a rat is required to localize a submerged platform, can be regarded as a task that predominantly measures spatial reference memory (Mundy, Barone & Tilson, 1990). Reference memory (RM) holds trial-independent information (Barnes, 1988b) about, for example, the position of the escape platform in the water tank. Young rats appear to acquire the reference memory version of the Morris water escape task faster than old rats (Aitken & Meaney, 1989; Gage, Dunnett & Björklund, 1984; Rapp, Rosenberg & Gallagher, 1987; but see also: Lindner & Schallert, 1988). In fact, impairments in RM performance occur at a relatively early age (e.g. 18 to 19 months, Steward, Mitchell & Kalant, 1989; 12 months, Aitken & Meaney, 1989; 16 months, Brandeis et al., 1990). The severity of the decrease in RM performance appears to be variable over studies and ranges from transient acquisition deficits (Rapp, Rosenberg & Gallagher, 1987) to permanent

^{*} Part of this chapter has been published previously: van der Staay, F.J. & de Jonge, M. (1993). Effects of age on water escape behavior and on repeated acquisition in rats. *Behavioral and Neural Biology*, **60**, 33-41.

impairments to locate and to escape onto, the submerged platform (Aitken & Meaney, 1989; Fong, Neff & Hadjiconstantinou, 1997).

Most Morris water escape experiments have compared the behavior of only two age groups. This approach addresses the question whether aging affects performance in the task, but does not provide information about the mechanisms underlying the effects of aging on water escape behavior (Barnes, 1990). Relevant information regarding the time course of the aging process and when age-related deficits first occur can be obtained from longitudinal studies or from cross-sectional studies with multiple age points (e.g. Ingram, 1996). The Morris water escape task appears to be less suited for longitudinal studies, because the spatial memory of rats for this task is preserved for several months (e.g. Pitsikas, Biagini & Algeri, 1991; Gyger, Kolly & Guigoz, 1992; van der Staay & Blokand, 1996b, and Chapter 2.3). Therefore, we performed two cross-sectional experiments to identify the age at which reliable age-associated impairments occur in the outbred Wistar rat strain (WISW: Bor). Rats of this strain have been used routinely for geronto(pharmaco)logical research in our laboratory. In the first experiment, we compared the acquisition of the standard Morris water escape task of 2-, 5-, 12- and 19-month-old WISW rats. In the second experiment, we trained 3- and 24-month-old Wistar rats to escape onto the submerged platform.

In addition to reference memory versions of the Morris water escape task, versions have been developed which allow the assessment of a working memory (WM) or short-term memory component. Whishaw (1985, 1987) described a repeated acquisition paradigm to test the formation of what he called a *place learning set* by rats. Within a daily training session of this repeated acquisition paradigm, each of four start positions (situated in the northern, eastern, southern, or western quadrant of the pool) is used randomly in each series of four trial pairs. Thus, rats are randomly started from each of the four starting positions per trial pair. From one daily session to the next, the escape platform is in a different quadrant, and each position is used once in a series of four consecutive sessions. Successful repeated acquisition is demonstrated when subjects have shorter latencies to find the platform during the second trial of a pair than during the first trial (one trial learning), i.e., when they show an improved WM performance. Contrary to Whishaw (1985, 1987), we consider performance in the repeated acquisition task to be a measure of WM (see also Grauer & Kapon, 1993).

We adopted the paradigm described by Whishaw (1985, 1987) to assess age-related changes in the ability to show one trial learning and to assess spatial WM. As we found that older WISW rats suffer from clear age-associated impairments of spatial RM in comparison with younger rats, we used 3- and 24-month-old rat to assess the effects of age on spatial WM, using the repeated acquisition paradigm. We expected that the performance of the 24-month-old WISW rats in the repeated acquisition task in the Morris water escape task would be worse than that of the younger rats.

Experiment 1: acquisition of the standard water escape task by 2-, 5-, 12-, and 19-month-old outbred Wistar rats.

Material and Methods

Animals: we used 32 male Wistar rats (WISW: Bor; Winkelmann, Borchon, FRG) of four different ages: 2, 5, 12, and 19 months (n = 8 per age-group). The breeder has renamed this strain twice HsdCpb: Wu, and subsequently HsdWin: Wu. The oldest rats were selected from a larger group. They appeared to

be healthy and showed no signs of abnormalities or physical impairments. Special care was taken to use only rats that were free of cataracts. The rats were group-housed (four animals per cage) in standard Makrolon[®] cages in a temperature (ca. 21.5°C)- and humidity (50%)-controlled vivarium. Lights were on from 7:00 to 19:00. When testing started, all rats were transferred to the experimental room where they were housed for the entire testing period. The light/dark regimen was the same as in the vivarium.

Apparatus: the Morris water tank consisted of a circular white tub (diameter: 135 cm, depth: 60 cm) filled with 41 cm of water at a temperature of approximately 22°C. The escape platform was a clear Plexiglas cylinder (diameter: 9 cm) that was submerged 1.5 cm below the surface of the water. The water was made opaque by addition of dried skimmed milk (Glücksklee, approximately 250 grams). A video camera, mounted in the center above the circular pool, provided a picture of the pool on a TV-monitor. Lines on the monitor defined quadrant boundaries and the position of the escape platform. Crossing a line, i.e. a quadrant entry, was scored when a rat moved across it with its whole body. The movements of the rat were registered manually and stored in an MS-DOS compatible microcomputer.

The water tank was situated in a room illuminated by daylight and white fluorescent strip lights. Abundant extra-maze cues were provided by the furniture in the room, which included desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that was playing softly.

Procedure: the animals received four trials a day for 7 days. A trial was started by placing a rat into the pool, facing the wall of the tank. Each of four starting positions (north, east, south, and west) was used once in a series of four trials; their order was randomized. The escape platform was always in the same quadrant. A trial was terminated as soon as the rat had climbed onto the escape platform or when 60 seconds had elapsed, whichever event occurred first. A rat was allowed to stay on the platform for 10 seconds. Then it was taken from the platform and the next trial was started. Rats that did not find the platform within 60 seconds were put on the platform by the experimenter and were allowed to stay there for 10 seconds.

After the fourth trial of the seventh session, an additional trial was given as a probe trial: the platform was removed, and the time a rat spent in the four quadrants was measured for 60 seconds. All rats started from the same start position (east) in the probe trial.

Statistical analysis

Two measures were analyzed:

- escape latency (s), that is, the time taken to find and escape onto the submerged platform (Morris, 1984), and
- the number of times quadrants were entered (Lalonde & Joyal, 1991). This measure can be taken as an index for the distance swum or, alternatively, as an index of the extent to which a rat explored the water tank.

The escape latencies and the number of quadrant entries within each session were averaged per rat. Age differences in the acquisition of the water escape task were assessed with an analysis of variance (ANOVA; Winer, 1971) with repeated measures over sessions. Where appropriate, the results of ANOVAs on differences between ages for particular sessions are included. Duncan's post hoc multiple range tests were performed to evaluate age differences in more detail.

Age differences in the swimming time during the probe trial were assessed with a repeated measures ANOVA over quadrants (time in the northern, eastern, southern, and western quadrant were considered as levels of the repeated measures factor), complemented by ANOVAs on the swimming times *per* quadrant, supplemented with Duncan's post hoc multiple range tests to evaluate age differences.

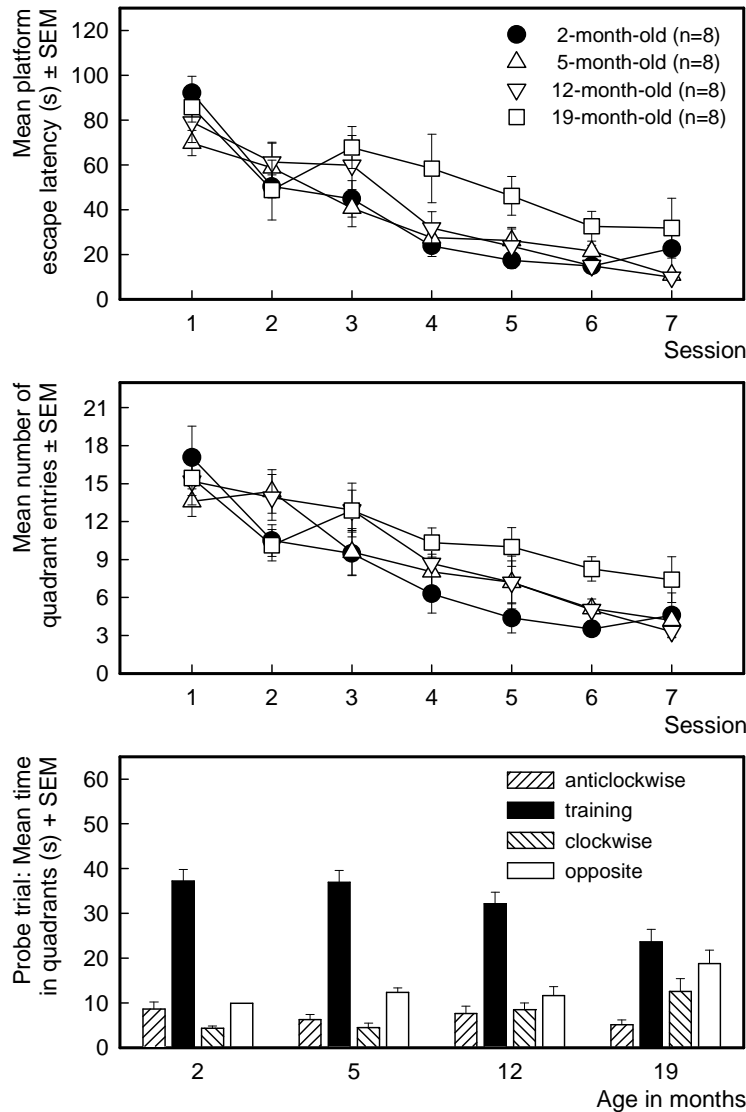


Figure 1. Acquisition of the Morris water escape task and performance in a probe trial of 2-, 5-, 12-, and 19-month-old Wistar rats. Session means and standard errors of the means (SEM) are depicted. Upper panel: latencies (s) to escape onto a submerged platform. Center panel: number of quadrant entries. Lower panel: time (s) spent in each quadrant of the circular pool during a 1-minute probe trial.

Results

Escape latencies (see Fig. 1, upper panel): averaged over all sessions, the 19-month-old rats had longer escape latencies than the other rats (General mean: $F_{3,28} = 4.2$, $p < 0.05$). Rats of all ages learned to localize the escape platform faster in the course of training (Sessions: $F_{6,168} = 33.2$, $p < 0.01$), but there was no difference in the rate of improvement between the different age groups (Age by Sessions: $F_{18,168} = 1.3$, n.s.). However, on a session basis, there was a statistically reliable

difference in the escape latency for the different age groups in sessions 4, 5, and 6 (all $F_{3,28} > 3.0$, with associated probabilities < 0.05). Post hoc analyses confirmed that the performance of the 2-, 5-, and 12-month-old rats was similar and that the 19-month-old rats had longer latencies than the rats in the three other groups.

Number of quadrant entries (see Fig. 1, center panel): averaged over all sessions, there was no difference between age groups in the number of quadrants entered (General mean: $F_{3,28} = 2.0$, n.s.). Rats of all age groups reduced the number of quadrant entries over the successive acquisition sessions (Sessions: $F_{6,168} = 29.8$, $p < 0.01$), but there was no difference in the rate of improvement (Sessions by Age: $F_{16,168} = 1.5$, n.s).

Probe trial (see Fig. 1, lower panel): the rats spent most time in the quadrant where the escape platform had been during the training sessions (Quadrant: $F_{3,84} = 111.3$, $p < 0.01$). The pattern of occupancy of quadrants, however, was different for the different age groups (Age by Quadrant: $F_{9,84} = 4.7$, $p < 0.01$). The oldest rats spent about one third of their time in the quadrant where the platform had been during the acquisition trials. In contrast, the 2-, 5-, and 12-month-old rats spent more than 50% of their time in this particular quadrant ($F_{3,28} = 5.8$, $p < 0.01$; and confirmed by post hoc analysis).

Experiment 2: acquisition of the standard water escape task by 3- and 24-month-old Wistar rats.

Material and Methods

Animals: we used ten 3-month-old and ten 24-month-old male Wistar (WISW:Bor) rats (Winkelmann, Borchon, FRG). The old rats were selected from a larger group. They appeared to be healthy and showed no signs of abnormalities or physical impairments. Special care was taken to use only rats that were free of cataracts. The young animals were group-housed in standard type III Makrolon[®] cages, the old rats were group-housed in type IV Makrolon[®] cages, both with five animals per cage. All other conditions were as in experiment 1.

Apparatus: the same apparatus was used as in the first experiment. However, to obtain an additional arbitrary measure for the distance swum, each quadrant was further subdivided by a pattern of lines (a 4 * 4 matrix of squares, corresponding to a distance between grid lines in the pool of 16.9 cm). Crossing a line was scored when a rat moved across it with its whole body.

Procedure: testing was performed as in experiment 1, with minor modifications. The animals received four trials a day for 5 days (there were seven sessions in experiment 1). Rats that did not find the platform within 90 seconds (in the first experiment: 60 seconds) were put on the platform by the experimenter and were allowed to stay there for 10 seconds.

Statistical analysis

In addition to escape latency and the number of times quadrants were entered, the number of line crossings and the swimming speed were analyzed. The number of line crossings can be taken as second index for the distance swum. The swimming speed was calculated as number of line crossings divided by the escape latency.

The measures were averaged per rat within each session. Age differences in the acquisition of the water escape task were assessed with an analysis of variance (ANOVA; Winer, 1971) with repeated

measures over sessions. In addition, age differences on particular session means were analyzed by *t*-statistics.

Results

Escape latencies (see Fig. 2, upper left panel): averaged over the acquisition sessions, the aged rats had longer escape latencies than the young rats (General mean: $F_{1,18} = 56.6$ $p < 0.01$). Both age groups learned to localize the escape platform faster in the course of training (Sessions: $F_{4,72} = 15.9$, $p < 0.01$). There was, however, no difference between ages for the rate of improvement (Age by Sessions: $F_{4,72} = 2.1$, n.s.). *t*-tests confirmed that the young rats reached the platform faster than the old rats during all sessions (all $t_{s18} > -2.4$, p 's < 0.05).

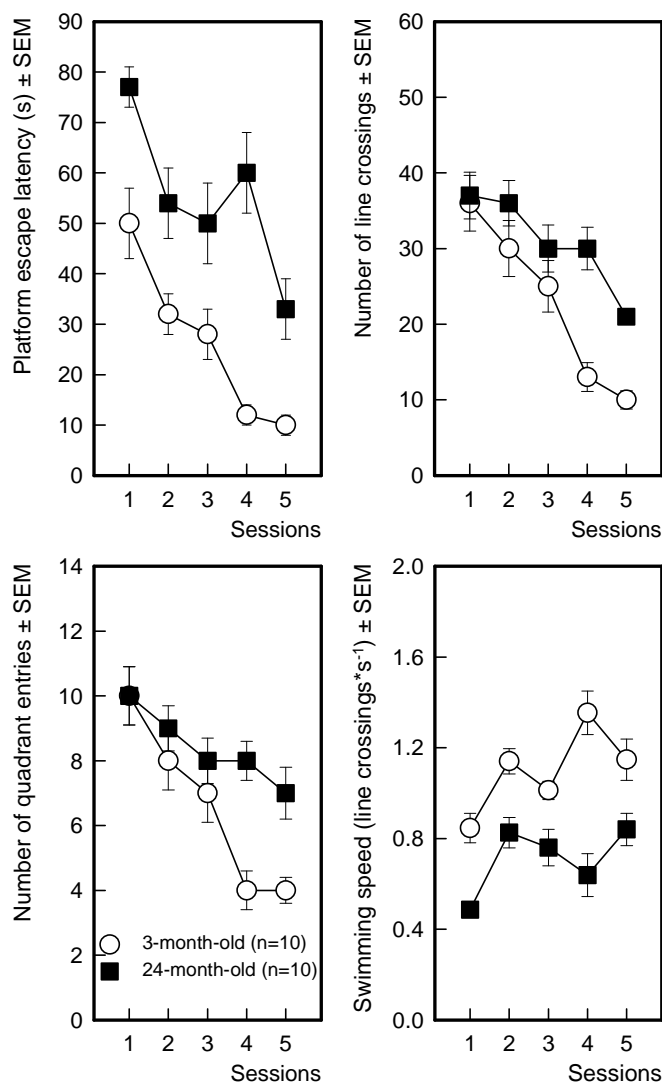


Figure 2. Acquisition of a water escape task in a circular pool by 3-, and 24-month-old Wistar rats. Session means and standard errors of the means (SEM) of latency (s) to escape onto a submerged platform (upper left panel), number of line crossings (upper right panel), number of quadrant entries (lower left panel), and swimming speed (lower right panel) are depicted.

Number of quadrant entries (see Fig. 2, lower left panel): averaged over all sessions, the aged rats made more quadrant entries than the young rats (General mean: $F_{1,18} = 9.1$, $p < 0.01$). In both age

groups, the number of quadrant entries decreased over the successive acquisition sessions (Sessions: $F_{4,72} = 12.7$, $p < 0.01$). There was a marginal difference in the rate of improvement (Sessions by Age: $F_{4,72} = 2.1$, $0.10 > p > 0.05$). Age differences were statistically reliable in the fourth ($t_{18} = -3.9$, $p < 0.01$) and fifth ($t_{18} = -3.4$, $p < 0.01$) session.

Number of line crossings (see Fig. 2, upper right panel): exactly the same results were found for number of line crossings. The aged rats made, on average, more line crossings before they reached the submerged platform (General mean: $F_{1,18} = 13.2$, $p < 0.01$). In both age groups, the number of line crossings decreased over the successive acquisition sessions (Sessions: $F_{4,72} = 18.6$, $p < 0.01$). The rate of improvement was only marginally different (Sessions by Age: $F_{4,72} = 2.3$, $0.10 > p > 0.05$). Again, age differences were statistically reliable in the fourth ($t_{18} = -4.9$, $p < 0.01$) and fifth ($t_{18} = -3.8$, $p < 0.01$) session.

Since the number of line crossings and the number of quadrant entries were highly correlated (young: $r_{PM} = 0.98$; old: $r_{PM} = 0.92$), they probably both provide a measure for the distance swum to reach the escape platform, and one may well dispense with one of the measures (Walsh & Cummins, 1976).

Swimming speed (see Fig. 2, lower right panel): the swimming speed of the young rats was consistently higher than that of the aged animals (General mean: $F_{1,18} = 78.8$, $p < 0.01$) and increased slightly over sessions (Sessions: $F_{4,72} = 7.41$, $p < 0.01$). The increase was slightly faster in the young animals (Sessions by Age: $F_{4,72} = 3.21$, $p < 0.05$). *t*-tests confirmed that the young rats swum to the platform faster than the old rats during all sessions (all $t_{s_{18}} > 2.7$, p 's < 0.05).

Experiment 3: acquisition of a repeated acquisition task in the Morris water maze by 3- and 24-month-old Wistar rats.

Material and Methods

Animals: ten 3-month-old and ten 24-month-old male Wistar rats (WISW: Bor) were supplied by Winkelmann (Borchen, Germany). The old rats were selected from a shipment consisting of 40 animals. They appeared to be healthy and showed no signs of abnormalities or physical impairments. Selection criteria were as in the first two experiments. Special care was taken to use only rats free of cataracts. Housing conditions were as described in the first experiment.

Apparatus: the same equipment as in the first experiment was used.

Procedure: the animals received pairs of trials, as described in the Introduction (see Fig. 3). The young and old rats were trained alternately. When all rats had completed a first trial pair, a second pair was given, etc., until all rats had received four trial pairs. The interval between trial pairs for each rat was 30 to 45 minutes. Within a daily session, the escape platform remained in the same position, and over a series of four daily sessions, the platform was moved once to each of the four quadrants (the order was always: south, west, east, north). On the first day of testing, only two trial pairs were run in order to allow the old subjects to adapt to swimming in the pool. On the second day, the platform was kept in the same place as on the first day. After each trial pair, rats were gently dried with crêpe paper and returned to their home cages. The animals were kept warm under an infrared bulb (Original Hanau Solilux, 150 W) fixed about 60 cm above the floor of the cage.

All animals were trained on 12 successive days (first day, 2 trial pairs, all other days, 4 trial pairs, to a total of 46 trial pairs). Only two measures were registered: platform escape latencies and number of quadrant entries.

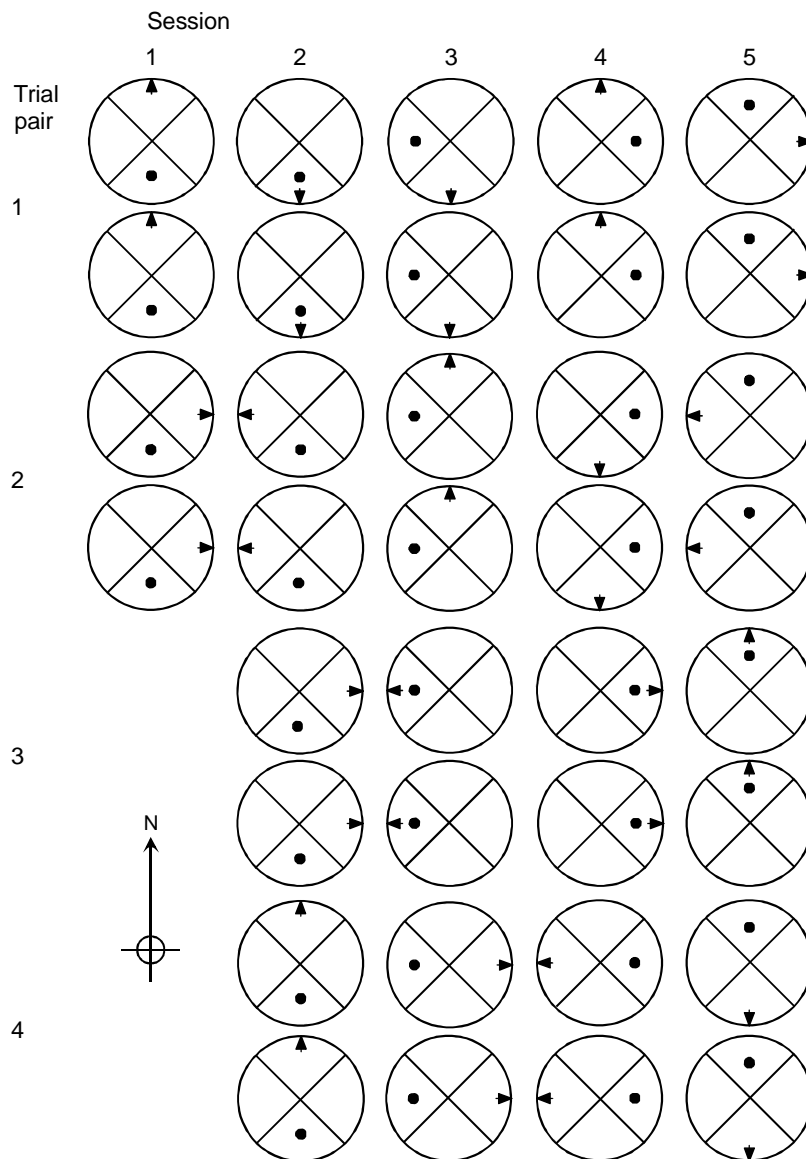


Figure 3. Schematic overview of the training procedure in the repeated acquisition task. Within a daily session, the escape platform remained in the same position, but was different in each of 4 successive daily sessions (the order was always: south, west, east, north). On the first day of testing, only two trial pairs were run in order to allow the old rats to adapt to swimming in the pool. On the second day, the platform was kept in the same place as on the first day. The start position at the rim of the pool is marked by an arrow, the platform position in the center of a quadrant is shown by '●'.

Statistical analysis

Two measures were analyzed: 1) escape latency (Morris, 1984), that is, the time taken to find and escape onto the submerged platform; and 2) number of times quadrants were entered (Lalonde and Joyal, 1991). The second measure might be taken as index for the distance swum to reach the platform.

Initial level of performance: in an analysis of performance during the first session, which consisted of only two trial pairs, we evaluated, using t-statistics, whether the level of performance of all age groups was the same at the start of the experiment.

Comparison of the average escape latencies of the first with that of the second trials in pairs over all acquisition sessions: analysis of the *entire* learning curves, based on individual trials, was not possible, because the resulting repeated measures ANOVA exceeded the capacity of our computer. Instead, for each rat, the time (in s) taken to escape onto the submerged platform and the number of quadrant entries were averaged per session separately for the first and second trials of the pairs [average of first swims: $(\text{trial}_{1,1} + \text{trial}_{2,1} + \text{trial}_{3,1} + \text{trial}_{4,1}) / 4$; average of second swims: $(\text{trial}_{1,2} + \text{trial}_{2,2} + \text{trial}_{3,2} + \text{trial}_{4,2}) / 4$; the first subscript represents the number of the trial pair within a session, the second subscript represents the trial within trial pairs]. The acquisition curve of the repeated acquisition task was analyzed further with a two-way ANOVA with the factors Age (young vs. old), and the repeated measures factors Sessions (sessions 2 to 12), and Trial Pairs (average of first vs. average of second trials within a session).

Comparison of the average escape latency of the second trials of the previous pairs with that of the first trials of the next pairs over all acquisition sessions: a within-session decline in escape latencies would produce results indicative of one trial learning, whereas this effect could be a statistical artifact. With a steady decline over trials, the second trial of a pair would have a shorter latency than the first trial by definition. In order to assess whether our data on escape latencies represent a real effect and not a statistical artifact, we performed an additional analysis. Instead of comparing the mean escape latencies of the first swims of the trial pairs within a session with the mean of the second swims, we analyzed whether the latencies decreased, by comparing the mean of the second swims in the previous pairs with the mean of the first swims of the next pairs [average of previous swims: $(\text{trial}_{1,2} + \text{trial}_{2,2} + \text{trial}_{3,2}) / 3$; average of next swims: $(\text{trial}_{2,1} + \text{trial}_{3,1} + \text{trial}_{4,1}) / 3$]. The data were analyzed with a two-way ANOVA with the factors Age (young vs. old), the repeated measures factors Sessions (sessions 2 to 12), and Trial Pairs (average of the second trial of the previous pairs vs. average of first trial of the next pairs).

Analyses based on individual trials over the second half of the learning curve: visual inspection of Fig. 4 revealed that both age groups had reached a stable performance by the sixth session. Therefore, we assessed separately whether one trial learning had occurred, *based on individual trials*, for sessions six to twelve. An Age (young vs. old) by Sessions (6 to 12) by Trial Pairs (1 to 4) by Trials Within Pairs (first versus second swim) by age ANOVA was performed with repeated measures on the last three factors.

All analyses were complemented with separate repeated measures ANOVAs *within* age groups.

Results

The results are summarized for escape latencies in Fig. 4, upper and center panel, and in Fig. 5. For quadrant entries, the results are depicted in Fig. 4, lower panel.

Initial level of performance: analysis of the first session confirmed that the two age groups started at the same level of performance (escape latencies: $t_{18} = 0.71$, n.s.; quadrant entries: $t_{18} = 0.14$, n.s.).

Comparison of the average escape latencies of the first with that of the second trials in pairs over all acquisition sessions (see Fig. 4, upper panel): the repeated measures analysis over sessions 2 to 12

revealed that, averaged over all sessions, the old animals took longer to find the submerged platform than the young rats (General mean: $F_{1,18} = 10.62$, $p < 0.01$). The escape latencies decreased in the course of training (Sessions: $F_{10,180} = 12.84$, $p < 0.01$) and the decrease was similar for young and old rats (Age by Sessions: $F_{1,18} = 1.08$, n.s.). The speed of learning was different for the two age groups (Age by Trial Pairs: $F_{1,18} = 10.38$, $p < 0.01$). Surprisingly, the decrease in escape latencies from the first to the second trials in pairs appeared not to increase with training (Trial Pairs by Sessions: $F_{10,180} = 0.82$, n.s.), nor was there any indication of a differential acquisition over sessions for the two age groups (Age by Trial Pairs by Sessions: $F_{10,180} = 1.18$, n.s.).

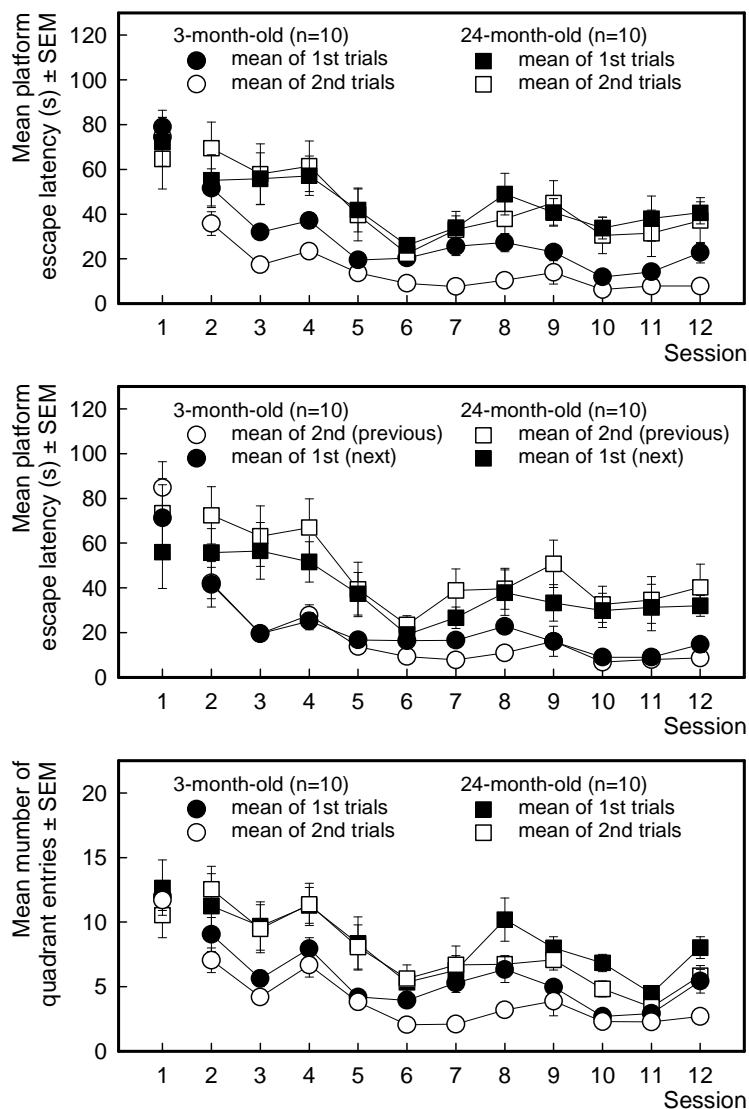


Figure 4. Repeated acquisition in 3- and 24-month-old Wistar rats. Session means and standard errors of the means (SEM) are depicted. Upper panel: latencies (s) of the first and second trial of pairs to escape onto a submerged platform. Center panel: latencies to escape onto a submerged platform (s) during the second swim of the previous pair and during the first swim of the next pair. Lower panel: number of quadrant entries of the first and second trial of pairs to find the platform.

In order to analyze the Age by Trial Pairs interaction further, separate repeated measures analyses within age groups were performed. These analyses confirmed that the young animals had, on average, shorter escape latencies on the second trial of a pair than on the first trial (Trial Pairs: $F_{1,9} = 85.85$, $p < 0.01$), whereas the old rats did not show such an improvement ($F_{1,9} < 1.0$, n.s.). However, the

mean escape latencies decreased over sessions in both age groups (Sessions: young rats, $F_{10,90} = 13.30$, $p < 0.01$; old rats, $F_{10,90} = 5.29$, $p < 0.01$).

Comparison of the average escape latency of the second trials of the previous pairs with that of the first trials of the next pairs over all acquisition sessions (see Fig 4, center panel): escape latencies decreased over sessions ($F_{10,180} = 12.80$, $p < 0.01$) to a similar extent in both age groups (Age by Sessions: $F_{10,180} = 1.55$, n.s.). An Age by Trial Pairs interaction indicated that swim latencies developed differently from the second trial of the previous pair to the first trial of the next pair ($F_{1,18} = 11.40$, $p < 0.01$).

Separate repeated measures analyses per age group revealed that the swim latencies of the young rats increased ($F_{1,9} = 10.34$, $p < 0.05$), whereas the old rats showed a very small, albeit statistically reliable, decrease in average swim latencies from the second swim of the previous trial pair to the first swim of the next trial pair ($F_{1,9} = 6.37$, $p < 0.05$). These results indicate that the one trial learning in young rats is not an artifact of a decrease in escape latencies over trials within sessions. They confirm that the escape latencies of old rats remain relatively constant within a session.

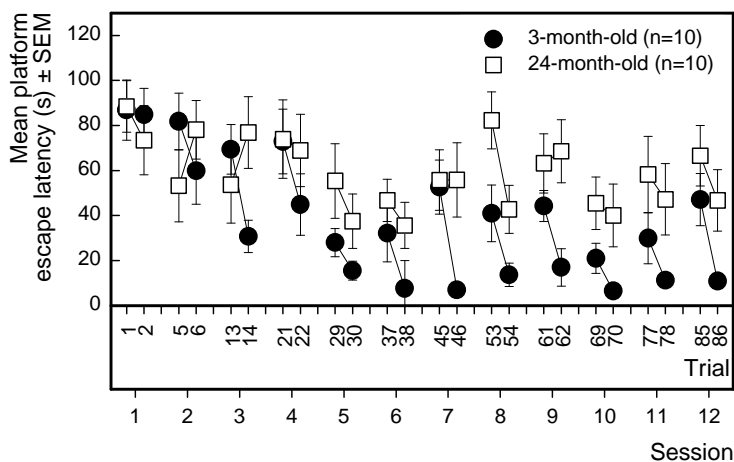


Figure 5. Mean latencies ($s \pm SEM$) of 3- and 24-month old Wistar rats to escape onto a submerged platform during the first and second trial of the first trial pair in sessions 1 to 12. The trial and session numbers are given on the horizontal axis.

Analyses based on individual trials over the second half of the learning curve: this analysis confirmed that the aged rats had longer escape latencies than the young animals (General mean: $F_{1,18} = 13.03$, $p < 0.01$). There were fluctuations in the escape latencies over sessions ($F_{6,108} = 3.01$, $p < 0.01$), which were probably because the escape platform was more difficult to localize in the northern quadrant than in the other locations. This was true for both ages (Age by Session interaction: $F_{6,108} = 1.11$, n.s.). Escape latencies also fluctuated over pairs ($F_{3,54} = 28.08$, $p < 0.01$), but these fluctuations were similar for the two age groups (Age by Pairs interaction: $F_{3,54} = 2.48$, n.s.). Escape latencies within pairs differed ($F_{1,18} = 14.86$, $p < 0.05$). Most importantly, an Age by Trials Within Pairs interaction ($F_{1,18} = 4.31$, $p < 0.05$) indicated that the reduction in escape latency from the first to the second trial within pairs was different for the two ages, even after performance had stabilized. Additional analyses within age groups revealed that aged rats showed no improvement within pairs ($F_{1,9} < 1.00$, n.s.), whereas young rats showed a clear reduction in escape latency on the second trial of a pair ($F_{1,9} = 84.92$, $p < 0.01$).

Practice effects within sessions could have biased the previous analyses. To obtain an estimate of the one-trial learning performance which excludes practice effects within daily sessions, an additional analysis was performed with the repeated measures factor Sessions (session 1 to 12) and Trials (first and second trial within session) and the factor Age ANOVA. This analysis considered only the first two trials per session. The results are summarized in Fig. 5.

The escape latency was, on average, shorter in young than in old rats (General mean: $F_{1,18} = 9.20$, $p < 0.01$). The escape latencies changed over sessions (Sessions: $F_{11,198} = 7.64$, $p < 0.01$), but this change was similar for the two age groups (Age by Sessions interaction: $F_{11,198} = 1.30$, n.s.). There was an effect of trials ($F_{1,18} = 20.13$, $p < 0.01$). An Age by Trials interaction ($F_{1,18} = 5.57$, $p < 0.01$), however, indicated that the decrease from the first to the second trial within the first trial pair of sessions was different for the age groups. The decrease in escape latency was not confirmed for the old rats when an ANOVA over Sessions 1 to 12 by Trials 1 to 2 within sessions was performed (Trials: $F_{1,9} = 1.05$, n.s.; Trials by Sessions interaction: $F_{11,99} = 1.22$, n.s.). For the young rats, on the other hand, this analysis confirmed that the escape latencies decreased from the first to the second trial within the first trial pairs of sessions (Trials: $F_{1,9} = 45.74$, $p < 0.01$). The magnitude of the reduction in escape latencies was not influenced by training over the 12 sessions (Trials by Sessions interaction: $F_{11,99} < 1.0$, n.s.)

Number of quadrant entries (see Fig. 4, lower panel): the statistical analyses of the number of quadrant entries revealed results highly similar to those performed on escape latencies, and are therefore, not presented here.

Discussion

The results of the three experiments confirm that aged rats have an impaired ability to acquire the standard Morris task, which predominantly relies on spatial RM, and for the repeated acquisition task, which is considered to measure spatial WM.

In the first experiment with 2-, 5-, 12-, and 19-month-old WISW rats we found that the 19-month-old rats needed more time to find the escape platform than the younger rats did, suggesting that the decrease in escape performance in the WISW:Bor strain occurs somewhere between 12 and 19 months of age; the 12-month-old animals performed as well as the younger rats. The decrease in escape latency in the 19-month-old animals was most likely due to a decrease in swimming speed, and does not necessarily indicate an age-associated impairment in spatial learning and memory, because we did not detect age-related differences in the number of quadrant entries, an index of the distance swum to reach the platform. In the WISW strain, age-related impairments in spatial discrimination thus seem to appear in rats older than 19 months. This contrasts with the findings of other investigators who reported an earlier onset of the age-associated decrease in performance (e.g. 18 to 19 months, Steward, Mitchell & Kalant, 1989; 12 months, Aitken & Meaney, 1989; 16 months, Brandeis et al., 1990).

The second experiment showed that the 24-month-old rats needed more time to reach the escape platform than the 3-month-old WISW rats, and that they swam a longer distance, measured as number of quadrant entries, before they located and escaped onto the platform. These data corroborate the findings of others who found that aging rats, compared with young animals, exhibit an impairment to

acquire the water escape task in a circular pool (e.g. Brandeis, et al, 1990; Pelley, Mounter, Smith & Gallagher, 1987; Rapp, Rosenberg & Gallagher, 1987; Steward, Mitchell & Kalant, 1989; see also Chapter 2.1). Combining the information obtained in the first two experiments, we suggest that the age-related impairments in spatial orientation learning in the Morris maze of WISW rats occur in the period between 19 and 24 months of age. We found repeatedly, and without exception, that the acquisition of the standard Morris water escape task of 24-month-old WISW rats was poorer than that of young rats (data not shown).

In the third experiment the 24-month-old rats were impaired in acquiring the repeated acquisition task. Because of the age-associated decrease in swimming speed the measure *time to escape onto the escape platform* might be less suited to assess age-related impairments in learning and memory in WISW rats. Distance swum, measured as number of quadrant entries, therefore, appears to be better suited to compare age-related changes in spatial RM in the standard water escape task than platform escape latency, due to the bias induced by differences in swimming speed. However, repeated acquisition is assessed within subjects. Thus, the occurrence of one-trial learning is basically independent of general differences in swimming speed between age groups. Although differences in swimming speed might complicate the interpretation of age differences, they do not interfere with interpretations concerning the occurrence of one-trial learning in the repeated acquisition paradigm. One trial learning is defined as a decrease in the time needed to find the platform from one trial to the next within a pair. This effect can be measured within age groups, irrespective of the basal swimming speed.

The young rats acquired the repeated acquisition task quickly, whereas one-trial learning was not found in the aged rats. The comparison of the average escape latency of the first trial with that of the second trial of a trial pair over all acquisition sessions, the analyses based on individual trials over the first and the second half of the learning curve, and the analyses considering only the first two trials per session yielded very consistent results, which support the notion that old rats are not able to learn the specific aspects of the repeated acquisition task. The consistency of the results also shows that the statistical approach used is valid to evaluate the performance of rats in the repeated acquisition task. However, it might be worthwhile to consider the possibility of giving only one trial pair per session (Grauer & Kapon, 1993; Youngblood et al., 1997), or of introducing a longer inter-trial pair interval (Petrie, 1995), or of increasing the number of different positions of the escape platform in future research (Youngblood et al., 1997), in order to decrease interference between trial pairs and to simplify the data analysis and the interpretation of results.

Young rats in fact very consistently needed less time and swam a shorter distance to escape onto the platform in the second than in the first trial of a trial pair from the first acquisition sessions onwards, with no further improvement when training progressed. Our data corroborate earlier observations made by Whishaw (1985, 1987) that one-trial learning already occurs within the first few trials of training. An additional analysis supported the notion that the improvement of the young rats within trial pairs does not represent a statistical artifact.

The improvement from the first to the second trial of trial pairs found for young rats might have been due to an effect of practice: within sessions, the rats always swam to the same platform location. However, the improvement on the second trial was also found when only the first trial pair (i.e. the first and second trial) per session was considered. Moreover, although the development of some response strategy cannot be excluded definitively, we do not have any indication that the young rats used a strategy other than spatial orientation to solve the task. The old rats did not improve their performance

on the second trial of pairs, although the trials within pairs were given in close succession. Nagahara and McGaugh (1992) found that young rats showed an improved performance in the second trial of a pair in a comparable place learning task; there was a similar reduction in escape latency from the first to the second trial in a session after a short (15 s) and after a long (15 min) retention interval. Considering these findings, our results suggest that the old rats showed a rather severe short-term memory deficit, because it was already noticeable with the very short interval between the trials of pairs used in the present study.

It is, however, not clear whether our data reflect an inability of aged WISW rats to acquire the procedural aspects of the task, or whether they really reflect an impaired WM performance. The occurrence of one trial learning depends upon procedural memory (M'Harzi et al., 1987), which in turn might be considered an aspect of spatial RM (Olton, Becker & Handelmann, 1979). In the repeated acquisition paradigm, one might consider the decrease in escape latencies over sessions to be an improvement in RM performance.

The RM performance of the old rats appears also to be impaired in the standard Morris water escape task. The question whether RM and WM are independent measures in the repeated acquisition paradigm cannot be answered. For the holeboard task (van der Staay, van Nies & Raaijmakers, 1990) and the cone-field task (van der Staay, Blokland & Raaijmakers, 1990), both of which allow the simultaneous assessment of WM and RM, the two spatial memory measures have been found to be independent of each other. In the repeated acquisition paradigm the acquisition of the procedural demands might be a prerequisite for an improvement in WM performance. In a study by Youngblood and colleagues (1997), sleep-deprived rats were tested in a repeated acquisition task in the Morris maze. They found sleep-deprived rats to be impaired in spatial RM, but not WM. This result suggests that the two memory components are dissociated in this task.

The clear age-associated deficits in the ability to show one trial learning in old rats, and the speed of acquisition of this task in young rats might make this task suitable to test pharmacological compounds believed to affect learning and memory, i.e. substances which might ameliorate experimentally induced or age-associated memory impairments (Whishaw, 1985; Cohn, MacPhail & Paule, 1996). In this context it might be of interest to distinguish between impaired and unimpaired old animals (e.g. Gallagher & Burwell, 1989; Rapp & Gallagher, 1996; Fong, Neff & Hadjiconstantinou, 1997; Abrous et al., 1997, Anisman et al., 1998). The higher standard errors of the old rats in the present experiments indicate that not all aged animals had an impairment in acquiring this task. The question whether the poorer performance of old rats to show one trial learning in the repeated acquisition task reflects predominantly impaired WM, or whether it is caused by deficits in RM or conceptual learning, however, needs further investigation.

2.3

Longitudinal assessment of spatial discrimination performance of aged rats in the Morris water escape task^{*}

Abstract

It is generally accepted that rodents suffer from an age-associated decrease in spatial discrimination performance. This impairment is usually seen in cross-sectional studies, in which the performance of naive young animals is compared with that of naive aged animals. However, a few studies with a longitudinal design have shown that spatial discrimination performance is sometimes preserved in the aged animal, if the animal has acquired the task at a younger age. We extended these findings and performed two experiments in which albino Wistar rats acquired the Morris water escape task for the first time at the age of 25 months. They re-acquired the task approximately 3 and 5 months after they had originally learned it.

After the rats had acquired the task in a first training series, we observed that the performance of these aged animals was not only preserved, but actually improved with repeated testing. Most of the improvement appeared in the first retention, about 3 months after the original acquisition. The effect was more pronounced in the first than in the second experiment. Possible reasons for the differences between experiments are discussed. Although the Morris water escape task may be suitable for the detection of age-related deficits in spatial learning performance in naive rats, we conclude that it is not suited for evaluation of age-associated decrements of spatial memory performance in old Wistar rats (up to an age of 30 months) in longitudinal studies.

Introduction

Age-related deficits in spatial discrimination tasks in rodents have been well documented in cross-sectional studies. Old rodents show impaired performance in various types of spatial discrimination tasks (Stone 14-unit maze: Goodrick, 1968, 1975; Michel & Klein, 1978; the circular platform and the radial maze: Barnes, 1979; Barnes, Nadel & Honig, 1980; Wallace, Krauter & Campbell, 1980a; Davis, Idowu & Gibson, 1983; van Gool, Mirmiran & van Haaren, 1985; the holeboard: van der Staay, Raaijmakers & Collijn, 1986; van der Staay et al., 1988; van der Staay, van Nies & Raaijmakers, 1990, and the cone field: van der Staay, Krechting, Blokland & Raaijmakers, 1990; van der Staay, Raaijmakers & Blokland, 1990). The Morris water escape task (Morris, 1984) has received much attention in aging research since Gage and coworkers reported a clear age-related performance deficit in this task (Gage, Dunnett & Björklund, 1984). This age-related deficit has since been found in cross-

^{*} Part of this chapter (experiment 1) has been published previously: van der Staay, F.J. & Blokland, A. (1996b). Repeated assessment of spatial discrimination performance of aged rats in the Morris water escape task. *Neurobiology of Learning and Memory*, **65**, 99-102.

sectional studies by different research groups (e.g. Rapp, Rosenberg & Gallagher, 1987; Aitken & Meaney, 1989; van der Staay & de Jonge, 1993), most of them being based on the comparison of one young and one aged group of rats.

As Ingram (1985) pointed out, no distinction can be made between age and cohort differences when a cross-sectional approach is used. Therefore, one should ideally investigate the effects of aging on the performance in learning and memory tasks by using a longitudinal design, i.e. animals should be tested at regular intervals throughout their entire lifespan. However, it is conceivable that, in longitudinal studies, the previous experience with the test influences the performance in later retention series. Ideally, a task intended to be used in longitudinal approaches should be sensitive to age-related decreases in cognitive performance and should not be influenced by previous experience. Unfortunately, only a few longitudinal studies on complex spatial learning and memory have been performed, and the results of these studies are not in accordance with the above-mentioned ideal situation.

In a longitudinal study Beatty, Bierley, and Boyd (1985) observed that Sprague Dawley rats showed a preserved acquisition and spatial memory performance up to 26 months of age in a radial maze task with a delay interval between the fourth and fifth trial. In additional studies, Bierley et al. (1986) found that spatial memory performance was unaffected in a radial maze task after a retention interval of 10 months between the last series of learning sessions and retention of the task at 21.5 months of age. Caprioli et al. (1991), who used a working memory version of the radial maze task, found that 24-month-old rats showed no signs of an age-related deficit when they had been trained at 4 and 13 months of age. By contrast, 25-month-old rats with no previous experience showed clear acquisition deficits in this task.

Using a longitudinal design, Gyger and colleagues (Gyger, Kolly & Guigoz, 1992) tested rats, which were kept under different feeding regimens, at 6, 12, 19 and 24 months of age in the Morris water escape task. The rats maintained their performance in the three training series of the task. Pitsikas and colleagues (Pitsikas, Biagini & Algeri, 1991) trained rats at 12 months of age in a Morris water escape task and re-tested these 'expert' rats when they were 24 months old. The learning curves of these rats were compared with those produced by 'naive' rats, i.e. rats which acquired the task for the first time at 24 months of age. The aged 'expert' rats performed strikingly better than the aged 'naive' rats and better than the 4-month-old rats which were also tested. These data show that age-related deficits in complex spatial learning tasks cannot be shown in rats which have previous experiences of the tasks.

The present study extends the assessment of the effects of previous learning on the retention performance in a standard Morris water escape task by old albino Wistar rats. These animals were trained for the first time on the Morris water escape task at the age of 25 months, an age at which Wistar rats usually show a spatial learning deficit (van der Staay & de Jonge, 1993; see Chapter 2.2). The rats were given two retention series consisting of five daily sessions each of the Morris water escape task approximately 3 and 5 months after they had originally learned it.

Material and Methods

Animals: outbred male Wistar rats (HsdWin:Wu) were supplied by Winkelmann, Borchen, Federal Republic of Germany at the age of 24 months. The animals were housed four to six in standard

Makrolon® type IV cages under an artificial 12 hour light/12 hour dark regimen (lights on from 7:00 to 19:00) in a temperature ($21.5^{\circ}\text{C} \pm 0.5^{\circ}\text{C}$) and humidity (50%) controlled animal room. Water and food were available ad libitum. Before testing, the animals were transferred to the experimental room where they were housed throughout the entire testing period. Housing conditions were similar to those in the animal room.

The data in the present study are from two untreated control groups of animals used in two independent studies in which the behavioral effects of chronic dietary enrichment with different test compounds were assessed. Nineteen of the original forty animals and thirteen of the original twenty-four animals completed the entire series of experiments in the first and second study, respectively.

Behavioral testing

Training: after approximately 3 to 4 months and 5 to 6 months, the rats were tested in a series of tests, consisting of the Morris water escape task, sensorimotor tests, and the open field test (see Table 1).

Table 1. *Experimental protocol of the two longitudinal studies with aged albino HsdWin:Wu rats. In the first study, testing was started with 40 rats. Nineteen rats survived till the end of all behavioral testing. In the second study, testing was started with 24 rats. Thirteen rats survived till the end of all behavioral testing. Only the data of these animals were used for statistical evaluations. The column 'Animals (n)' shows the number of animals in a particular phase of both studies.*

First study			Second study		
Age in months/ weeks	Event	Animals (n)	Age in months/ weeks	Event	Animals (n)
24/0	Arrival at our laboratory	40	24/0	Arrival at our laboratory	24
24/3 - 25/1	Acquisition of standard Morris water escape task	35	24/3	Acquisition of standard Morris water escape task	24
			24/4	Open field	
			25/1	Sensorimotor tests	
28/0 - 28/2	First retention of standard Morris water escape task	31	27/1	First retention of standard Morris water escape task	19
28/3	Sensorimotor tests		27/2	Open field	
29/0	Open field		27/3	Sensorimotor tests	
30/1 - 30/3	Second retention of standard Morris water escape task	23	29/3	Second retention of standard Morris water escape task	13
31/2	Open field		29/4	Open field	
31/2	Sensorimotor tests	19	30/1	Sensorimotor tests	13

The Morris water escape performance was assessed in a water tank which consisted of a circular black tub (Material: polyethylene; inner dimensions: diameter at top 153 cm, diameter at bottom 143 cm, depth 63 cm), filled with clear tap water at a temperature of approximately 22°C. The escape platform consisted of a black polyethylene cylinder (diameter 10.8 cm), submerged 1.5 cm below the surface of the water. The water was not made opaque, because a black escape platform is virtually invisible in a black tank. The water tank was situated in a room illuminated by daylight and white fluorescent strip lights. Abundant extra-maze cues were provided by the furniture in the room, which

included desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that was playing softly. All testing was done between 8:00 and 15:00.

A video camera, mounted in the center above the circular pool, provided a picture of the pool on a TV monitor. Lines on the monitor defined quadrant boundaries and the position of the escape platform. In addition, to obtain an arbitrary measure of the distance swum, each quadrant was further subdivided by a pattern of lines (a 4 * 4 matrix of squares). Crossing a line was scored when a rat moved across it with its whole body. The movements of the rat were registered manually and stored in an MS-DOS compatible microcomputer.

The animals received four trials during five daily acquisition sessions. A trial was started by placing a rat in the pool, facing the wall of the tank. Each of four starting positions (north, east, south, and west) was used once in a series of four trials; their order was randomized. The escape platform was always in the same quadrant. A trial was terminated as soon as the rat had climbed onto the escape platform or when 90 seconds had elapsed, whichever event occurred first. A rat was allowed to stay on the platform for 30 seconds. Then it was taken from the platform and the next trial was started. Rats that did not find the platform within 90 seconds were put on the platform by the experimenter and were allowed to stay there for 30 seconds. After completion of the fourth trial the rat was gently dried with crêpe paper and returned to its home cage. The animals were kept warm under an infrared bulb (Original Hanau Solilux, 150 W) fixed about 60 cm above the floor of the cage.

Probe trial: after the fourth trial of the fifth daily session of the original learning period and of the two retention series, an additional trial was given as a probe trial. The platform was removed, and the time a rat spent in the four quadrants was measured for 30 seconds in experiment 1 and for 60 seconds in the second experiment. In the probe trial, all rats started from the same start position, opposite to the quadrant where the escape platform had been positioned during acquisition.

Statistical analysis

Training: only the data of the rats which completed the entire series of testing were analyzed statistically. Three measures of the acquisition sessions were analyzed: 1) escape latency (Morris, 1984), that is, the time taken to find and escape onto the submerged platform, 2) the number of line crossings, and 3) the swimming speed (number of line crossings divided by escape latency). The second measure can be taken as an index for the distance swum to reach the escape platform. The measures were averaged per rat within each session. The effects of the repeated acquisition of the water escape task were assessed with an analysis of variance (ANOVA; Winer, 1971) with repeated measures over the factors Training series (original learning and retentions approximately 3 and 5 months after the original learning for the first and second study, respectively) and Sessions within Training series.

Probe trial: the effects on the bias toward the training quadrant were assessed by a within subjects ANOVA over the repeated measures Quadrants and Training series.

Results

First study

The results of this Morris water escape experiment are summarized in Fig. 1, and are depicted as curves with open symbols.

Training: the escape latency, i.e. the time taken to find and escape onto the submerged platform, decreased over the training series ($F_{2,36} = 64.02$, $p < 0.01$). Within Training series, the escape latencies decreased in the course of training ($F_{4,72} = 7.91$, $p < 0.01$). A Training series by Sessions within Training series interaction ($F_{8,144} = 2.66$, $p < 0.05$), however, indicated that the learning curves were different for the three training series. The rats found the platform faster in the two retentions than they had done during the acquisition.

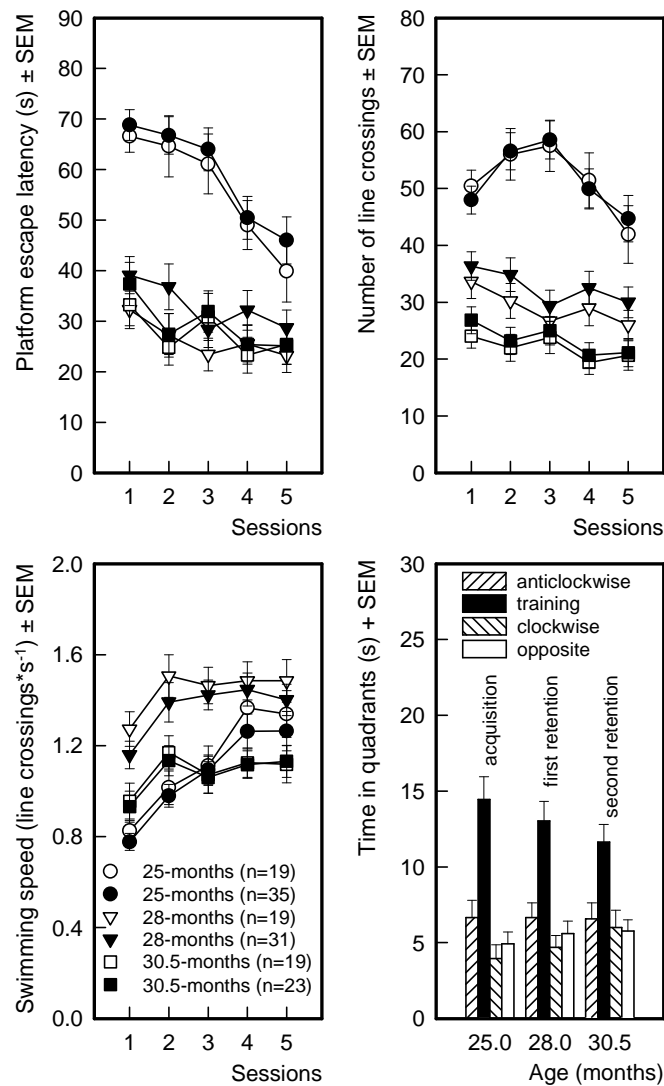


Figure 1. Escape latencies (s; upper left panel), number of line crossings (upper right panel), and swimming speed (line crossings* s⁻¹; lower left panel) of HsdWin:Wu rats in the first Morris water escape experiment at the age of approximately 25, 28, and 30.5 months. Session means and standard errors of the means (SEM) for the rats used for statistical analysis are shown as learning curves with open symbols. Session means and SEMs for all rats that completed a particular phase of the study are depicted as learning curves with filled symbols. Note that the number of animals decreased as the study progressed. The lower right panel shows the performance of aged Wistar rats in a probe trial, carried out after the last trial of the original acquisition and of the first and second retention. Means and SEMs for the time (s) spent in the four quadrants are depicted.

The number of line crossings decreased over training series ($F_{2,36} = 65.55$, $p < 0.01$). The same holds true for the Sessions within Training series, where the rats learned to reduce the distance swum to find the platform ($F_{4,72} = 3.41$, $p < 0.05$). The learning curves within Training series were similarly shaped, although there was weak statistical support for the impression that the learning curve of the first acquisition was steeper than were those of the two subsequent training series (Training series by Sessions within Training series interaction: $F_{8,144} = 1.94$, $0.10 > p > 0.05$).

The measure *swimming speed* changed from one training-series to the other (Training series: $F_{2,36} = 32.18$, $p < 0.01$). The rats increased the swimming speed over Sessions within Training series ($F_{4,72} = 3.41$, $p < 0.05$), the increase being different within the different training series (Training series by Sessions within Training series interaction: $F_{8,144} = 2.78$, $p < 0.05$).

Probe trial: the rats had a clear bias for the training quadrant (Quadrants: $F_{3,162} = 29.24$, $p < 0.01$; see Fig. 1). There were no changes in the extent of this bias over the training series (Quadrants by Training series interaction: $F_{6,162} < 1.0$, n.s.).

Second study

The results of this experiment are summarized in Fig. 2, and are depicted as curves with open symbols.

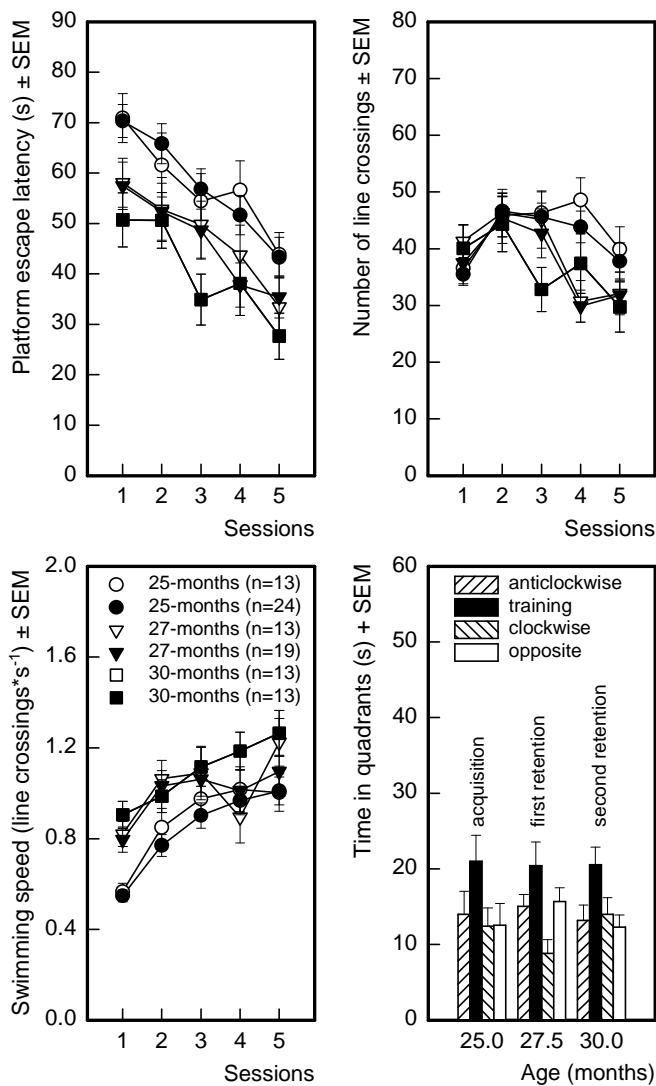


Figure 2. Escape latencies [(s); upper left panel], number of line crossings (upper right panel), and swimming speed (line crossings*s⁻¹; lower left panel), and performance in the probe trials (lower right panel) of HsdWin:Wu rats in the second Morris water escape experiment at the age of approximately 25, 27.5 and 30.0 months. For further explanations see the legend of Fig. 1. Note that the symbols ■ and □ represent identical curves since all rats that were tested during the last training series survived the entire study.

Training: the *escape latency*, i.e. the time taken to find and escape onto the submerged platform, decreased over the training series ($F_{2,24} = 7.63$, $p < 0.01$). Within Training series, the escape latencies decreased in the course of training ($F_{4,48} = 13.44$, $p < 0.01$). The learning curves were similar for the three training series (Training series by Sessions within Training series interaction: $F_{8,96} < 1.0$, n.s.).

There was a tendency to a decrease in the *number of line crossings* over the training series ($F_{2,24} = 2.91$, $0.10 > p > 0.05$). Analysis of the Sessions within Training series revealed that the number of lines crossed changed with repeated testing ($F_{4,48} = 4.20$, $p < 0.01$). The learning curves within Training series were different (Training series by Sessions within Training series interaction: $F_{8,96} = 3.54$, $p < 0.05$).

A different picture emerged for *swimming speed*. This measure changed from one training series to the other (Training series: $F_{2,24} = 6.16$, $p < 0.01$). The rats increased their swimming speed over Sessions within Training series ($F_{4,48} = 13.60$, $p < 0.01$), but within the different training series the increase in swimming speed was similar (Training series by Sessions within Training series interaction: $F_{8,96} = 1.90$, n.s.).

Probe trial: the rats had a clear bias for the training quadrant (Quadrants: $F_{3,108} = 5.76$, $p < 0.01$; see Fig. 2). There were no changes in the extent of this bias over the training series (Quadrants by Training series interaction: $F_{6,108} < 1.0$, n.s.).

Discussion

In both experiments, the ages at which the rats were tested and the intervals between acquisition and retention series of the task were very similar. The rats that took part in the last training series can be considered as 'old', as they had reached or exceeded the age at which survival in the normal aging population is 50 percent or less. In 30-month-old rats age-associated cognitive deficits would be expected.

Both experiments showed that previous experience of the standard Morris water escape task facilitated and improved retentions of the task by old Wistar rats after retention intervals of approximately 3 and 5 months. This effect was clearest in the first experiment, where the first retention started at a performance level that was indistinguishable from the performance level reached at the end of the original acquisition, i.e. the 28-month-old rats did not forget the task over a retention period of 3 months. This was also true for the 30-month-old rats: they showed no signs of cognitive impairments when their performance was compared to that of the previous two acquisitions. These results are in agreement with those of other studies (Beatty, Bierley, & Boyd, 1985; Bierley et al., 1986; Caprioli et al., 1991; Pitsikas, Biagini & Algeri, 1991, Gyger, Kolly & Guigoz, 1992), although this preservation of cognitive performance has not been observed in the age range that was used here.

In addition, the present study showed that the rats that learned a task at an age at which a dramatic acquisition impairment can be usually observed (van der Staay & de Jonge, 1993) were able to preserve this spatial memory up to an age of 30 months. The probe trials in both experiments revealed that the rats acquired the position of the platform, because they showed a clear quadrant bias. The bias for the training quadrant remained similar with repeated testing, giving further support the notion that spatial memory was preserved in these old rats.

We observed that the performance of the old rats in different sensorimotor tests (square bridge, paw test, footprint test; data not shown), which were also part of the present studies, became worse with repeated testing. This indicates that there is an age-related decline in sensorimotor functions. Such a decline has already been well documented by others (e.g. Marshall, 1982; Gage, Dunnett & Björklund, 1984, 1989). This deterioration of sensorimotor function did not influence the cognitive performance in the Morris task, in agreement with previous findings (Gage, Dunnett & Björklund, 1984, 1989; Gallagher & Burwell, 1989). This suggests that aging is not a homogeneous process but can be differentiated on the basis of individual processes (Markowska et al., 1989; van der Staay, Blokland & Raaijmakers, 1990). Moreover, the present data suggest that repeated training in sensorimotor tasks does not lead to improved sensorimotor skills, whereas repeated testing in a learning task appears to enhance cognitive performance in very old rats.

In a cone field task, which permits the automatic and simultaneous assessment of spatial working-memory and reference memory, two successive retention series after 4-month retention intervals were found to produce learning curves similar to those of naive adult Wistar rats trained at 4-, 8 and 12 months of age, with no signs of either improvement or impairment from one acquisition to the next (van der Staay, Krechting, Blokland & Raaijmakers, 1990). Thus, even at a young age, rats did not retain this complex task. This finding contrasts with the data of the present study and does not corroborate the results of other studies which evaluated the spatial memory in longitudinal studies (Beatty, Bierley & Boyd, 1985; Bierley et al., 1986). It has been suggested that the cone field task is more complex than the Morris task (Blokland, Honig & Raaijmakers, 1994), and thus the preservation of the spatial memory might be related to the complexity of the task (i.e., rules and/or complexity of the spatial configuration of cues and locations).

Differences between the two studies

Cohort differences in longevity might be an explanation for the different results of the two studies. The second experiment was performed approximately half a year after the first experiment. In this time period there may have been genetic drift (see Chapter 2.4), or changes in the health status of the population from which the samples were derived.

The survival characteristics of populations are under genetic control. The Senescence Accelerated Mouse (SAM: Miyamoto et al., 1986) shows that survival characteristics respond to genetic selection. Further evidence for genetic factors in aging is provided by the fact that inbred strains of mice (Russell, 1972) show considerable differences in the mean and distribution of their lifespans. Similar differences between survival characteristics have been reported for inbred rat strains (e.g. Burek, 1978).

The heritability of life expectancy in the male outbred Han:WIST rat strain, for example, has been estimated to be 51%. As a consequence of random combination of animals from sub populations of the outbred strain, a strong heritability may lead to pronounced shifts in the life expectancy of the offspring (Deerberg, 1991). The breeders of the rats in the present study 'refresh' or 're-vitalize' the outbred breeding nuclei at periodic intervals by introducing males of a sub-population of the same strain, which have been kept elsewhere. If this sub population is genetically different, and even more markedly if genetic drift took place in this sub population, then the life expectancy of the strain will be influenced considerably. This might be a cause for the differences between the animals used in the two studies.

Life expectancy and cognitive performance

Loss of animals in the course of an experiment introduces a complicating factor, the implications of which are not yet well understood. Statistics are based on the data of the survivors only, and it is not clear whether the loss of animals (approximately half of the animals died before the last retention was finished) produced 'biased' groups, as the less vital rats (in terms of survival characteristics) did not complete the study. It is conceivable that two groups of animals existed: one group of impaired rats, and one group of unimpaired rats (cf. Goodrick, 1972). If the health status and consequently the longevity were strongly related to the degree of impairment in spatial discrimination tasks, then the unimpaired animals would have been the ones that survived, and the impaired rats would have died. The selective loss of impaired rats would lead to an improved mean performance level of the surviving sample.

To get an impression whether this hypothesized relationship between health status, survival, and cognitive performance level might explain the improved mean performance levels during the first and the second retention, we plotted the learning curves of all animals that completed a particular phase of the experiment (curves with filled symbols in Figures 1 and 2, respectively). The learning curves were virtually identical. Thus, the longevity of an individual rat does not predict its learning performance in the Morris water escape task. If, however, the mean performance level during the first retention would have been lower than that calculated using only the data of the animals which also finished the second retention, then the data would suggest a relationship between longevity and learning performance.

The above-mentioned hypothesis cannot be tested experimentally because animals die during experiments. Aging rats that are well below the 50% survival age might be better suited to assess the possible relationship between health status and cognitive performance in more detail. In this context, Bronson (1990) suggests that both healthy and ill-appearing animals should be used in aging studies and that the effects of aging should be distinguished from those induced by pathological changes, as determined by pathological analysis of the animals.

Conclusions

In conclusion, the standard Morris water escape task appears not to be suited as a tool to assess the age-related deterioration in spatial memory in Wistar rats in longitudinal studies, in which the same rats are tested repeatedly over a longer period. Although this is not favorable for longitudinal studies aimed at evaluating putative cognition enhancing drugs, this observation may be useful for toxicological studies in which the potential disruptive effects of chronic treatment with test compounds on cognitive performance is evaluated. Old rats preserved their performance over retention intervals of about 3 months. They even slightly improved their performance from retention to retention and did not show any loss of *spatial memory* up to an age of 30 months. By contrast, cross-sectional studies assessing *spatial learning* consistently show an age-related impairment.

2.4

Shift in the performance of 24-month-old Wistar rats in the Morris water escape task: a comparison across thirty-six experiments*

Abstract

Spatial discrimination learning in aged rats serves as an animal model of cognitive aging. We assessed the replicability of spatial discrimination performance in the standard Morris water escape task. To this end, the learning curves and the performance in a probe trial of 24-month-old outbred Wistar (HsdWin:Wu) control rats from 36 experiments were compared. These experiments had been performed at our laboratory under strictly controlled conditions over a period of 71 weeks. There was a very high variability in the learning curves between experiments. The initial performance level, i.e. the performance during the first session, did not change systematically across the 36 experiments. In contrast, the final performance level, i.e. the level reached in the fifth training session, decreased over the 71 week period, when the platform escape latency and the distance swum to reach the platform, measured as number of line crossings, were considered. In the last experiments of the series, learning curves were no longer seen: the rats did not improve their performance across the acquisition sessions.

By contrast, the swimming speed and, in the probe trial, the bias for the quadrant where the platform had been positioned during training, did not change. This indicates that a decrease across experiments occurred predominantly with respect to *spatial orientation learning*, whereas the *motor performance* appeared to be unchanged. Explanations for this observation, such as differences in viability between shipments and the possible occurrence of genetic drift, are discussed.

Introduction

Changes in learning and memory in old rats are considered to reflect cognitive aging (Campbell, Krauter & Wallace, 1980; Ingram, 1985; Schuurman et al., 1986; Raaijmakers, Blokland & van der Staay, 1993). Deficits in spatial discrimination tasks in aged rodents, predominantly rats, are well documented in cross-sectional studies. Old, experimentally naive rats show impaired performance compared with young conspecifics in various types of mazes such as the Stone 14-unit maze (Ingram, 1985; Goldman et al., 1991), the circular platform (Barnes, 1979; Barnes et al., 1990) and the radial maze (aversively motivated radial water maze: Pitsikas & Algeri, 1992; appetitively, i.e. food-motivated, radial maze: Wallace, Krauter & Campbell, 1980a; Marczyński, Artwohl & Marczyńska; 1994; Arendash, Sanberg & Sengstock, 1995; Levin & Torry, 1996), the holeboard (van der Staay, van Nies

* This chapter is based on the publication: van der Staay, F.J. (1997). Shift in performance of 24-month-old Wistar rats in the Morris water escape task: a comparison across 36 experiments. *Behavioural Brain Research*, **87**, 213-222.

& Raaijmakers, 1990; Markel et al., 1995), and the cone field (van der Staay, Krechting, Blokland & Raaijmakers, 1990).

The Morris water escape task (Morris, 1984) has become one of the most frequently used testing paradigms in aging research since Gage and colleagues reported a clear age-related performance deficit in this task (Gage, Dunnett & Björklund, 1984). This age-related deficit has since been confirmed in cross-sectional studies by different research groups (e.g. Rapp, Rosenberg & Gallagher, 1987; Aitken & Meaney, 1989; van der Staay & de Jonge, 1993).

Using the standard Morris water escape task and a short-term memory version of the task, we found clear deficits in the acquisition rate and in the performance in the probe trial of 24-month-old outbred Wistar (HsdWin:Wu, previous names WISW:Wu and HsdCpb:Wu, respectively) rats, when compared with young-adult rats (van der Staay & de Jonge, 1993). For several years, we used 24-month-old rats of this Wistar strain as a model of aging and age-related impairments in gerontopharmacological studies. The rats were tested under strictly controlled and standardized experimental conditions. However, we had the strong impression that, over the years, the learning curves in this Wistar strain became less pronounced, and in the end were even absent.

One of the assumptions underlying the strictly standardized and controlled studies (e.g. Runkel & McGrath, 1972) is that they strongly increase the replicability of results. Our file of data from experiments in which the standard Morris water escape task was used provided the opportunity to address the question of replicability and of the stability of results over a longer period of time. To this end, we compared the learning curves and the initial (first day of testing) and final (fifth day of testing) performance of aged control rats of the HsdWin:Wu Wistar strain from 36 experiments, carried out over a period of 71 weeks.

Material and Methods

Animals

Outbred male Wistar rats (HsdWin:Wu) were supplied by Winkelmann, Borcheln, Federal Republic of Germany, at the age of 24 months. They were housed four to six in standard Makrolon[®] type IV cages under an artificial 12 hour light/12 hour dark regimen (lights on from 7:00 to 19:00) in a temperature (21.5°C ± 0.5°C) and humidity (50%) controlled vivarium. Water and food were available ad libitum. Before testing, the rats were transferred to the experimental room where they were housed throughout the entire testing period. Housing conditions were similar to those in the animal room.

The data in the present study are from the control groups of animals used in 36 independent studies in which the behavioral effects of different test compounds were assessed. The control groups consisted of 8 to 12 animals each. The animals were given a tylose suspension orally, 30 minutes before each of the daily acquisition sessions.

Behavioral testing

Training: the Morris water escape performance was assessed in a water tank which consisted of a circular black tub (Material: polyethylene; inner dimensions: diameter at top 153 cm, diameter at bottom 143 cm, depth 63 cm), filled with clear tap water at a temperature of approximately 22°C. The escape platform consisted of a black polyethylene cylinder (diameter 10.8 cm), submerged 1.5 cm

below the surface of the water. The water was not made opaque, because a black escape platform is virtually invisible in a black tank.

The water tank was situated in a room illuminated by daylight and white fluorescent strip lights. Abundant extra-maze cues were provided by the furniture in the room, which included desks, computer equipment, a second water tank, the presence of the experimenter, and by a radio on a shelf that was playing softly. All testing was done between 8:00 and 15:00.

A video camera, mounted in the center above the circular pool, provided a picture of the pool on a video monitor. Lines on the monitor defined quadrant boundaries and the position of the escape platform. In addition, to obtain an arbitrary measure of the distance swum, each quadrant was further subdivided by a pattern of lines (a 4 * 4 matrix of squares). Crossing a line was scored when a rat moved across it with its whole body. The movements of the rat were registered manually and stored in an MS-DOS compatible microcomputer.

Table 1. Overview of the Morris water escape experiments analyzed. The week number and the number of animals in an experiment are indicated. Note that in some weeks, two experiments (Exp. 1, Exp. 2) were run simultaneously.

Week no.	Exp. 1	Exp. 2	Week no.	Exp. 1	Exp. 2	Week no.	Exp. 1	Exp. 2
1	8		25	8		49		
2	8		26	8		50		
3			27	8		51		
4			28			52		
5			29	8	8	53	8	8
6			30			54	8	8
7			31			55	8	
8			32	7	6	56	8	
9	12		33	8		57	8	
10			34			58		
11			35			59	8	
12	12		36			60		
13			37			61	8	
14	8		38			62	8	8
15			39	8	8	63		
16			40	8		64		
17			41			65	8	
18	8		42	8		66	8	
19			43			67		
20			44	8		68	7	
21			45			69		
22			46			70	8	
23			47			71	8	
24			48	8				

The animals received four trials during five daily acquisition sessions. A trial was started by placing a rat in the pool, facing the wall of the tank. Each of four starting positions (north, east, south, and west) was used once in a series of four trials; their order was randomized. The escape platform was always in the same quadrant. A trial was terminated as soon as the rat had climbed onto the escape platform or when 90 seconds had elapsed, whichever event occurred first. A rat was allowed to stay on the platform for 30 seconds. Then it was taken from the platform and the next trial was started. Rats that did not find the platform within 90 seconds were put on the platform by the experimenter and were allowed to stay there for 30 seconds. After completion of the fourth trial the rat was gently dried with crêpe paper and returned to its home cage. The animals were kept warm under an infrared bulb (Original Hanau Solilux, 150 W) fixed about 60 cm above the floor of the cage.

Probe trial: after the fourth trial of the fifth daily session, an additional trial was given as a probe trial. The platform was removed, and the time a rat spent in the four quadrants was measured for 30 seconds. In the probe trial, all rats started from the same start position, namely opposite the quadrant where the escape platform had been positioned during acquisition.

Statistical analysis

Some rats were discarded from an experiment because they showed sensorimotor deficits, or because they were never able to find the escape platform. Thus the number of rats in some control groups was reduced to 7 or, in one case, to 6 animals (see Table 1).

Acquisition: four measures of the acquisition sessions were analyzed: 1) the number of line crossings, 2) the number of quadrant entries (Lalonde & Joyal, 1991), 3) the swimming speed (number of line crossings divided by escape latency), and 4) escape latency (Morris, 1984), that is, the time taken to find and escape onto the submerged platform. The first two measures can be taken as an index for the distance swum to reach the escape platform. The measures were averaged per rat within each session.

Probe trial: the time a rat spent in the quadrant where the platform had been during the training sessions was determined per rat as a percentage of the duration of the probe trial (30 seconds) and was taken as a measure for the rat's bias for this part of the water tank.

Correlations

Pearson product moment correlation coefficients (r_{PM}) were calculated between the general means (average performance across five acquisition sessions) of line crossings, quadrant entries, swimming speed, escape latency, and percent time spent in the previous training quadrant in the probe trial, across the 292 24-month-old control Wistar rats from the 36 Morris water escape experiments.

Analyses of variance

The replicability of the results of the water escape task across the 36 experiments was assessed with an analysis of variance (ANOVA; Winer, 1971) with the factor Experiments (i.e. the 36 experiments) and repeated measures factor Sessions (i.e. the five acquisition sessions within experiments). In addition, the performance on the first and fifth (last) day of acquisition was analyzed by ANOVA, with the factor Experiments.

Probe trial: the replicability of the bias toward the previous training quadrant in the probe trial across the 36 experiments was assessed by an ANOVA with the factor Experiments.

Regression analyses

Acquisition: regression analyses were based on the 292 control animals in the 36 experiments. Estimates of intercept and slope were calculated for the first and fifth (last) session, respectively, with number of line crossings, number of quadrant entries, swimming speed and escape latency as dependent variables, and week number (see Table 1) as regressor (SAS/STAT REG-Procedure; SAS Institute, 1990).

The regression across the first sessions was taken as an index of a change in initial performance over the 71-week period in which the experiments were performed. The regression across the fifth sessions was taken as an index for a shift in the performance level reached by the rats on the last day of training.

Probe trial: the regression of the bias for the quadrant in which the platform had been situated during training was also determined, with week number as regressor. In addition, it was determined whether the rat spent more time than would be expected to occur by chance in the previous training quadrant. It was assumed that the bias for the training quadrant was at chance level when the rats spent about 25 percent of the duration of the probe trial in that particular quadrant. Above chance level was calculated as percent time spent in the previous training quadrant minus 25. Per experiment it was determined by *t*-statistics whether this difference score deviated from zero.

Results

Correlation analysis

The correlation coefficients between the number of line crossings, the number of quadrant entries, the swimming speed, the escape latency, and the percent time spent in the training quadrant in the probe trial are summarized in Table 2.

Table 2. Pearson product moment correlation coefficients (r_{PM}) and associated probabilities between the general means (average performance across the five acquisition sessions) of line crossings, quadrant entries, swimming speed, and escape latency during acquisition, and of the percent time spent in the previous training quadrant during the probe trial, calculated across the 292 24-month-old control Wistar rats from 36 Morris water escape experiments.

Measure		Lines crossed	Quadrant entries	Swimming speed	Escape latency	Probe trial
Lines crossed (mean performance)	r_{PM}	1.000				
	$p <$	-				
Quadrant entries (mean performance)	r_{PM}	0.915	1.000			
	$p <$	0.01	-			
Swimming speed (mean performance)	r_{PM}	0.289	0.191	1.000		
	$p <$	0.01	0.01	-		
Escape latency (mean performance)	r_{PM}	0.697	0.677	-0.395	1.000	
	$p <$	0.01	0.01	0.01	-	
Probe trial: percent time in training quadrant	r_{PM}	-0.181	-0.185	0.207	-0.336	1.000
	$p <$	0.01	0.01	0.01	0.01	-

Acquisition: the platform escape latency showed a strong, positive correlation with both the number of quadrant entries ($r_{PM} = 0.677$) and the number of line crossings ($r_{PM} = 0.697$), respectively. There was a moderate, negative correlation between platform escape latency and swimming speed ($r_{PM} = -0.395$). Swimming speed showed a very weak, but statistically reliable positive correlation with the number of quadrant entries ($r_{PM} = 0.191$) and the number of line crossings ($r_{PM} = 0.289$).

Previously, we had found that the number of quadrant entries and the number of line crossings are highly correlated (van der Staay & de Jonge, 1993: young Wistar rats: $r_{PM} = 0.98$; old Wistar rats: $r_{PM} = 0.92$). The correlation analysis performed for the present study fully corroborated our earlier findings ($r_{PM} = 0.915$). Since the number of line crossings and the number of quadrant entries were highly correlated, they both provide a measure for the distance swum to reach the escape platform, and one may well dispense with one of the measures (Walsh and Cummins, 1976). Therefore, only the results on number of line crossings will be considered further.

Probe-trial: weak, but statistically reliable, correlations were found between the four measures of acquisition and the bias of the animals for the previous platform position. Interestingly, there were very weak negative correlations between percent in this quadrant on one hand, and number of quadrant entries ($r_{PM} = -0.185$), number of line crossings ($r_{PM} = -0.181$), and latency to escape onto the platform ($r_{PM} = -0.336$), on the other. The rats with the shorter escape latencies and with the shorter distances swum to find the escape platform had a stronger bias toward the previous platform position in the probe trial. The swimming speed was positively correlated ($r_{PM} = 0.207$) with the percent time spent in the previous training quadrant.

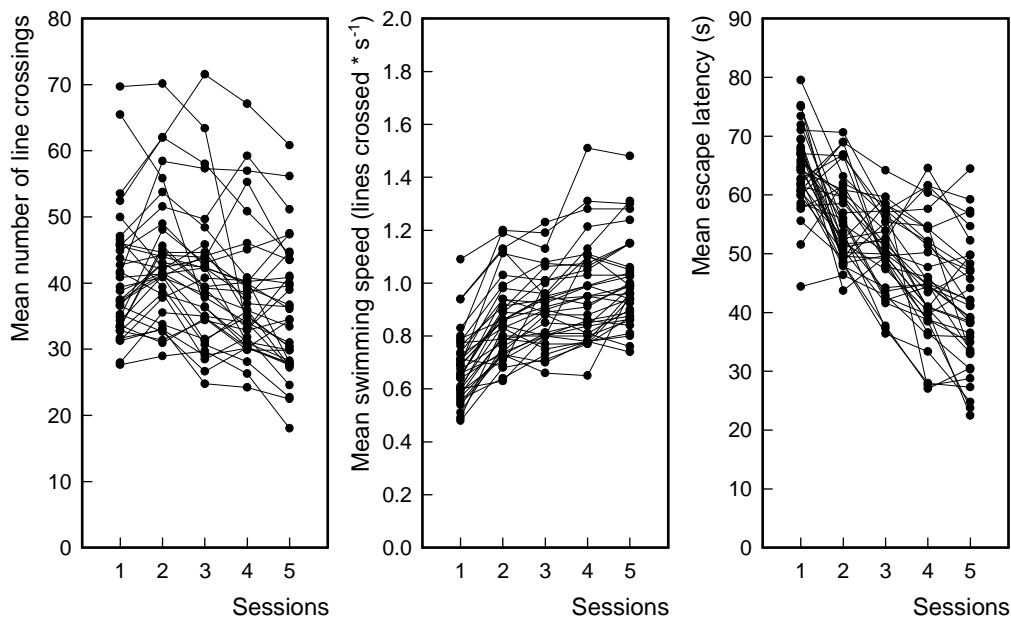


Figure 1. Acquisition of a water escape task in a circular pool by 24-month-old HsdWin:Wu rats. Session means of the control groups from 36 experiments of the distance swum (i.e. number of line crossings; left panel), swimming speed (i.e. line crossings * s^{-1} ; center panel), and latency (s) to escape onto a submerged platform (right panel) are depicted.

Analyses of variance

Number of line crossings (see Fig. 1, left panel):

Acquisition curves: averaged over the five sessions within experiments, the number of line crossings was different (Experiments: $F_{35,256} = 4.01$, $p < 0.01$). Within experiments, the number of line crossings in general decreased over sessions (Sessions: $F_{4,1024} = 15.42$, $p < 0.01$). The acquisition curves, however, had a different shape across the experiments (Experiments by Sessions interaction: $F_{140,1024} = 1.57$, $p < 0.01$).

First acquisition session (see Fig. 2): the number of line crossings on the first day of the acquisition of the task was different between experiments (Experiments: $F_{35,256} = 4.93$, $p < 0.01$).

Fifth acquisition session (see Fig. 2): the performance level reached by the fifth acquisition session was different in the 36 experiments (Experiments: $F_{35,256} = 2.43$, $p < 0.01$).

Swimming speed (see Fig. 1, center panel):

Acquisition curves: averaged over the five sessions in the 36 experiments, the swimming speed was different (Experiments: $F_{35,256} = 6.51$, $p < 0.01$). Within experiments, the swimming speed in general increased across sessions (Sessions: $F_{4,1024} = 143.60$, $p < 0.01$). This increase, however, was different in the 36 experiments (Experiments by Sessions interaction: $F_{140,1024} = 1.43$, $p < 0.01$).

First acquisition session (see Fig. 3): the swimming speed of the rats in the first acquisition session was different between experiments ($F_{35,256} = 5.58$, $p < 0.01$).

Fifth acquisition session (see Fig. 3): the performance level reached by the fifth acquisition session was different in the 36 experiments (Experiments: $F_{35,256} = 3.60$, $p < 0.01$).

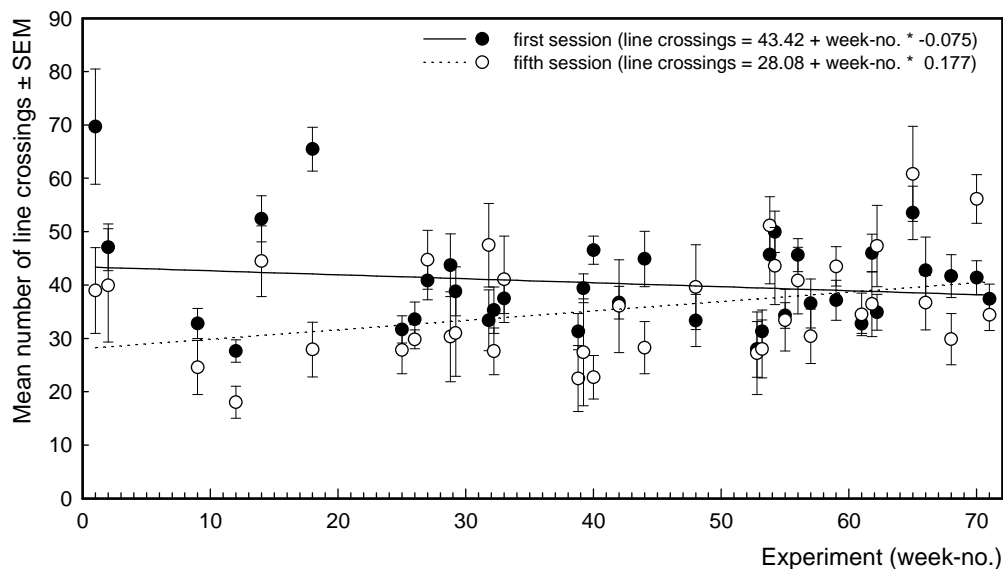


Figure 2. Number of line crossings of 24-month-old HsdWin:Wu rats in the Morris water escape task. Session means and standard errors of the means (SEM) are depicted. Estimates for intercept and slope of the regression equations based on the 292 control animals in the 36 experiments with line crossings of the first session and of the fifth (last) session as dependent variable, and week number as regressor, are also shown.

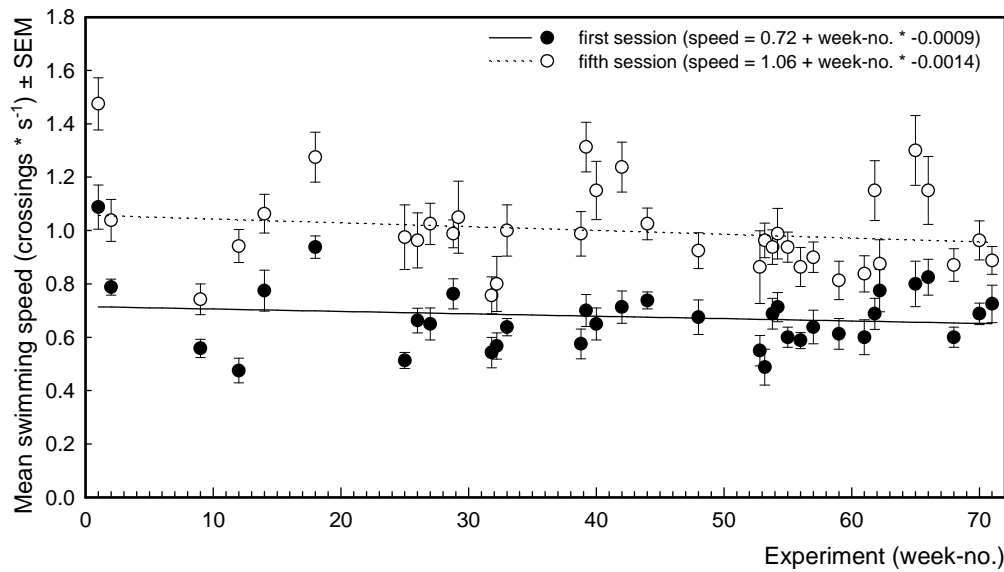


Figure 3. Swimming speed (line crossings * s^{-1}) of 24-month-old HsdWin:Wu rats in the Morris water escape task. Session means and standard errors of the means (SEM) are depicted. Estimates for intercept and slope of the regression equations based on the 292 control animals in the 36 experiments with swimming speed of the first session and of the fifth (last) session as dependent variable, and week number as regressor, are also shown.

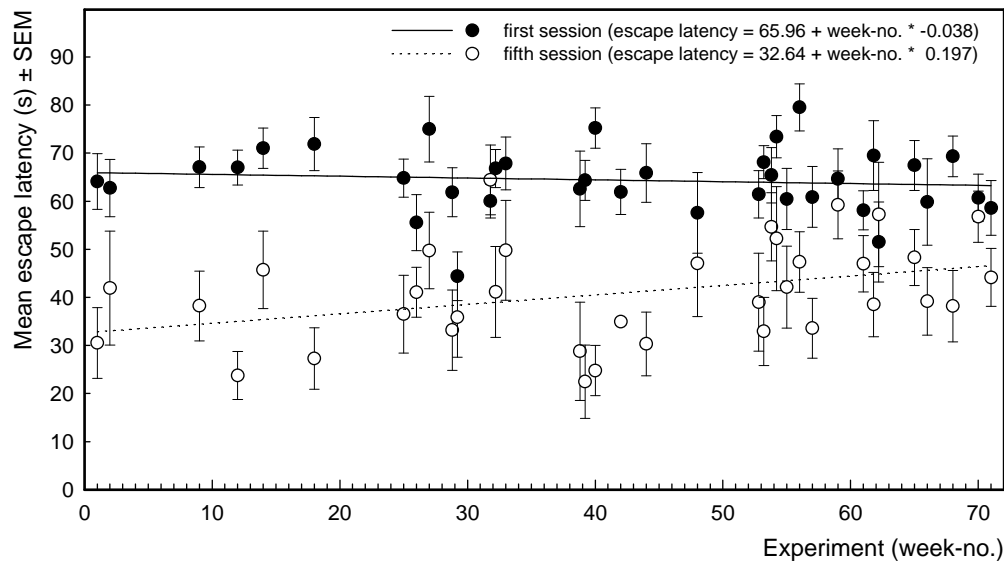


Figure 4. Escape latencies (s) of 24-month-old HsdWin:Wu rats in the Morris water escape task. Session means and standard errors of the means (SEM) are depicted. Estimates for intercept and slope of the regression equations based on the 292 control animals in the 36 experiments with escape latencies of the first session and of the fifth (last) session as dependent variables, and week number as regressor, are also shown.

Escape latency (see Fig. 1, right panel)

Acquisition curves: averaged over the five sessions within experiments, the escape latency, i.e. the time taken to find and escape onto the submerged platform, was similar (Experiments: $F_{35,256} = 1.22$, n.s.). Within experiments, the escape latency in general decreased over the training sessions

(Sessions: $F_{4,1024} = 82.61$, $p < .01$). The acquisition curves, however, had a different shape across the experiments (Experiments by Sessions interaction: $F_{140,1024} = 1.28$, $p < 0.05$).

First acquisition session (see Fig. 4): the performance of the rats in the first acquisition session was marginally different between experiments (Experiments: $F_{35,256} = 1.46$, $0.10 > p > 0.05$).

Fifth acquisition session (see Fig. 4): the performance level reached by the fifth acquisition session was different in the 36 experiments (Experiments: $F_{35,256} = 1.75$, $p < 0.01$).

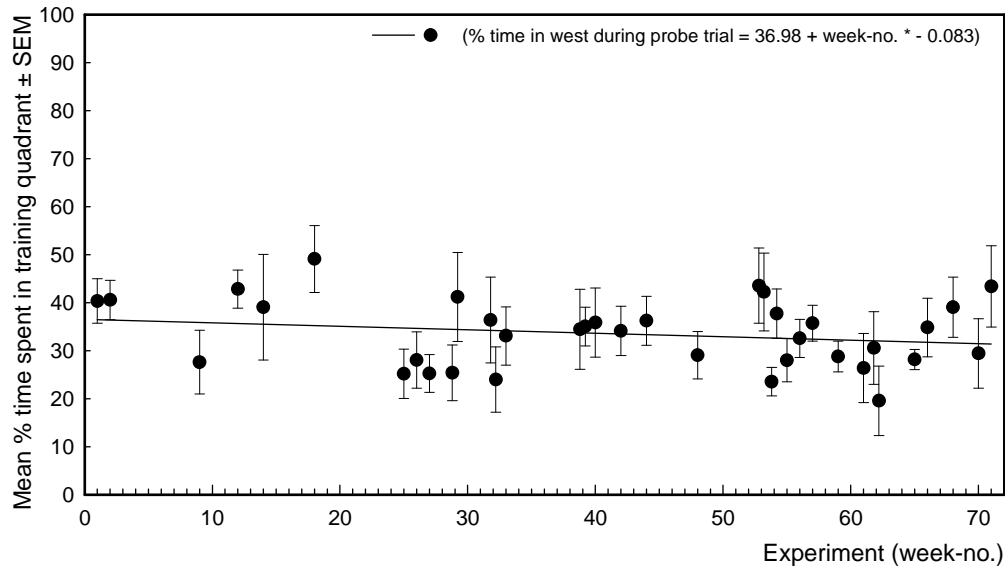


Figure 5. Percent time spent in the training quadrant during the probe trial of 24-month-old HsdWin:Wu rats in the Morris water escape task. Session means and standard errors of the means (SEM) are depicted. Estimates for intercept and slope of the regression equations based on the 292 control animals in the 36 experiments with percent time in the training quadrant during the probe trial as dependent variable, and week number as regressor, are also shown.

Probe trial (see Fig. 5): the percent time spent in the training quadrant during the probe trial was not different in the 36 experiments (Experiments: $F_{35,256} = 1.24$, n.s.). In only 8 of the 36 experiments did the rats spend more time in the previous training quadrant than would be expected to occur by chance.

Regression analyses (see Table 3, and Figures 2 to 5): there were no systematic shifts in the performance in the first acquisition session across the 71 weeks in which the 36 experiments were performed. However, analysis of the performance level reached on the fifth day of training revealed that for the number of lines crossed and for the escape latency, there was a shift toward a poorer performance. In contrast, the swimming speed appeared to be relatively constant across the 71-week period. The same was true for the bias of the rats in the probe trial.

Table 3. Estimates for intercept and slope of the regression equations for line crossings, swimming speed, escape latency of the first session and of the fifth (last) session of the acquisition, and percent time spent in the previous training quadrant during the probe trial for 292 control animals across 36 Morris water escape experiments. The regressor in this analysis was the week number in which an experiment was performed.

Measure	Parameter	Estimate	SEM	t-value	p t
Lines crossed during the first session	intercept	43.4166	1.944	22.333	< 0.01
	slope	-0.0747	0.042	-1.756	0.10 > p > 0.05
Lines crossed during the fifth session	intercept	28.0833	2.561	10.963	< 0.01
	slope	0.1765	0.056	3.151	< 0.01
Swimming speed during the first session	intercept	0.7150	0.026	27.271	< 0.01
	slope	-0.0009	0.000	-1.569	n.s.
Swimming speed during the fifth session	intercept	1.0577	0.037	28.051	< 0.01
	slope	-0.0014	0.000	-1.737	0.10 > p > 0.05
Escape latency during the first session	intercept	65.9623	2.169	30.412	< 0.01
	slope	-0.0379	0.047	-0.800	n.s.
Escape latency during the fifth session	intercept	32.6384	3.084	10.582	< 0.01
	slope	0.1968	0.067	2.918	< 0.01
Probe trial: percent time spent in training quadrant	intercept	36.9815	2.393	15.454	< 0.01
	slope	-0.0831	0.052	-1.588	n.s.

Discussion

We assessed the replicability of the standard Morris water escape task using aged outbred Wistar (HsdWin:Wu) rats. The three major observations of the present study are:

First, there was a very high variability in the acquisition curves between experiments (compare Fig. 1). This was also true for the experiments which were run in parallel (compare Table 1; and Figs. 2 to 5). In general, the old rats in the 36 experiments showed a very poor bias for the previous training quadrant during the probe trial.

Second, the measures for acquisition of the Morris water escape task (quadrant entries, line crossings, escape latencies), averaged over the five training sessions (general means) were fairly highly correlated. By contrast, the correlations of these measures with the bias for the previous training quadrant during the probe trial were very weak. In fact, only between 3 and 11 percent of the variance of the performance in the probe trial can be predicted from the variance in the measures for the acquisition of the Morris task (calculated as $r_{PM}^2 * 100$; e.g. Ferguson, 1971).

Third, whereas regression analysis revealed that the initial performance, i.e. the performance during the first session, did not change systematically across the 36 experiments, there was a decrease in the final performance level reached in the fifth training session across the 71-week period in which the experiments were performed. This shift in performance was statistically reliable for the platform escape latency and for the number of line crossings. In fact, in the last experiments of the series, the rats no longer improved their performance across the five training sessions, i.e. they no longer produced learning curves.

No shift was found for the swimming speed and for the bias in the probe trial for the quadrant where the platform had been positioned during training. This indicates that predominantly *spatial orientation learning* decreased across experiments, whereas *motor performance* appeared to be unchanged. Whether this shift in spatial orientation performance has been caused by a shift in learning ability, or

whether other factors are involved, cannot be decided from our data. For example, the alternative explanation that not learning ability *per se*, but motivation to escape from the water had changed, cannot be rejected.

Experimenter-induced differences

The experiments were performed by seven highly experienced and well-trained technicians. Using the video-tracking system EthoVision® (Noldus Information Technology b.v., Wageningen, The Netherlands; Sams-Dodd, 1995; Spruijt, Hol & Rousseau, 1992) in parallel with the observation procedure reported in this paper, we found that the data obtained with our observation procedure, are highly reliable. They were correlated strongly with the corresponding measures obtained using EthoVision® ($r_{PM} > 0.90$ for all measures in all sessions; unpublished results). Therefore, it is unlikely that the differences between experiments are due to the resolution of the observation method used.

Environmental changes

It is accepted that the phenotypes assessed in behavioral studies are the result of an interaction between the genotype and the environment (e.g. Strickberger, 1976, pp. 182-201). Moreover, genotype-environment interactions have been shown to affect aging and mortality (e.g. Ordy, 1975). Environmental changes, therefore, might act as strong sources of phenotypic change, even when the genotype is stable and does not change over time. However, environmental changes are an unlikely cause of the observed strong variability in the results from experiment to experiment on one hand, and of the gradual shift in performance across experiments, on the other.

All animals were from the same outbred Wistar strain, bred by the same breeder. All rats were housed in the animal facilities of the breeder in the same room until they had reached the age of 24 months. Then, they were transported to the our laboratory, where they were always housed in the same animal room. The rats were allowed to habituate for one to three weeks before the Morris water escape experiment was performed. All testing was done in the same laboratory. Thus, the environmental conditions in the breeder's animal house, and in the animal room and in our laboratory, where behavioral testing occurred, were highly stable and strictly standardized across experiments.

Age-related sensorimotor impairments and age-associated pathological changes

The animals were always selected from a larger group of rats. The rats appeared to be healthy and showed no signs of abnormalities or physical impairments. Special care was taken to use only rats free of cataracts. However, the number of rats that fulfilled the criteria was different between shipments. Although animals which did not pass the inclusion criteria of the visual health check for behavioral studies were not included in the studies, there were differences between cohorts for the number of rats which were found to be acceptable for Morris water escape experiments. It is conceivable that although no overt abnormalities and physical impairments were seen in the rats used in our experiments, the general health state might have been different between shipments. Consequently, the differences between experiments might have been due to differences in animal health characteristics.

If swimming speed *per se* is considered as an index of motor performance, then it is unlikely that the shift in learning performance was due to decreased sensorimotor abilities, as the regression analysis did not reveal any systematic shift in performance over the 71-week period. Moreover, Gage and co-workers found that the age-associated deterioration of sensorimotor function did not influence the cognitive performance of rats in the Morris task (Gage, Dunnett & Björklund, 1984, 1989). We made similar observations in a longitudinal study with Wistar rats which started when the rats were about 25 months old and ended when the animals had reached the age of 30 months (van der Staay & Blokland,

1996b; Chapter 2.3). These findings suggest that aging is not a homogeneous process but can be differentiated on the basis of individual processes (Campbell, Krauter & Wallace, 1980; van der Staay, Blokland & Raaijmakers, 1990). It should be noted that the rats of the Wistar strain used in the present study are considered 'old' when they are the age of about 30 months, at which survival in the normal population is 50 percent or less (van der Staay & Blokland, 1996b). There may have been considerable differences between cohorts which went undetected, because the animals were generally tested at 24 months of age, that is, long before overt signs of age-related sensorimotor impairments and age-associated pathological changes occurred. Such processes, however, could have influenced rat's behavior in a subtle manner.

We did not perform pathological analyses at the end of the experiments, and consequently, the contribution of differences in health to the variability of the results cannot be estimated. Bronson (1990) suggests using both healthy and ill-appearing animals in aging studies and to separate the effects of aging from those induced by pathological changes, based on pathological analyses of all animals used in the study. Although this appears to be a good idea from the scientific point of view, it will be impractical in most cases. Instead, it might be more feasible to select rat strains which are relatively free of specific pathologies, or in which the incidence of age-specific pathologies can be considerably reduced.

Genetic drift, or changes in gene frequency due to the dispersive process

Although there was a high variability between experiments, the shift toward impaired performance across the 71 weeks was statistically reliable. The Wistar strain used was an outbred strain in which the genome is not fixed, as is the case in inbred strains and their F_1 crosses. The decrease in performance on the fifth acquisition session might therefore have been due to genetic drift.

The breeding nucleus used to maintain a line represents only a small sub-population of the original random bred population. In large populations the gene frequencies are inherently stable. By contrast, in small (sub)-populations changes in gene frequency due to random drift from generation to generation might occur in an erratic manner, without returning to that of the original larger population (Falconer & Mackay, 1996, pp. 48-64). Thus, if random drift was in effect, then it is conceivable that even the variability between experiments might have been caused by this process. In the (sub)-population of the Wistar strain used, the gene(s) which are responsible for the decrease in learning performance might, in the long run, have increased. Consequently, across experiments, there was a change toward poorer performance.

Alternative approaches

In those situations where it is mandatory to rely on highly reproducible and generalizable samples, one might use samples obtained from a heterogeneous stock or from a 'mosaic population' (van Zutphen, 1993). A heterogeneous stock consists of the F_1 hybrids from crossings between a selected number of inbred strains. In a 'mosaic population', inbred animals are also included. Because these stocks are based on inbred strains, they can be reconstructed whenever needed, and as long as the inbred strains involved are available (van Zutphen, 1993).

Reproducibility of samples from heterogeneous stocks or from 'mosaic populations' can be attained by assigning F_1 hybrids (and inbreds) to a sample in a predefined, fixed proportion. The inbred strains and the hybrids can be selected to provide samples which cover a wide genotypic and phenotypic range. The generalizability of results might profit from a broad phenotypic range. Festing (1993), however, states that the phenotypic variability in heterogeneous stocks is usually lower than expected, and that

where the variation is too large, the probability of false positive or false negative results might increase. Instead, he suggested using samples of animals from different inbred strains.

The problem of replicability and generalizability is of special relevance if one wishes to establish a standard against which new results can be evaluated. Outbred rat strains in these cases might not provide the model of choice (Festing, 1993; 1999). An outbred Wistar strain was used in all experiments of the present study. In general, the use of outbred strains increases the generalizability of results, provided the strains are maintained under a breeding schedule that slows down, or prevents, inbreeding. At the same time, it reduces the reproducibility and predictability of results, due to the heterogeneity of genotypes, and consequently phenotypes within the strain. It should be noted, however, that genotypic differences do not always show up as phenotypic differences (Festing, 1993). In contrast, when using inbred rats, or F₁ hybrids from crosses between inbred strains, the generalizability of results might be lower, whereas the reproducibility and predictability increase (Russell, 1972; McClearn & Hofer, 1999). This is a consequence of the reproducibility of individuals within specific inbred strains, and of F₁ hybrids from crosses between inbreds.

Conclusion

These data support the notion that well-defined animal strains should be used to improve the reproducibility of results and to minimize the probability of genetic drift, which might strongly affect the replicability of results. The replicability and generalizability of results could be increased by using samples from a heterogeneous stock or from a 'mosaic population'. To our knowledge, however, no information is available on the feasibility of such alternative approaches, especially when it concerns the availability of animals for use in aging research and gerontopharmacology.

2.5

Age-related changes in learning and memory in rats, assessed with the Morris water escape task: discussion and conclusions

The aging rodent is an established animal model of human aging. Although the proportion of aged people in the population is steadily increasing (Martin, 1991; Olshansky, Carnes & Cassel, 1993; Holden, 1996; Butler, 1997), the processes underlying aging are still poorly understood. In the present study, we addressed a number of questions concerning the Morris water escape task (Morris, 1984) as a tool to assess age-related changes in cognitive functioning in rodents. This task is one of the test paradigms that has consistently been found to be sensitive to age-associated decline in spatial orientation performance (Gage, Dunnett & Björklund, 1984; van der Staay & de Jonge, 1993; Blokland, Honig & Raaijmakers, 1994; Socci, Sanberg & Arendash, 1995), although the severity of the deficits differs considerably between studies and might be only transient (e.g. Rapp, Rosenberg & Gallagher, 1987). The standard Morris water escape task is believed to rely predominantly on spatial reference memory (RM).

The first question concerned differences in the performance of young or adult rats, and aged conspecifics of different strains in the standard Morris water escape task. In a series of three experiments, described in Chapter 2.1, we compared the spatial discrimination performance of adult and aged outbred Janvier Wistar (WISRJ) rats, young and old inbred Fischer 344 (F344), and hybrid Fischer 344*Brown Norway (FBNF1) rats. The aged rats of the WISRJ and FBNF1 strains had a poorer acquisition of the platform escape behavior, and a weaker bias for the previous platform position in the probe trial than their younger counterparts. The aged rats of the Fischer 344 strain were unable to acquire the task.

The second question addressed in more detail the age at which clear performance deficits in the Morris water escape task become apparent. In the first two experiments of Chapter 2.2, we assessed the acquisition of the standard Morris water escape task by 2-, 5-, 12-, and 19-month-old Winkelmann Wistar rats (WISW). We extended this assessment of age-related differences in the acquisition of this task by WISW rats by comparing the performance of 3-, and 24-month old animals in the second experiment of Chapter 2.2. These experiments revealed that, in this strain, clear age-related impairments in the acquisition of the task appear between 19 and 24 months of age.

The third question addressed whether the age-related differences in the RM performance of WISW rats extend to spatial working memory (WM). In the third experiment of Chapter 2.2, we used 3-month-old and 24-month-old WISW rats to assess the effects of age on WM in the repeated acquisition modification of the Morris task. In this task, which was originally designed by Whishaw (1985, 1987), one of the four start positions in the pool was chosen at random for each of the four trial pairs that constituted a session. The escape platform was in a different position on each daily session. The decrease in escape latency and distance swum to reach the escape platform from the first to the second trial within a trial pair was considered as a measure of spatial WM.

The young rats acquired the task within the first sessions. In contrast, the 24-month-old animals did not acquire the task, even after 12 daily training sessions. It was not clear, however, whether this poor performance of the old rats on the repeated acquisition task reflected impaired WM or whether they did not acquire the procedural aspects of the task. Earlier results obtained with the standard Morris task, however, showed that aged WISW rats can acquire the escape response, although they never reached the performance level of their young counterparts.

Most studies on aging are cross-sectional, i.e. animals of different ages are tested in parallel in order to assess the effects of aging on, for example, cognitive performance. However, when comparing age groups in cross-sectional designs, one is also comparing different cohorts and, consequently, groups of animals with a different history (Barnes, 1990) and even with different survival characteristics (Mos & Hollander, 1987). A more elegant approach would be to study the process of aging in longitudinal experiments. Such studies have shown that spatial discrimination performance is sometimes preserved in aged animals, if they acquired the task at a younger age (e.g. Beatty, Bierley & Boyd, 1985). Therefore, we addressed the question whether a longitudinal approach can be used, with the standard (RM) Morris task, to assess the effects of aging *in old rats* up to the age at which about 50% of the individuals of the rat strain used die a natural death (Chapter 2.3). Two experiments were performed in which WISW rats acquired the Morris water escape task for the first time at the age of 25 months and then retention performance was tested approximately 3 and 5 months later.

The performance of the aged animals was not only preserved, but actually improved in the retentions. The clearest improvement was seen about 3 months after the original acquisition, and the effect was more pronounced in the first than in the second experiment. A factor that could be responsible for the differences between experiments is genetic drift in the rat strain used, as discussed in Chapter 2.4. Although age-related deficits in spatial learning and memory performance in naive rats are consistently found in cross-sectional studies using the Morris water escape task (experiments 1, 2, and 3 in Chapter 2.1, experiments 1, and 2 in Chapter 2.2), this task appears to be unsuited for the evaluation of age-associated deficits of spatial memory performance in old Wistar rats (up to an age of 30 months) in longitudinal studies.

Because there appeared to be an undesirably large variations in the performance of WISW rats between experiments, we compared the results of 36 experiments which had been performed with aged WISW rats over a period of 71 weeks in order to determine whether the age-associated impairments in the Morris water escape task are replicable. These experiments had been performed at our laboratory under strictly controlled conditions. Results showed there to be a very high variability in the learning curves between experiments. The initial performance level, i.e. the performance during the first session, did not change systematically across the 36 experiments. In contrast, the final performance level, i.e. the level reached in the fifth training session, decreased over the 71-week period, when the platform escape latency and the distance swum to reach the platform, measured as number of line crossings, were considered. In fact, in the last experiments of the series, it was not possible to establish learning curves: the rats did not improve their performance across the acquisition sessions.

By contrast, the swimming speed and, in the probe trial, the bias for the quadrant where the platform had been positioned during training, did not change. This indicates that *spatial orientation learning* decreased across experiments, whereas the *motor performance* appeared to be unchanged. The most obvious explanation for these differences between experiments is that the cohorts (shipments of rats) were different.

Mos and Hollander (1987) found a wide variation in the survival characteristics of the inbred WAG/Rij and Brown Norway (BN/BiRij) strains. In their 5-year study they observed short- and long-living cohorts, but there was no consistent trend. The failure to find a trend is consistent with the fact that the WAG and the BN strains are *inbred*. By contrast, our regression analyses support the notion that genetic drift had occurred in the *outbred* WISW rat strain, as reflected by the shift in performance of the aged rats in the Morris water escape task.

Factors which might affect the measures used to evaluate spatial RM (and WM) in the Morris task(s)

Ability to swim (neurological changes in swimming behavior)

Age affects the ability of rats to move (Marshall and Berrios, 1979; Gage, Dunnett & Björklund, 1984, 1989; Gallagher & Burwell, 1989), and the response speed is reduced in old rats (e.g. van der Staay, Blokland & Raaijmakers, 1990; van der Staay, van Nies & Raaijmakers, 1990). In experiments 2 and 3 of Chapter 2.1, we found that the aged FBNF1 hybrids and the aged inbred F344 rats swam slower than their young counterparts when searching for the submerged escape platform. A reduced swimming speed might be the consequence of age-related, neurological impairments, which need not *per se* be correlated with the decline in cognitive function. For example, deterioration of sensorimotor functions was found not to influence the cognitive performance in the Morris task (Gage, Dunnett & Björklund, 1984, 1989; Gallagher & Burwell, 1989; Bickford et al., 1992; van der Staay & Blokland, 1996b; see also Chapter 2.3). These observations suggest that aging is not a homogeneous process (van der Staay, Blokland & Raaijmakers, 1990; Blokland & Raaijmakers, 1993a), but that the time course of age-related changes is different for distinct behaviors and their underlying processes. Nevertheless, it makes sense to assess the swimming behavior of aged rats that are going to be tested in the Morris task in order to be able to estimate the putative contribution of age-related *neurological* changes to the decline in measures which are believed to reflect spatial learning and memory. The procedure described by Marshall and Berrios (1979; see also Chapter 3.3) can be used for this purpose.

The role of age-related impaired thermoregulation

Old rats have a lower body temperature than younger conspecifics and thermoregulation is impaired: aged rats are unable to maintain normothermia after a short exposure to water at room temperature (21°C; Lindner & Gribkoff, 1991). Lindner and Gribkoff (1991) then investigated the role of the loss of thermoregulatory control on the spatial performance of aged rats in the standard Morris task. They found that warming 23-month-old rats in a warm water bath (38°C) before exposure to the Morris tank improved the performance of some rats, but not that of others. It is conceivable that the magnitude of 'old age' hypothermia is different for different rat strains. As we did not measure body temperature in our experiments, we cannot determine the contribution of possible hypothermia to the age-related differences in performance.

Visual impairments

Old rats might suffer from a reduced visual acuity which could conceivably interfere with successful acquisition of spatial learning tasks, such as the Morris water escape task. Recently, Lindner and colleagues (1997) reported that blind rats are able to acquire the spatial version of the standard Morris task, i.e. the version with a submerged platform. Even more surprising, blind rats were also able to acquire the cued version with great efficiency: their performance could not be distinguished from that of rats with normal vision. In an earlier study, Lindner and Gribkoff (1991) showed that visual acuity in 16- to 18-month-old F344 rats was not correlated with their poor spatial learning in the Morris task. This

result, however, does not exclude the possibility that such a relation emerges later in life, when visual acuity might be compromised more severely. It is not yet clear what implications these findings have for the Morris water task as such and for the age differences we found. Perhaps, blind rats and learning-impaired rats compensate for their deficits by adopting non-visual strategies, for example by adopting egocentric cognitive maps (Moghaddam & Bures, 1996). In the case of the blind rats the strategies adopted appear to be highly efficient.

Strategies to find the platform

In food-search tasks, win-shift strategies appear to predominate over win-stay strategies (e.g. Olton & Schlosberg, 1978). Once a rat takes the food from a particular location, it is highly unlikely that the same location will contain food if the time between two successive visits is too short. By contrast, in tasks where an escape route must be found, a win-stay strategy might be more appropriate. In its natural habitat it is important for survival that an animal learns the shortest, and consequently, fastest route to escape from an aversive situation, such as an open area, as under laboratory conditions is provided by, for example, the Barnes circular maze, or the water in the Morris task. This might be one of the reasons why the Morris water escape task is acquired rapidly by young rats.

However, aged rats and rats treated with 'amnesic' compounds, such as scopolamine, had an impaired ability to acquire the Morris task. One explanation might be that they do not switch to an active exploration strategy and instead spend more time hugging the wall (Puumala et al., 1996; Yau, Morris & Seckl, 1994). As a consequence, they do not negotiate the center of the pool as frequently as young or non-scopolamine-treated rats do. The measure *distance to platform* (Gallagher, Burwell & Burchinal, 1993) was greater for old rats in the three experiments of Chapter 2.1, which supports the notion that aged rats continued to swim at a larger distance from the platform, i.e. near the edge of the pool. As Yau and colleagues (1994) state, it remains to be determined whether this difference between young and aged rats reflects cognitive impairments or whether other factors play a role.

Role of acquiring the procedural demands of the task for the WM modification of the Morris task

As mentioned above, aged rats do not acquire the procedural demands of the standard Morris task as efficiently as young rats do, yet this is considered a pre-requisite for a good performance in the WM version of the Morris task. Unfortunately, from the data available (Chapter 2.2) we cannot determine how much the poor RM performance of old rats contributed to their very poor WM performance. The aged rats were able to reduce the escape latencies and the distance swum to reach the platform across sessions, which suggests that they learned the demands of the task, i.e. to escape onto the platform, albeit less efficiently than their young counterparts. It remains to be determined whether the measures for spatial WM and RM, as operationalized in the Morris task, are independent from one another, as has previously been shown, for example, for the spatial holeboard discrimination task (van der Staay, van Nies & Raaijmakers, 1990).

Experimenter-induced variability

The scoring method used, e.g. manual scoring versus automatic scoring using a video tracking system such as EthoVision[®] might affect the 'resolution' and the replicability of the results obtained. For example, in the experiments of Chapter 2.1 we used a video tracking system, whereas in the other experiments of Chapter 2 we scored performance manually. It is conceivable that manual scoring introduced experimenter-related variation. We addressed this problem in a series of studies and analyses (results not shown). When performance was scored manually and with EthoVision[®] (Noldus Information Technology b.v., Wageningen, The Netherlands; Spruijt, Hol & Rousseau, 1992; Sams-

Dodd, 1995), we obtained very similar data. The data collected manually were strongly correlated with the corresponding measures obtained by using EthoVision® ($r_{PM} > 0.90$ for all measures in all sessions; unpublished results). Therefore, manual scoring by an experienced experimenter can be considered as a reliable tool for the assessment of rats' behavior in the Morris task. We consider it highly unlikely that the manual scoring method introduced variation in the results which could explain the differences observed between age-groups or the differences between successive experiments.

However, since video tracking systems operate automatically, any experimenter-related bias is effectively eliminated. In addition, more parameters (e.g., swim path, average distance to platform) can be obtained with video tracking systems such as EthoVision®, which thus allow a more detailed analysis of the behavior of rodents in the Morris task. For example, response strategies such as swimming at a fixed distance from the edge of the pool can be analyzed better with a video tracking system, because the position of the rat in the pool (X-Y-coordinates) is available for (re)-analysis.

The Morris water escape task to assess age-associated cognitive decline in longitudinal studies.

Ingram (1985) emphasized the potential value of longitudinal analyses in animal models of aging. Unfortunately, there have been only a few longitudinal studies on complex learning and memory in animal research (e.g. Beatty, Bierley & Boyd, 1985; Bierley et al., 1986). Our data show that the standard Morris task is not suited to assess the aging of spatial learning and memory in a longitudinal design. Rats tested repeatedly over a long period of their life do not show the age-related changes which are normally found in cross-sectional studies.

Thus, although the Morris water escape task is one of the most frequently used test paradigms in aging research since Gage and colleagues reported a clear age-related performance deficit in this task (Gage, Dunnett & Björklund, 1984), some aspects of the task still have not been investigated in depth, and some results are inconclusive. For example, it is very important to understand the role of strategies, vision and extra-maze cues in orientation, and age-associated non-cognitive changes, such as sensorimotor impairments, when interpreting of age-related decreases in Morris water escape performance. For this reason, it makes sense to register behavior as precisely as possible, preferably using a video tracking system, so that different alternative hypotheses for the impairments found can be tested (e.g. wall hugging strategies: Puumala et al., 1996; egocentric strategies: Moghaddam & Bures, 1996). Although the Morris water escape task can be modified to assess either spatial WM or RM, it is still not clear, whether these two components of spatial memory really measure something different.

The standard Morris task appears to be unsuited to investigate age-associated changes in spatial learning and memory in longitudinal designs. It remains to be determined how reproducible age-related impairments in the Morris task are. This aspect is addressed in more detail in Chapter 5.

Given that currently available animal models often lack any true analogy to the human disease state they are supposed to model (Gamzu, 1985), a situation that may change with the development of transgenic rodent strains, the model of the aged rodent may be useful for evaluating the potential of pharmacological interventions in the aged (Mohs, 1988). Small rodents, especially, possess a number of advantages: they have a relatively short life span of 2 to 3 years, their environment can be strictly controlled, and they show clear age-related impairments of learning and memory (e.g. Elias & Elias, 1976; Gage, Dunnett & Björklund, 1984; Rapp, Rosenberg & Gallagher, 1987; Aggleton, Blindt & Candy, 1989; Gallagher & Burwell, 1989; van der Staay & de Jonge, 1993; Ingram, 1988; Raaijmakers, Blokland & van der Staay, 1993). The short life span provides the opportunity to test the possible

prophylactic or therapeutic effects of drugs on the aging process in a short period of time (Mervis, 1981).

However, when selecting a specific rat strain one should be aware of the performance of adult rats and performance under normal conditions (Jucker & Ingram, 1997). If the performance in a certain learning task is poor during adulthood, the window for age effects is small or absent. This clearly holds true for shock-motivated tests with BN and F344*BN rats, two genotypes which are frequently used in aging research. They perform very poorly in shock-motivated tasks at all ages (van der Staay & Blokand, 1996a). As a consequence, it may be necessary to select more than one strain for gerontological and gerontopharmacological studies and to use particular strains only in those tasks in which they have shown an age-related decline.

Some investigators, for example Weindruch and Masoro (1994), are concerned about the overuse of one genotype, the F344 rat, in aging research. Instead, they recommend that every effort should be made to “(...) *maximize the separation of the study of aging from that of diseases and to do so in a variety of animal models*” (p. B88). In this context, Bronson (1990) suggests that both healthy and ill-appearing animals should be used in order to separate the effects of aging from those induced by pathological changes, based on pathological analysis of all animals used. However, it might be more feasible to select rat strains which are relatively free from specific pathologies or in which the incidence of age-specific pathologies can considerably be reduced. This, for example, appears to be the case in F344 rats which, when fed with standard casein based rat chow, show a high prevalence of severe nephropathy. A soy protein-based diet, eventually in combination with caloric restriction, dramatically reduces the occurrence of this pathology in F344 rats (Shimokawa et al., 1993).

Research on the processes and the consequences of normal aging, using animal models, has already contributed significantly to our understanding of human aging. Many factors contribute to the learning and memory performance seen in the aged animal. The heterogeneity of cognitive strategies, behavioral habits, and pathology-related effects, however, complicates the interpretation of age-related changes (van der Staay, Blokland & Raaijmakers, 1990). For this reason the tasks used to assess these changes must permit detailed analysis of the rat behavior. The Morris water escape task with its different modifications appears to fulfill this requirement.