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Specific Behavioral Effects Related to Age and Cerebral Ischemia in Rats

MAIBRITT B. ANDERSEN,* JENS ZIMMER† AND FRANK SAMS-DODD*

*Pharmacological Research, H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark
†Department of Anatomy and Cell Biology, Institute of Medical Biology, Odense University, Winsløwparken 19, DK-5000 Odense, Denmark

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ANDERSEN, M. B., J. ZIMMER AND F. SAMS-DODD. *Specific behavioral effects related to age and cerebral ischemia in rats.* PHARMACOL BIOCHEM BEHAV **62**(4) 673–682, 1999.—Rats at 4, 14, and 20 months of age were subjected to permanent occlusion of the left middle cerebral artery (MCAO) and the effects of age and ischemia assessed in tests for spatial learning (Morris' water maze), social behavior, olfactory learning, exploratory behavior, and motor function. Furthermore, the extent of ischemic damage to the brain of rats of 5 and 19 months of age was studied. An age-related decline in water-maze performance was observed, and aged rats were less agile, less explorative, and less frequently engaged in social interactions than young rats. After ischemia, mild memory impairment was observed in old rats, while changes in some exploratory behaviors were observed in young rats. Neuropathological analyses revealed a variable and limited degree of infarction in the piriform cortex and the insular cortex with no difference between age groups. In conclusion, the present study confirmed and extended current data on behavioral differences between young and old rats. MCAO had limited influence on the tested behaviors. © 1999 Elsevier Science Inc.

MCAO Focal ischemia Age Behavior Learning Morris' water maze Social interaction

THROMBOEMBOLIC stroke and vascular dementia (VD) are more frequent in elderly than in young people. Nonetheless, most animal models used in ischemia research are based on the neuropathological, neurological, or behavioral outcome of experimental cerebral ischemia in young adult rodents. Aged animals differ from young animals in a number of ways—in physiology, neurochemistry, and behavior. In rats, an age-related decline has been demonstrated in regional cerebral blood flow (18) and glucose utilization (4). Receptor sensitivity and regulation (21,26,39) and transmitter levels (37) may be altered in aged rats, and a decline in brain plasticity (1,21) has been observed. Age-related changes in learning and memory have been demonstrated, for example, a decline in spatial memory (8,16) and working memory (3,28,29). Considering the vast number of observed differences between young and old subjects, animal models of vascular dementia based on cerebral ischemia in aged rats should be more relevant to the clinical situation than the currently used models based on young rats.

The purpose of the present study was to investigate age-related differences in specific rat behaviors and to compare the behavioral outcome of focal cerebral ischemia in rats of three age groups. Furthermore, the possible differences in the extent of ischemic brain damage was studied in the youngest and the oldest group.

Focal cerebral ischemia was produced in rats by left middle cerebral artery occlusion (MCAO), which is a well-documented experimental model of thromboembolic stroke in humans. The artery was occluded distal to the origin of the striatal branch to avoid major ischemia-induced neuropathology and behavioral deficits in young rats.

The rats were tested for ischemia-induced deficits in several models based on natural behaviors. Spatial memory was assessed in the Morris water maze. Social behavior was studied in the social interaction test. Explorative behavior was examined in a brightly lit and a dark area using the black and white box test, and olfactory recognition of distinct unfamiliar odors was assessed in the olfactory learning test. The rats

Requests for reprints should be addressed to Dr. Maibritt B. Andersen, Novo Nordisk A/S, Novo Nordisk Park, Maaloev, DK-2760, Denmark.

were further tested for any major impairment in motor function on a vertical lattice. These behaviors were considered to be relevant to the clinical manifestations of vascular dementia where memory deficits, a reduction in social activities, and reduced interest in the outside world are frequently observed.

METHOD

Animals

The experimental procedures carried out in this study were in compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

Male Wistar rats (Iffa Credo breeding center, France) in three age groups, 4–5 months, 14 months, and 19–20 months, were used in these experiments. The rats were housed in groups of three in Macrolon type III (4 to 5 months old) or Macrolon type IV cages (14 to 20 months old) at standard housing conditions (temperature: 21 ± 2°C, relative humidity: 55 ± 5%, air exchange: 16 times/h, and 12 h light/dark cycle: lights on at 0700 h). The rats were either bought shortly before start of the experiments (youngest group) and maintained on ad lib food (RM1 pellets, Special Diets Services, Cambridgeshire, UK) and water, or they were bought 1 year before the experiments and maintained on a restricted diet (140 g RM1 pellets per cage three times a week) and ad lib water.

Occlusion of the Middle Cerebral Artery

The rats were anesthetized with 4% halothane in 30% oxygen and 70% nitrous oxide. During the operation the halothane concentration was reduced to 2.5 ± 0.5%. To avoid hypothermia, body temperature was maintained at 37.5 ± 0.5°C with a heating pad connected to a rectal thermometer (CMA/150, CMA/Microdialysis, Sweden). The left side of the head was shaved, and a vertical skin incision was made behind the left eye. By microscopical guidance the skull was exposed by cautiously parting the temporal muscle, and a hole drilled in the skull by a dental drill (Fig. 1A). The middle cerebral artery was located and the dura mater removed in a small area. This procedure was identical in all animals.

For the occlusion of the left middle cerebral artery a small segment of the artery was electrocauterized in a position between the olfactory tract and the rhinal sulcus with a pair of current-carrying forceps (Fig. 1B). The artery was subsequently cut in the center of the cauterized area to prevent reestablishment of the blood flow. In sham-operated rats the artery was exposed, but not cauterized.

A small sterile sponge (Spongostan Dental, Ferrosan, Denmark) was placed over the occlusion site, and the temporal muscle sutured with 4-0 vicryl suture. A 4-0 silk suture was used for the skin. All animals were injected SC with 0.03 ml/100 g b.w.t Spiramycin (H. Lundbeck A/S, Valby, Denmark) to prevent infections. The sutured wounds were treated with a local anesthetic (Xylocain Adrenalin, Astra, Sweden) and sprayed with sterile liquid plaster. The animals were kept under 100-W infrared light bulbs for a few hours after the operation to prevent a postoperative decrease in body temperature.

Motor Function Test

For the motor function test the rat was placed on a vertical lattice facing upwards. It was noted whether the rat climbed up or down or fell off. Climbing up was defined as all four paws moved and forepaws on top of the lattice. Climbing down was defined as turning the body and climbing or jumping down on the table.

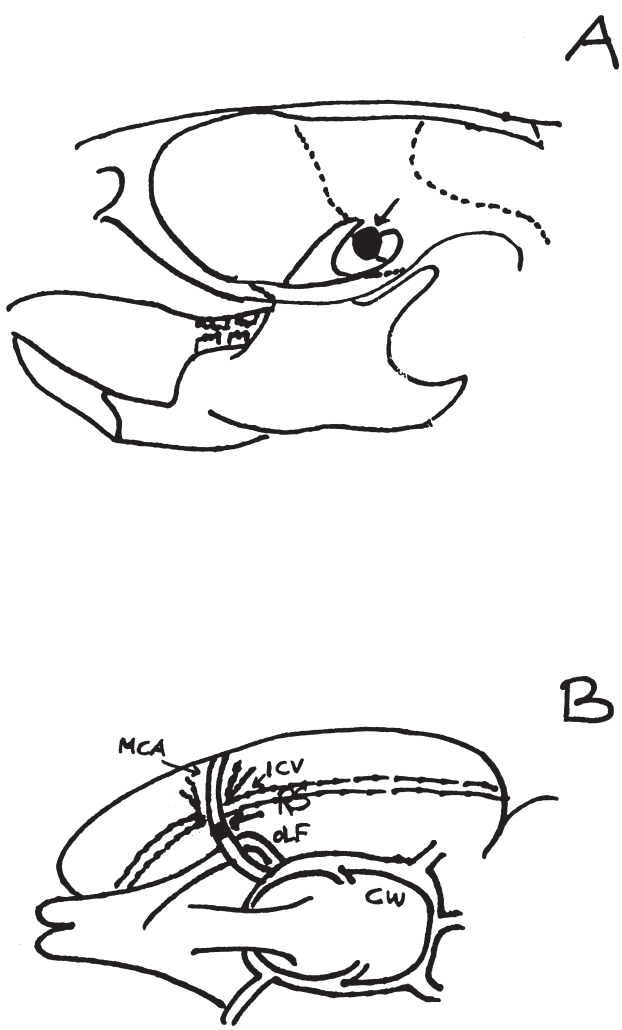


FIG. 1. (A) Left side of rat skull. The black spot shows the location of the drilled hole in the temporal bone anterior to the zygomatic process. (B) Rat brain seen from the ventral side. The middle cerebral artery (MCA) was cauterized in a position between the olfactory tract (OLF) and the rhinal sulcus/inferior cerebral vein (RS/ICV). CW, circle of Willis. A was adapted from Popesko et al. (30). B was adapted from Bederson et al. (5).

Morris Water Maze

The Morris water maze is a well-documented test for spatial learning deficits. The general design of the water maze test was adapted from Morris (24,25), and the present design was adapted from Skarsfeldt (34). The water maze consisted of a circular pool made of white perspex (diameter = 1.00 m, height = 0.50 m) filled with opacified water (temperature = 18°C, depth = 35 cm). A circular escape platform (diameter = 8 cm) was placed in the maze with its top surface submerged 5 cm below the water surface. The platform was placed in a fixed position in the northwest quadrant of the pool throughout the experiment. A video camera was placed over the maze and connected to a tape recorder that recorded the rats'

movements. The signal was transmitted to an image analyzer (HVS VP112). All rats were dyed on top of the head with black hair color (Polycolor 890, Henkel Cosmetic, Germany) at least 3 days before start of the water maze test to facilitate discrimination from the background. In the test room were extra maze cues in permanent positions.

The rats achieved three successive training trials per day for 5 consecutive days. On the first training day, the test rat was placed on the platform for 15 s before start of the training session. Then it was placed in the water facing the pool wall in one of three fixed starting positions (west in the first trial). The rat immediately started swimming, and if it found the platform it would climb onto this to escape from the water. It was then allowed to rest on the platform for 15 s before the next trial was initiated. Thereafter, it was placed in the water in the next starting position (north). This procedure was repeated for the third starting position (east). If the rat did not find the platform within the 60-s trial, it was gently placed on the platform and stated as a nonfinder in that trial.

The following parameters were recorded: 1) escape latency: latency (s) to find and climb onto the hidden platform; 2) wall factor: % time spent within 5 cm of the pool edge; 3) path length: swim distance (cm) before end of the trial (platform found or 60 s passed); and 4) swimming speed: mean swimming speed (cm/s).

Escape latency and path length were used to measure spatial memory whereas swimming speed was used to evaluate motor function.

Normally, when the rat was placed in the water for the first time it would swim along the walls of the maze. During subsequent trials it would gradually develop a search strategy, first crossing back and forth, then narrowing the search area, and finally swim directly to the platform. The wall factor was thus believed to reflect the rat's search strategy.

Social Interaction Test

The general design of the social interaction test was adapted from File (12), with the present design developed by Sams-Dodd (31). The test was performed in three open arenas (l,w,h: 150 × 100 × 40 cm) made of clear Perspex covered with a black, nonreflecting material. The bottoms were covered with gray gravel. To maintain a constant odor level in the arenas the gravel had been preexposed to other rats, and was not changed between tests.

The test rat and an unfamiliar young control rat were placed in the unfamiliar arena for 10 min. The behavior of the rats was recorded by video cameras (Cohou Model 4722-2000 with Ernitec 6 mm/1.2 lens) mounted above the arenas and connected to video recorders (JVC Model HR-5000SH). Lighting in the room consisted only of a dim red light that was diffuse to minimize shadows in the arena. There was no visual contact between the arenas. The video tapes were analyzed off line by a trained observer using the software program The Observer 3.0® (Noldus Information Technology, Holland), and the rater was unaware of age and treatment of the rats. The total duration of the following social and nonsocial parameters was analyzed for the test rats: 1) investigation (social): sniffing part of the control rats body; 2) rearings (nonsocial): raised on hind legs, sniffing in the air; 3) exploration (nonsocial): moving around, sniffing at bedding, walls, and in the air; and 4) inactive (nonsocial): sitting inactively.

Other behavioral parameters were rated as well (e.g., some additional social parameters, aggressive behaviors, and grooming) but because these were only rarely exhibited or varied much between individuals, they were not included in the analysis.

Black and White Box Test

The general design of the black and white box test was adapted from Colpaert et al. (9), with its present design developed by Sánchez (32,33).

The test was performed in brightly lit white Perspex arenas (l,w,h: 80 × 65 × 33 cm). The floors were covered with a thin layer of wood shavings. Each arena was connected to a closed black box (l,w,h: 39 × 25 × 22 cm) by an opening (10 ×10 cm) in the center of one of the short side walls. A row of 18 light sources and photo cells in the longitudinal direction (5.5 cm above the cage floor) detected horizontal locomotor activity. The photocells were connected to a computer, and the test system was fully automated. Four arenas were run in parallel. There was no visual contact between the arenas.

The test rat was placed in the center of the arena and its movements were registered for 10 min. For the analysis the floor of the arena was divided into nine squares. The total time spent in the brightly lit arena, and the number of line crossings in the arena and in the black box were recorded.

Olfactory Learning Test

The olfactory learning test is based on the natural exploratory behavior of rats. The general design was adapted from Hunter and Murray (19). In three consecutive trials the test rat is allowed to investigate the odor of a dried herb. The odor has no biological significance to the rat, and it is assumed that the time spent investigating the odor will decrease over the three trials if the odor is recognized by the rat. In a fourth trial a distinctly different odor is presented. As this is new to the rat, it is assumed that the time spent by odor investigation will increase in the fourth trial compared to the third trial.

The test was performed in clean Macrolon type IV housing cages (59 × 38 × 20 cm) with a bedding of wood shavings. In one end wall there was a circular hole. Cylindrical metal containers (diameter = 3 cm) with one end covered with a wire mesh could be fitted into the holes. The rats could smell the herb in the container through the wire mesh but were prevented from licking and sniffing inside the container.

Before start of the test the rat was placed in the test cage for 15 min. Then the test, consisting of five 2-min trials with intertrial intervals of 20 min, was initiated. The first trial was a habituation trial with an empty container fitted into the hole. In the remaining four trials there was a dried herb in the container (mint or thyme). The same herb was presented in the first three trials and replaced by the other in the fourth trial. The duration of investigation of the container (nose within 1 cm of the container) was registered manually over a period of 2 min in the four test trials. In the intertrial intervals the rats were placed in their home cages. The test was conducted in red light to minimize aversive stimuli.

Histology

The rats were deeply anesthetized with sodium pentobarbital and perfused transcardially with 4% paraformaldehyde in 0.15 M Sørensen Buffer (pH 7.3). The fixed brains were then vibratome sectioned at 50 μm in the frontal plane. One series of sections was stained by toluidine blue, which stains nucleic acids in the nucleus and the cytoplasm in shades of blue. In a second series astroglial cells were visualized by immunocytochemical staining for intermediate filament protein

glial fibrillary acidic protein (GFAP) (6,7,14,20), which is present in normal and reactive astroglial cells (6,13). A third series of sections were stained immunohistochemically using the OX42 antibody against the microglial complement type 3 receptor (CR3) (13,20), which is present in both normal and activated microglial cells, though upregulated in the activated cells (13).

Statistics

The results in the motor function test were analyzed using the χ^2 test.

The general linear models procedure (SAS®, SAS Institute Inc., Cary, NC) was used to analyze ranked data for wall factor, path length, and swimming speed in the Morris water-maze test. Mean values for the 15 trials were used to analyze effects of age and ischemia. Furthermore, effects of ischemia were analyzed separately by age groups. Least-square means were used to analyze contrasts between the age groups. The escape latency data were expressed as percent nonfinders (i.e., the percentage of trials that lasted >60 s) in each age and treatment group and were analyzed for each age group using the χ^2 test.

Duration or frequency of the behavioral parameters in the social interaction test and the black and white box test were analyzed using the ANOVA test with two between subjects factors (age × ischemia) on ranked data. Newman–Keul's post hoc test was used to compare mean values between the age groups.

In the olfactory learning test the duration of odor investigation was analyzed using the ANOVA test on ranked data with two between subjects factors (age × ischemia) and one within subjects factor (trial, repeated measures). The four trials were compared within each age and treatment group using the Wilcoxon's signed ranks test for matched pairs. For all analyses, except of water maze data, the software package Crunch 4.0® (Crunch Software® Corporation, USA) was used.

Comparison of horizontal extension of ischemic brain lesion in Experiment 2 was made using the nonparametric Kruskall–Wallis test.

In all tests the accepted level for statistical significance was $p < 0.05$.

Experimental Series

Experiment 1. Four, 14, and 20 months old rats were used with 12–14 rats per group. Three days after MCAO, the motor abilities of the rats were examined. The olfactory learning test was performed 3 weeks after surgery, the social interaction test (manual analysis) 3.5 weeks, the Morris water maze 4 weeks, and the black and white box test 5 weeks after surgery.

Experiment 2. Ten rats at 5 and nine rats at 19 months of age were used. Seven days after surgery the rats were per-

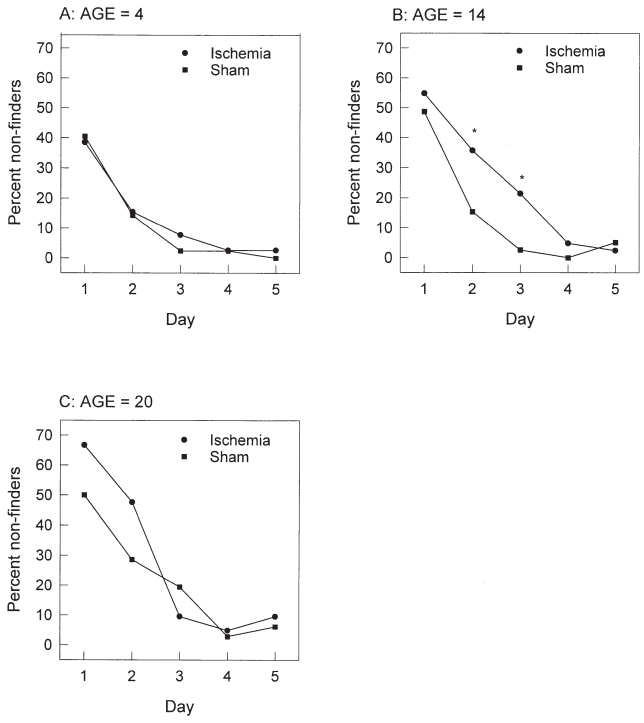


FIG. 2. Effect of permanent occlusion of the left middle cerebral artery on spatial navigation in the Morris water maze on rats in three age groups. Percent nonfinders, i.e., the percentage of trials where the animals did not find the hidden platform within 60 s, is shown. The rats were tested for 5 successive days, 4 weeks after MCAO or a sham operation. Ischaemia vs. sham: $*p < 0.05$, χ^2. Group sizes were 12–14.

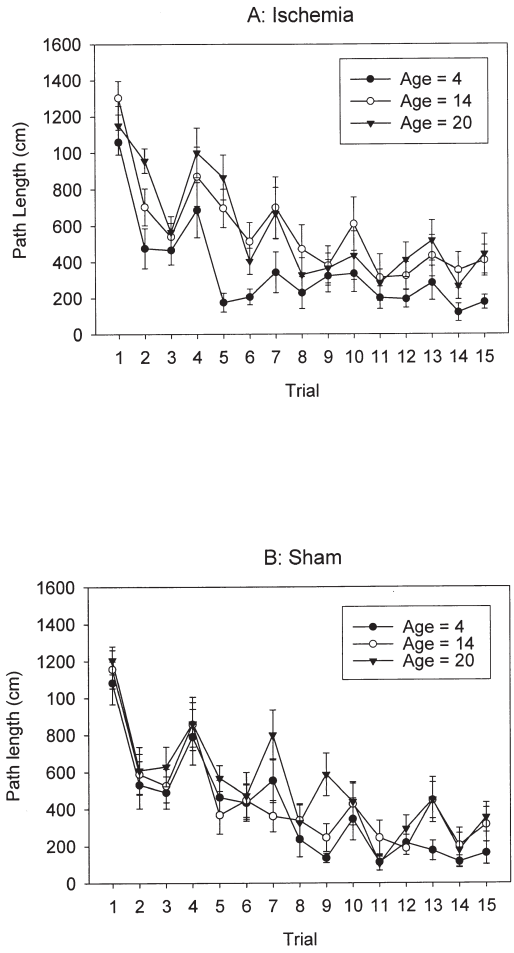


FIG. 3. Effect of MCAO in rats in three age groups on the swimming distance (path length) (mean + SEM) until platform was found or trial ended (60 s) in the Morris water maze. The rats were tested three times a day for 5 successive days, 4 weeks after permanent MCAO or a sham operation. Group sizes were 12–14.

fused and the brains histologically processed as described. Lesion size was rated in the toluidine stained sections by counting the number of sections with ischemic damage.

RESULTS

Experiment 1

In general, the ability to climb the lattice in the motor function test was not affected by the MCAO. There was an effect of age, however, which was significant only in the MCAO group. In the MCAO group more rats in the 20-month group compared to the younger rats fell off when placed on the vertical lattice (41.7% in the 20-month groups, compared to 7.7 and 16.7%, respectively, in the 4 and 14 months groups, $\chi^2 = 7.24$, $p < 0.05$). A similar, but nonsignificant difference between age groups was observed in the sham group.

In the Morris water maze the age of the rats did not effect the latency to find the hidden platform, expressed as percentage of nonfinders. In the 4-month group there was no difference between ischemia and sham groups (Fig. 2A). The 14-month-old rats performed equally on the first training day, but the improvement over training days was slower in the ischemia group than in the sham group (Fig. 2B). On day 2 and day 3, the differences were significant [day 2: $\chi^2(1) = 4.04$, $p < 0.05$; day 3: $\chi^2(1) = 4.58$, $p < 0.05$]. In the 20-month group there were more nonfinders in the ischemia group than in the sham-operated group on the first two training days, but the difference did not reach statistical significance.

The swimming distance before the platform was found, expressed by the path length, increased with age [Fig. 3A–C, $F(2, 76) = 14.67$, $p < 0.0001$] and in both of the old age groups (sham and MCAO) the rats swam longer than the rats in the youngest group before the platform was found ($p < 0.001$ for both). There were no overall effects of age or ischemia on the search strategy expressed by the wall factor (data not shown), or on the swimming speed (data not shown). Contrast analyses nonetheless revealed that for both parameters the 20-month group differed significantly from the 4-month group ($p < 0.05$ for both). Thus, the old rats swam slower and closer to the side walls than the young rats. Separate analyses for effects of ischemia revealed no significant differences in path length, wall factor, or swimming speed in any age group, and there were no interactions between age and ischemia.

When tested in the social interaction test 3.5 weeks after MCAO (Fig. 4), the age of the rats affected most of the recorded parameters. An age-dependent decrease in the duration of the social parameter investigation was seen [Fig. 4A, $F(2, 71) = 8.51$, $p < 0.001$] and both of the older age groups differed from the 4-month group (4 vs. 14: $p < 0.01$; 4 vs. 20: $p < 0.001$). The frequency of rearings (Fig. 4B) decreased with age, $F(2, 71) = 15.11$, $p < 0.0001$, with the 20- month groups differing from the two younger age groups (4 vs. 20: $p < 0.0001$; 14 vs. 20: $p < 0.001$). An ischemia-induced increase in

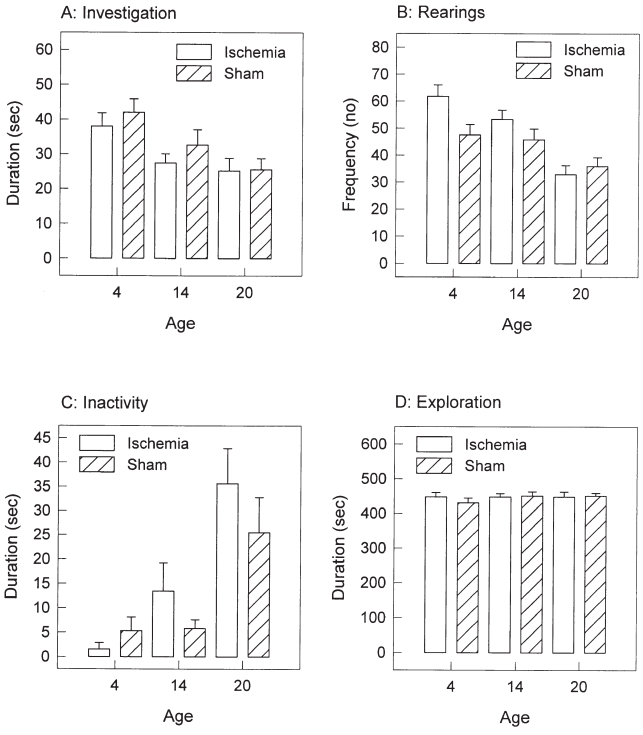


FIG. 4. Effect of MCAO in rats in three age groups on their behavior in the social interaction test. Duration (mean + SEM) of the behavioral parameters is shown. The rats were tested against untreated juvenile control rats 3.5 weeks after permanent MCAO. Group sizes were 12–14.

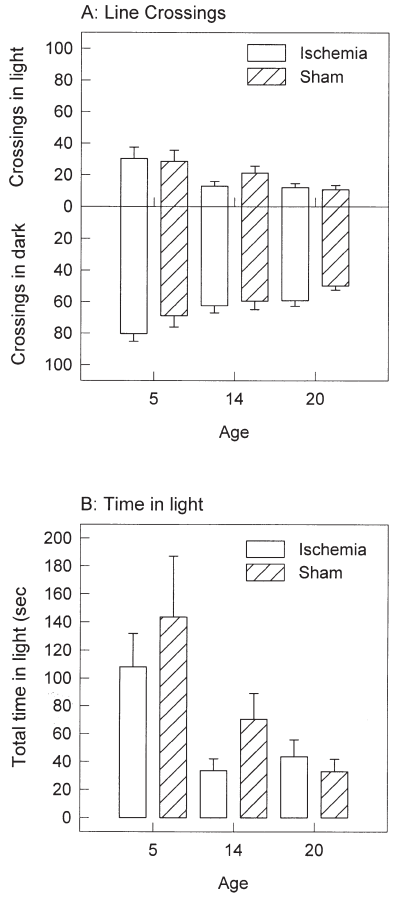


FIG. 5. Effect of MCAO in rats in three age groups on their behavior in the black and white box test. (A) Mean number (+ SEM) of line crossings in the brightly lit arena (upper part) and the dark box (lower part). (B) Total time spent in the brightly lit arena. The rats were tested for 10 min, 5 weeks after MCAO or a sham operation. Group sizes were 12–14.

the frequency of rearings was seen in the 4-month group ($p < 0.05$). This was the only effect of ischemia observed in the social interaction test. The duration of inactivity (Fig. 4C) increased gradually with age, $F(2, 71) = 20.41$, $p < 0.0001$, and the duration of exploration (Fig. 4D), which was by far the most commonly displayed parameter, did not vary between groups.

Figure 5 A summarizes the data for line crossings in the black and white box test showing an age-dependent decrease in the number of line crossings in both areas [white: $F(2, 73) = 3.43$, $p < 0.05$; black: $F(2, 73) = 10.39$, $p < 0.0001$] as well as an ischemia-induced increase in crossings in the black box, $F(1, 73) = 4.43$, $p < 0.05$. No interaction between age and ischemia was found in either area. As shown in Fig. 5B the total time spent in the brightly lit arena decreased with age, $F(2, 73) = 7.53$, $p < 0.01$.

In the olfactory learning test (Fig. 6A–C) an overall reduction in odor investigation was seen after ischemia, $F(1, 72) = 4.04$, $p < 0.05$, and with age, $F(2, 72) = 21.24$, $p < 0.0001$. The shape of the learning curve was preserved in all groups, with a significant decrease in odor investigation in trials 2 and 3 compared to trial 1, followed by a significant increase in trial 4 compared to trial 3. However, an interaction between trial and age, $F(6, 216) = 3.15$, $p < 0.01$, suggested that the difference between the four trials were smaller in the middle-aged and old-age groups than in the youngest group.

Experiment 2

The MCAO-induced infarcts were generally rather small and confined to the piriform cortex and the insular cortex (Fig. 7A and B and Fig. 8) within close range of the occlusion site, identifiable by a small local lesion. Two rats in the 5-month group and one rat in the 19-month group had infarcts extending into the parietal cortex as well (Fig. 7A, bottom, Fig. 8). Two rats in the 19-month group and one rat in the 5-month group had no visible ischemic lesion in the brain. No significant difference between age groups was found in horizontal extension of the lesions, $H(1) = 2.06$, $p = 0.15$. Location of reactive astrocytes (GFAP) and microglial cells (OX42) corresponded with the location of the infarcts as revealed by toluidine blue (for an example, see Fig. 7B).

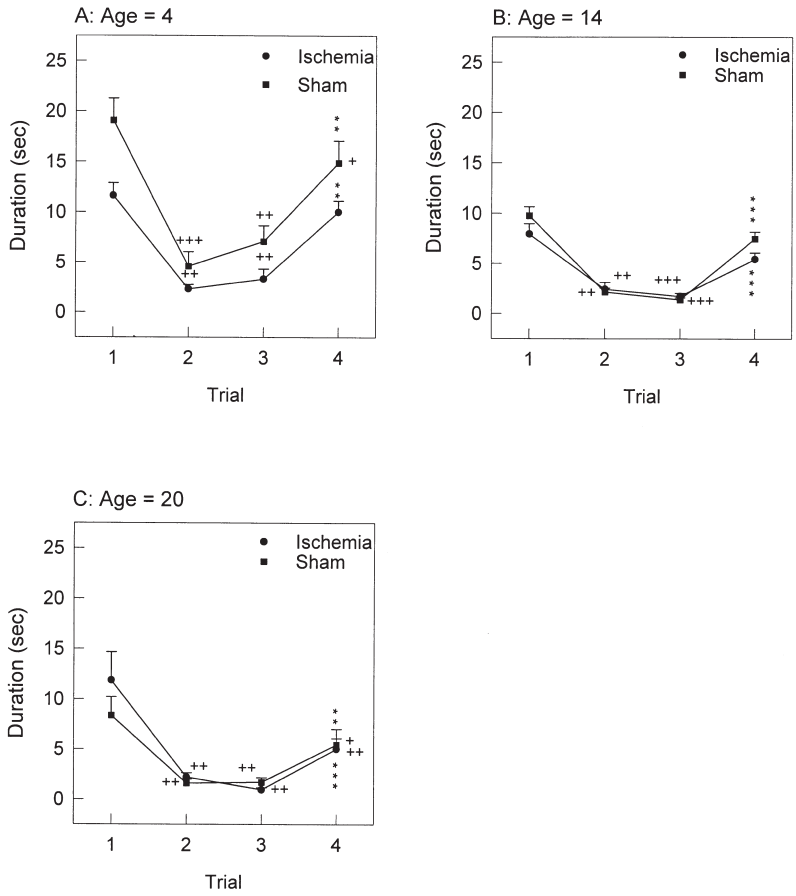
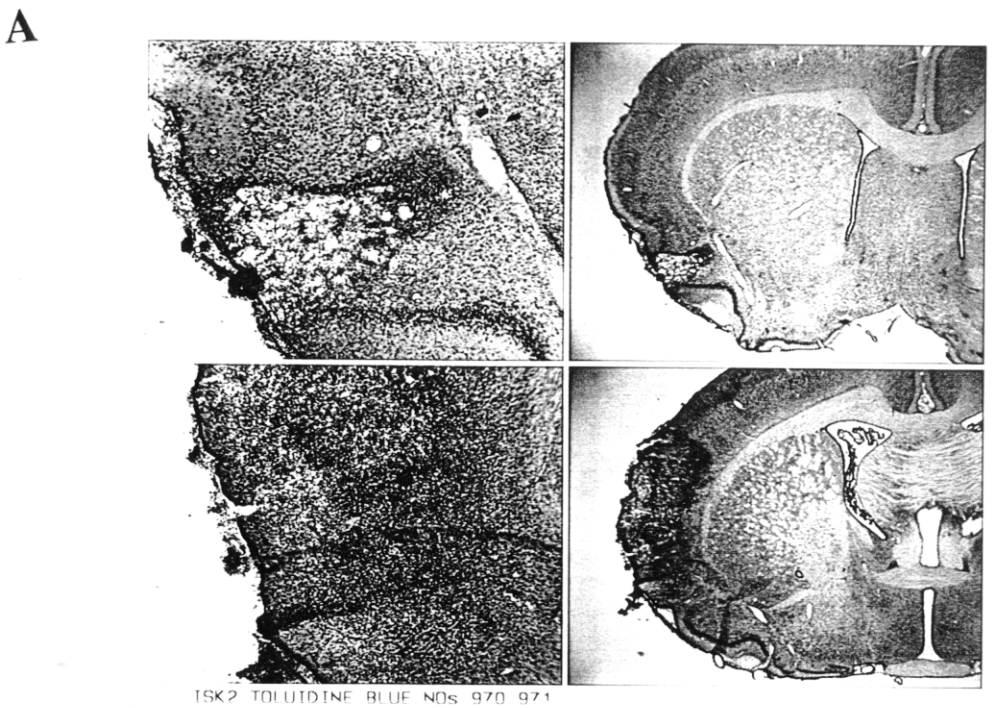


FIG. 6. Effect of MCAO in rats in three age groups on duration (mean + SEM) of odor investigation in the olfactory learning test. The rats were tested 3 weeks after permanent MCAO or a sham operation. The same odorant was presented in the initial three 2-min trials and was replaced by a different in trial 4. (A) 4 months; (B) 14 months; (C) 20 months. Stars represent significant difference of trial 4 from trial 3 and crosses represent significant differences from trial 1 in the Wilcoxon's signed-ranks test for matched pairs. $+p < 0.05$, **/++ $p < 0.01$, ***/+++ $p < 0.001$. Group sizes were 12–14.



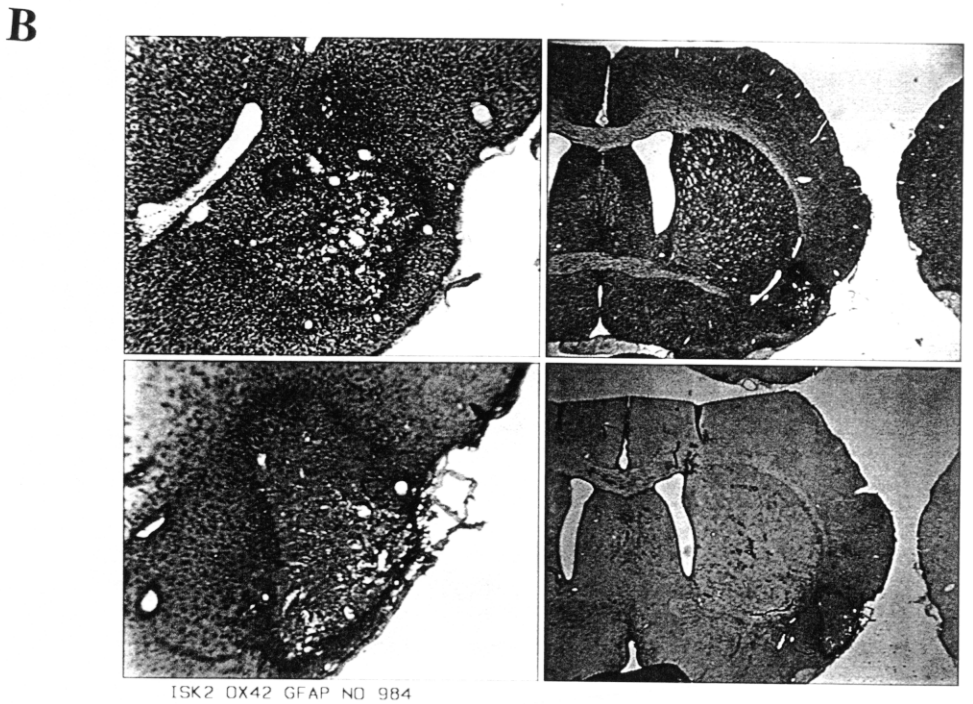


FIG. 7. Photomicrographs of ischemic lesions in the brains of rats subjected to MCAO. (A) Toluidine blue, the micrographs in top panel and bottom panel are from two different rats (Nos. 7 and 8, 5 months old). The left panel is a magnification of the right panel. (B) Microglial and astroglial cells visualized by OX42 (top panel) and GFAP (bottom panel) immunocytochemistry. The four micrographs are from the same rat (No. 18, 19 months old). Magnifications: left: 22.5×, right: 5.6×.

DISCUSSION

Age

Age-dependent changes were observed in several behavioral parameters. The Morris water maze test revealed a reduction in spatial learning expressed as an increase in path length and wall factor with increasing age. Furthermore, old rats swam slower than young rats. An age-dependent reduction in the frequency of rearings was observed in the social interaction test. Old rats were more inactive than young rats and the duration of social behavior decreased with age. The duration of social investigation in low light is a parameters commonly used to identify anxiogenic effects of various treatments. At first our results suggest an increase in anxiety with age. However, the decrease in social investigation corresponded with the increase in inactivity and decrease in rearings. This decrease in activity probably accounted for the lower level of social investigation in old rats. In the black and white box test old rats were likewise less active and spent less time in the brightly lit arena than young rats. A decrease in time spent in the arena would be seen if old rats were more anxious than young rats, but held together with the general decrease in activity and exploration found in several tests, we do not find that any conclusions should be drawn concerning anxiety. Also, in the olfactory learning test a decrease in exploration with age was found in that the level of odor investigation was lower in old rats than in young rats. Finally, more rats in the oldest age groups fell off the lattice in the motor function test. This may have been due partly to a decrease in motor function and partly to an age-dependent increase in mean body weight [Kruskall–Wallis: $H(2) = 60.11$, $p < 0.0001$]. In summary, an age-related decline in spatial learning was observed and generally, aged rats were less agile and less active and explorative than young rats and they did not engage as frequently in social interactions with other rats.

Ischemia

In a pilot study, middle-aged (13-month) as well as old (19-month) ischemic rats had a significantly slower learning curve compared to sham-operated rats when tested in the water maze. These results could not be fully replicated in the present study where the MCAO effect was significant only in the 14-month group. To further examine age differences in sensitivity to ischemia it would be of relevance to test young and aged rats subjected to MCAO in a maze with a large diameter (2 m). This size is the standard choice in many laboratories [e.g. (35)], and the present test in the small maze (1 m) seemed fairly easy for the rats. On the fourth training day there were at most 10% nonfinders in all groups. As it is considerably more difficult for the rats to navigate in a large maze, deficits in spatial navigation would be more easily revealed here.

In the social interaction test the frequency of rearings was increased in young, but not old ischemic rats compared to sham-operated rats. A nonsignificant increase in horizontal activity (exploration) was observed in the young ischemia group as well. Furthermore, in the black and white box test ischemia increased the number of crossings in the black box. It has been shown that global ischemia increases the level of locomotor activity in gerbils and this has been related to hippocampal neurone loss (2,17,23,27). The MCAO does not cause neuron loss in the hippocampus, but neuronal damage in other brain areas may produce a comparable effect. The increase in rearings was seen in the young group and partly in the middle-aged group. Thus, the activity of the oldest rats was not affected by ischemia. The relatively large body weight of these rats combined with an age-related decline in motor abilities may have prevented a possible ischemia-induced stimulation of rearing activity in this group.

In the olfactory learning test, odor investigation was decreased following ischemia in the youngest age group. The

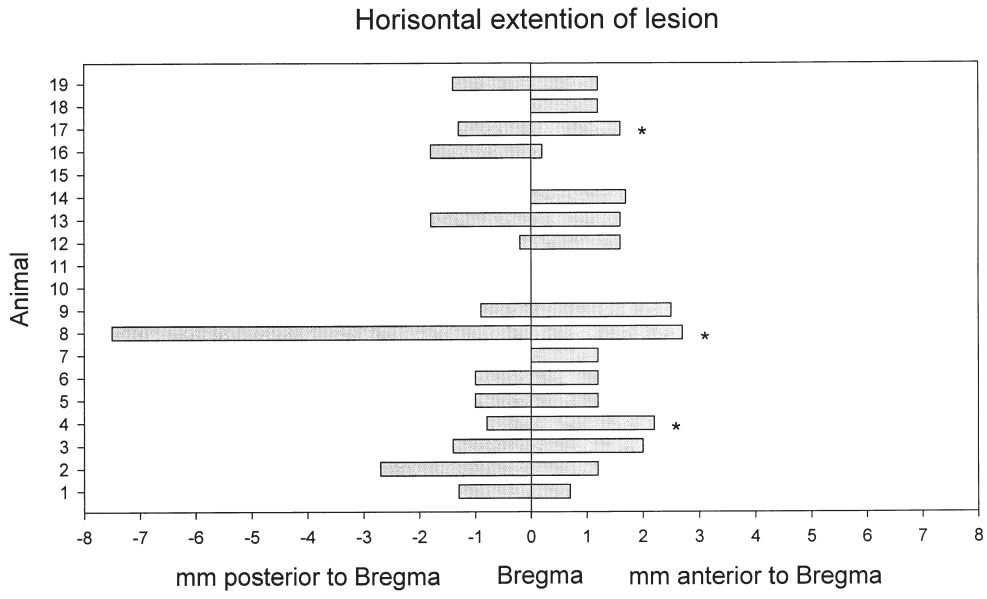


FIG. 8. One-dimensional horizontal extension of ischemic lesions in the insular and piriform cortices in brains of rats subjected to MCAO. The lesions were rated in frontal brain sections stained by toluidine blue. Asterisks represent rats with lesions extending into the parietal cortex. Animal numbers 1 to 10 were 5 months of age, numbers 11 to 19 were 19 months of age.

level was accordingly the same as in the older age groups. This may reflect an ischemia-induced decrease in olfactory sensitivity. The location of the occlusion was close to the lateral olfactory tract, which may have been partly damaged in some rats and further, infarctions were invariably found in the piriform cortex, which is a primary structure in the olfactory system. It has been shown that lesions in piriform cortex interferes with olfactory learning (36). Another possibility is that the MCAO-induced ischemia interacted with motivation. This would only be apparent in young rats because the basal duration of odor investigation was rather low in old rats.

In summary, apart from the water maze, the effects of MCAO were mainly found in the youngest age group. As discussed, these effects mainly concerned the locomotor activity of the rats and various physiological factors in the old rats may explain why these were not affected.

Histology

No significant differences between age groups were found concerning the size of the infarcts. Although the neuropathological differences were negligible or nonexisting, the susceptibility of the central nervous system may be altered in old rats. It has been shown that brain plasticity is reduced in old rats (1,21), and the ability for behavioral compensation for moderate neurone loss may be reduced even in middle-aged rats.

The lesions were variable and quite small in most animals. This was due partly to the location of the occlusion and partly to the choice of rat strain. By making the occlusion distal to the striatal branch of the middle cerebral artery, major motor deficits due to striatal infarction was avoided. It has been shown by others that MCAO in a distal position does not produce motor deficits in young rats [e.g., (10)]. However, at least in the Wistar strain that we used, the ischemic damage turned out to be too small to have any major impact on the behavior in any age group. The distal branching pattern of the middle cerebral artery is rather variable. Fox et al. (15) recorded six distinct patterns in Sprague–Dawley rats that may produce a variable histological outcome and photographs taken of the brains from the present study (Experiment 1—data not shown) revealed a varying branching pattern in the Wistar rats as well. To reduce variation future studies may be conducted using proximal occlusion or a rat strain more sensitive to ischemic damage to the brain. Duverger and MacKenzie (11) compared the occlusion-induced infarct size after focal ischemia in five rat strains and found that there was considerable variation in the size and distribution of infarcts in Wistar Kyoto rats. Larger and less variable infarcts were observed in Sprague–Dawley rats and especially in Fischer-344 rats. They also compared the infarct size in young and old Fischer-344 rats (3, 9, and 20 months). The infarct size did not differ between age groups. They did not test the behavior of the animals, so no data on differences in behavioral sensitivity to the occlusion was obtained. The most promising strategy for our purpose—to compare the behavioral sensitivity of young and old rats to focal ischemia—may be to use a distal occlusion in a more sensitive rat strain, for example, Fischer-344. Deficits in water maze learning has been shown to correlate with massive infarcts in young rats after a proximal occlusion of the MCA (22,38). Using a distal occlusion, major neuropathology and behavioral deficits will be avoided in young rats, and potential differences between age groups will be more easily revealed.

In summary, the present experiments confirmed and extended current data on behavioral differences between young and old rats. We observed an age-related decline in spatial learning, and aged rats were less agile and less active and explorative than young rats and did not engage as frequently in social interactions with other rats. The data from the water maze suggested that old rats may be more sensitive than young rats to memory disrupting effects of MCAO lesions. The question of age-related differences in behavioral sensitivity to ischemia is important, and has a strong clinical relevance. More experiments will be needed with careful selection of rat strain, ischemia technique, and behavioral tests.

REFERENCES

1. Agnati, L. F.; Benfenati, F.; Solfrini, V.; Biagini, G.; Fuxe, K.; Guidolin, D.; Carani, C.; Zini, I.: Brain aging and neuronal plasticity. Ann. NY Acad. Sci. 673:180–186; 1992.
2. Andersen, M. B.; Zimmer, J.; Sams-Dodd, F.: Post-ischaemic hyperactivity in the Mongolian gerbil correlates with loss of hippocampal neurons. Behav. Neurosci. 111:1–12; 1997.
3. Ando, S.; Ohashi, Y.: Longitudinal study on age-related changes of working and reference memory in the rat. Neurosci. Lett. 128:17–20; 1991.
4. Bassant, M. H.; Jazat, F. R.; Lamour, Y. A.: Age-related alterations in local cerebral glucose utilisation in the Sprague–Dawley rat. Neurodegeneration 3:243–250; 1994.
5. Bederson, J. H.; Pitts, L. H.; Tsuji, M., Nishimura, M. C.; Davis, R. L.: Rat middle cerebral artery occlusion: Evaluation of the model and development of a neurologic examination. Stroke 17:472–476; 1986.
6. Bignami, A.; Dahl, D.: Astrocyte-specific protein and neuroglial differentiation. An immunoflourescence study with antibodies to the glial fibrillary acidic protein. J. Comp. Neurol. 153:27–37; 1974.
7. Castellano, B.; Gonzáles, B.; Jensen, M. B.; Pedersen, E. B.; Finsen, B. R.; Zimmer, J.: A double staining technique for simultaneous demonstration of astrocytes and microglia in brain sections and astroglial cell cultures. J. Histochem. Cytochem. 39:561–568; 1991.
8. Cavoy, A.; Delacour, J.: Spatial but not object recognition is impaired by aging in rats. Physiol. Behav. 53:527–530; 1993.
9. Colpaert, F. C.; Meert, T. F.; Niemegeers, C. J. E.; Janssen, P. A. J.: Behavioural and 5-HT antagonist effects of ritanserin: A pure and selective antagonist of LSD discrimination in rat. Psychopharmacology (Berlin) 86:45–54; 1985.
10. Coyle, P.: Middle cerebral artery occlusion in the young rat. Stroke 13:855–859; 1982.
11. Duverger, D.; MacKenzie, E.: The quantification of cerebral infarction following focal ischaemia in the rat: Influence of strain, arterial pressure, blood glucose concentration, and age. J. Cereb. Blood Flow Metab. 8:449–461; 1988.
12. File, S. E.: The use of social interaction as a method for detecting anxiolytic activity of chlordiazepoxide-like drugs. J. Neurosci. Methods 2:219–238; 1980.
13. Finsen, B.: Neuro-glial-immune interactions. An experimental, neural transplant and lesion study. Copenhagen: Lægeforeningens forlag; 1995.
14. Finsen, B.; Sørensen, T.; Castellano, B.; Pedersen, E. B.; Zimmer, J.: Leukocyte infiltration and glial reactions in xenografts of mouse brain tissue undergoing rejection in the adult rat brain. A light and electron microscopical immunocytochemical study. J. Neuroimmunol. 32:159–183; 1991.
15. Fox, G.; Gallacher, D.; Shevde, S.; Loftus, J.; Swayne, G.: Anatomic variation of the middle cerebral artery in the Sprague–Dawley rat. Stroke 24:2087–2093; 1993.
16. Frick, K. M.; Baxter, M. G.; Markowska, A. L.; Olton, D. S.;

Price, D. L.: Age-related spatial and working memory deficits assessed in the water maze. Neurobiol. Aging 16:149–160; 1995.
17. Gerhardt, S. C.; Boast, C. A.: Motor activity changes following cerebral ischaemia in gerbils are correlated with the degree of neuronal degeneration in hippocampus. Behav. Neurosci. 102: 301–303; 1988.
18. Goldman, H.; Berman, R. F.; Gershon, S.; Murphy, S. L.; Altman, H. J.: Correlation of behavioural and cerebrovascular function in the aging rat. Neurobiol. Aging 8:409–416; 1987.
19. Hunter, A. J.; Murray, T. K.: Cholinergic mechanisms in a simple test of olfactory learning in the rat. Psychopharmacology (Berlin) 99:270–275; 1989.
20. Jørgensen, M. B.; Finsen, B. R.; Jensen, M. B.; Castellano, B.; Diemer, N. H.; Zimmer, J.: Microglial and astroglial reactions to ischaemic and kainic acid-induced lesions of the adult rat hippocampus. Exp. Neurol. 120:70–88; 1993.
21. Lynch, G.: Animal models for the study of age-related changes in brain and behavioural plasticity. Aging 19:201–206; 1982.
22. Markgraf, C. G.; Green, E. J.; Hurwitz, B. E.; Morikawa, E.; Dietrich, W. D.; McCabe, P. M.; Ginsberg, M. D.; Schneiderman, N.: Sensorimotor and cognitive consequences of middle cerebral artery occlusion in rats. Brain Res. 575:238–246; 1992.
23. Mileson, B. E.; Schwartz, R. D.: The use of locomotor activity as a behavioural screen for neuronal damage following transient forebrain ischaemia in gerbils. Neurosci. Lett. 128:71–76; 1991.
24. Morris, R.: Spatial localization does not require the presence of local cues. Learn. Motiv. 12:239–260; 1981.
25. Morris, R.: Development of a water maze procedure for studying spatial learning in rats. J. Neurosci. Methods 11:47–60; 1984.
26. Nisticò, G.; De Sarro, G. B.; Bagetta, G.; Mollace, V.: Altered sensitivity of a_2-adrenoceptors in the brain during aging in rats. Ann. NY Acad. Sci. 673:206–213; 1992.
27. Nurse, S.; Corbett, D.: Direct measurement of brain temperature during and after intraischaemic hypothermia: Correlation with behavioural, physiological, and histological endpoints. J. Neurosci. 14:7726–7734; 1994.
28. Ohta, H.; Ni, X.-H.; Matsumoto, K.; Watanabe, H.: Working memory deficit in aged rats in delayed nonmatching to position task and effect of physostigmine on performance in young and aged rats. Jpn. J. Pharmacol. 56:303–309; 1991.
29. Ordy, J. M.; Thomes, G. J.; Volpe, B. T.; Dunlap, W. P.; Colombo, P. M.: An animal model of human-type memory loss based on aging, lesion, forebrain ischaemia, and drug studies with the rat. Neurobiol. Aging 9:667–683; 1988.
30. Popesko, P.; Rajtová, V.; Horák, J.: A colour atlas of anatomy of small laboratory animals, vol. 2: Rat, mouse, hamster. England: Wolfe Publishing Ltd; 1992.
31. Sams-Dodd, F.: Distinct effects of *d*-amphetamine and phencyclidine on the social behaviour of rats. Behav. Pharmacol. 6:55–65; 1995.
32. Sánchez, C.; Arnt, J.; Costall, B.; Domeney, A. M.; Kelly, E.; Naylor, R. J.: Sertindole: A limbic selective neuroleptic with potent anxiolytic effects. Drug Dev. Res. 34:19–29; 1995.
33. Sánchez, C.: 5-HT_{1A} receptors play an important role in modulation of behaviour of rats in a two-compartment black and white box. Behav. Pharmacol. 7/8:788–797; 1996.
34. Skarsfeldt, T.: Differential effect of antipsychotics on place navigation of rats in the Morris water maze. A comparative study between novel and reference antipsychotics. Psychopharmacology (Berlin) 124:126–133; 1996.
35. Smith, S. E.; Hodges, H.; Sowinski, P.; Man, C. M.; Leach, M. J.; Sinden, J. D.; Gray, J. A.; Meldrum, B. S.: Long-term beneficial-effects of bw619c89 on neurological deficits, cognitive deficits and brain-damage after middle cerebral-artery occlusion in the rat. Neuroscience 77:1123–1135; 1997.
36. Staubli, U.; Schottler, F.; Nejat-Bina, D.: Role of dorsomedial thalamic nucleus and piriform cortex in processing olfactory information. Behav. Brain Res. 25:117–129; 1987.
37. Tanila, H.; Taira, H.; Piepponen, T. P.; Honkanen, A.: Effect of sex and age on brain monoamines and spatial learning in rats. Neurobiol. Aging 15:733–741; 1994.
38. Yonemori, F.; Yamada, H.; Yamaguchi, T.; Uemura, A.; Tamura, A.: Spatial memory disturbances after focal cerebral-ischaemia in rats. J. Cereb. Blood Flow Metab. 16:973–980; 1996.
39. Zaia, A.; Viticchi, C.; Piantinelli, L.: Differential regulation of brain b-adrenoceptor subpopulations in aging rodents. Ann. NY Acad. Sci. 673:201–205; 1992.