Attention and memory in aged rats: Impact of lifelong environmental enrichment

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Abstract

Aged rodents exhibit memory and attention dysfunctions. Environmental enrichment (EE) attenuates memory impairments. Whether it may reduce attention deficits is not known. At the age of 1 month, Long–Evans female rats were placed in standard or EE conditions and tested after 3 (young), 12 (middle-aged) or 24 (aged) months of differential housing. Spatial reference memory was assessed in a water-maze task. Attention performance was evaluated in the five-choice serial reaction time (5-CSRT) task. EE improved spatial memory at all ages, but did not ameliorate 5-CSRT performance in young and middle-aged rats; it prevented, however, the degradation of attention performances detected in aged rats. The number of ChAT (+30 to +64%) and p75NTR-positive (+35 to +44%) neurons was higher in the basal forebrain of aged enriched vs. standard rats, suggesting their EE-mediated protection. The weaker deficit of attention found in aged EE rats might be linked to a better survival in the very long term of neurons in the basalo-cortical system.

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1. Introduction

Aged rodents, as also well described in aged humans, exhibit alterations in cognitive functions, particularly when memory and attention are concerned (e.g., Gallagher and Burwell, 1989; Jones et al., 1995; Muir et al., 1999). Evidence suggests that this cognitive aging is the consequence of multiple alterations, rather than dysfunctions in (or of) a particular anatomically and/or neurochemically defined network or system. The discovery of the causes of such alterations in animals is one of the challenges that basic research in neurobiology of aging has dealt with for over more than half a century now. This research area also relied on the identification of environmental factors that might significantly contribute to the age-related decline in cognitive function. Among these factors, housing conditions might be of importance. Indeed, in the adult rodent, numerous studies have shown that, relative to standard laboratory housing conditions, exposure to an enriched environment, providing enhanced sensory, motor and cognitive stimulations as well as sustained social interactions, modified many aspects of behavior and improved learning and memory in a variety of hippocampal-dependent tasks such as object recognition (Bruel-Jungerman et al., 2005; Tang et al., 2001), contextual fear conditioning (Barbelivien et al., 2006; Duffy et al., 2001; Rampon et al., 2000; Tang et al., 2001) and spatial learning (Leggio et al., 2005; Schrijver et al., 2002; Williams et al., 2001). Even if the precise neurobiological bases of
the behavioral effects of an enriched environment are not fully understood, these effects may rely on the morphological, biochemical and functional changes induced by such environment in the central nervous system. For example, enriched environment increases dendritic branching, stimulates synaptogenesis (for reviews see, Mohammed et al., 2002; van Praag et al., 2000) and promotes the survival of newly formed neurons in the hippocampus (Kempermann et al., 1998; Nilsson et al., 1999). At the neurochemical level, environmental enrichment produces functional changes in several neurotransmitter systems and also increases levels of trophic factors in certain areas of the brain, especially in the hippocampus (for reviews see Mohammed et al., 2002; van Praag et al., 2000).

Although many studies have demonstrated that a short period (few weeks) of environmental enrichment is able to reduce some age-related learning and memory deficits (e.g., Bennett et al., 2006; Fernandez et al., 2004; Frick and Fernandez, 2003; Harburger et al., 2007), Kobayashi et al. (2002) showed that an exposure to an enriched environment over the lifespan can prevent the age-related decline of cognitive functions. Indeed, aged rats that were housed in enriched environment from the pre-weaning period until the age of 25 months performed better in the Hebb–Williams maze task than their counterparts housed in standard conditions (Kobayashi et al., 2002). Moreover, in contrast to middle age subjects (15 months), in which an enrichment either starting after weaning or covering the last 3 months before testing onset induced comparable effects, in aged subjects the beneficial effect of a short period of environmental enrichment was lower than that induced when the enrichment was started post-weaning (Kobayashi et al., 2002). Altogether, these data suggest that environmental enrichment may slow down or prevent the age-related decline of memory functions.

Aging does not only affect memory functions. Beside other deficits, aged rodents also show impaired visual attention (Jones et al., 1995; Muir et al., 1999; McGaughy and Sarter, 1995; Sarter and Turchi, 2002). Attentional performances are known to be preferentially sensitive to cholinergic alterations in the basalo-cortical system (e.g., Harati et al., 2008; Lehmann et al., 2003; McGaughy et al., 1996, 2002) and the decline of attention processes with age may be related, at least partially, to a neuronal loss/dysfunction in this system (Grottick et al., 2003; Jones et al., 1995). Although, environmental enrichment is known to impact the cholinergic system (Fordyce and Farrar, 1991; Park et al., 1992), there is no study having investigated if environmental enrichment could result in beneficial effects on age-related alterations of attentional processes.

The aim of this study was to determine if environmental enrichment during the whole post-weaning life could prevent aged-related alterations of memory and attentional functions. To this end, female rats were placed in either standard or enriched housing conditions at the age of 1 month and were tested in different behavioral tasks after 3, 12 or 24 months. Memory was evaluated in the Morris water maze, a hippocampal-dependent spatial reference memory task, which is sensitive to both age and enrichment (Bennett et al., 2006; Gallagher et al., 1993; Harburger et al., 2007). Subsequently, sustained visual attention was assessed in the five-choice serial reaction time task (5-CSRT; for review see Robbins, 2002). Locomotor activity was also monitored. Finally, aged rats which went through the 5-CSRT were euthanized for immunostaining using antibodies against choline acetyltransferase (ChAT), the low-affinity neurotrophin receptor (p75NTR), and parvalbumine (Parv) in the basal forebrain and the striatum.

2. Materials and methods

2.1. Subjects and housing conditions

Eighty-five female Long–Evans rats (Centre d’Elevage René Janvier, Le Genest-St-Isle, France) were used. They arrived at the age of 4 weeks in the laboratory and were randomly assigned to one of two different housing conditions. Non-enriched (standard) rats were housed in pairs in transparent Makrolon cages (46 cm × 26 cm × 15 cm). Enriched rats were housed in groups of 10–12 in two contiguous wire-mesh cages (112 cm × 40 cm × 40 cm) connected by two openings: various objects (tunnels, toys, chains, etc.) were placed in the cage and changed five times a week. Food and water were available ad libitum, but in the enriched condition, their location was changed when the objects were replaced. For both housing conditions, the cages were placed in a temperature (22 ± 1 °C)- and humidity (55 ± 5%)-controlled room under a 12–12 h light–dark cycle (lights on at 8:00 a.m.). After a period of differential housing lasting 3, 12 or 24 months, the rats were placed in individual transparent Makrolon cages (42 cm × 26 cm × 15 cm) for 1 week before the onset of behavioral testing.

Thus, the behavioral tests started when the young (Y) rats (13 enriched/12 standard) were 4 months old and had spent 3 months in the enriched/standard housing conditions, when the middle-aged (MA) rats (18 enriched/14 standard) were 13 months old and had spent 12 months in the enriched/standard housing conditions, and when the aged (AG) rats (16 enriched/12 standard) were 25 months old and had spent 24 months in the enriched/standard housing conditions.

2.2. Behavioral tests

The order of behavioral tests was not counterbalanced due to the fact that the duration of the three tests was very different (2 days for assessing locomotor activity, 6 days for testing water-maze performance, including the probe trial, but about 17 weeks for the 5-CSRT task). The study being mainly focused on age-related effects, priority was given to comparable ages at the moment of each test.

2.2.1. Locomotor activity in the home cage

One week after the end of differential housing, spontaneous locomotor activity of the rats was measured in their home cage. The cages were placed on shelves located in a dedicated room. Each cage was traversed by two infrared light beams targeted on two photocells, 4.5 cm above the floor level and 28 cm apart. The number of cage crossings (successive interruptions of light beams in 1-h bits) was recorded by a microcomputer over two consecutive days. For analysis, three periods were distinguished. For the first period, termed “habituation” hereafter, the scores were recorded over the first 3 h of the first testing day. During this period, the activity scores, which are influenced by some novelty due to unfamiliar room and cages, and novel sawdust (e.g., Galani et al., 2001), are usually larger than the baseline diurnal activity scores, which are reached after 2 or 3 h. In the second and third periods, termed “diurnal” and “nocturnal” hereafter, activity was recorded during the light phase (12 h) and the nocturnal phase (12 h) of the second day. All rats were then weight and gently handled for 1 min on each of the three following days.

2.2.2. Morris water-maze test

Place acquisition was tested in the Morris water maze. The Morris water maze consisted of a circular pool (diameter 160 cm, height 60 cm) filled with water to half the height. The water (20 °C) was made opaque with powdered milk. The pool was located in an experimental room with many extra-maze cues (e.g., chair, computer, desk, cages, lights, pictures on the wall, fan, etc.) and was virtually divided into four equal quadrants. A circular platform, 11 cm in diameter, was placed in the pool, 1 cm underneath the water surface. For each trial, the rat was placed in the pool, facing the wall, at a randomly designed starting point among eight possibilities from where it was released and given a maximum of 60 s to reach the submerged platform. When the rat had climbed onto the platform, it was left on it for 10 s, then removed, and passed to the next trial. When the rat failed to find the platform within 60 s, it was gently guided to it by the experimenter and was left there for 10 s. Each day, the rats were given four consecutive trials for which they were released from a different starting point in a randomized order. During five consecutive days, the platform was placed at the same location. This testing procedure is generally considered to provide a measure of acquisition (reflecting learning of a spatial reference memory task). The day after the last acquisition session, the platform was removed and all rats were given a probe trial for 30 s as previously done in aged rats (Cassel et al., 2005). This probe trial is considered to measure the strength of spatial learning. On the two following days, the rats were tested with a visible platform in order to evaluate possible age-related motivational or sensory-motor biases. On each day, the platform (Plexiglas colored black to enhance its visibility; 11 cm in diameter), which protruded 1 cm above the water surface, was placed in another location; four successive daily trials were conducted as previously described over two consecutive days (e.g., Cassel et al., 2007).

Using a video-tracking system (Noldus, The Netherlands), we recorded the latency and the distance to the platform, as well as the swim velocity and the thigmotaxis (defined as the time spent swimming in close contact with the pool borders, i.e., within an annulus of less than 10 cm from the pool wall).

For the acquisition phase, the following variables were analyzed: the distance swum to reach the platform corrected according to the method described by Lindner (1997), the percent thigmotaxis = [(time spent in the periphery/total latency to reach the platform) × 100], and the mean velocity. The latencies to reach the platform were recorded, but are not reported, because they are usually considered more sensitive than the distances to non-cognitive biases such as altered motivation or sensory-motor impairments (Lindner, 1997). In general, however, they yielded similar conclusions as those supported by analyses of the distances. For the probe trial, the following variables were collected: the time spent in the target quadrant (in which the platform was located during the acquisition phase) and the number of crossings of the annulus (which is a virtual 10-cm wide annulus surrounding the platform). For the visible platform task, distances swum to reach the platform were analyzed.

2.2.3. Five-choice serial reaction time task

After the Morris water-maze test, rats were subjected to the five-choice serial reaction time task. During this test requiring food restriction, the rats were weighted every day and, over 2 weeks before the start of testing, their body weight was progressively reduced to 85% of its free-feeding value. It was maintained at this level throughout the experiment by providing an individually adjusted amount of food after each daily testing session. Water was available ad libitum, except during testing.

2.2.3.1. Apparatus. Eight five-choice operant chambers (Bioseb, BP 89 92370 Chaville, France) placed in sound-attenuated and ventilated enclosures were used to test visual attention. Each chamber consisted of an alluminium enclosure (252 (W) × 280 (D) × 240 (H) mm) illuminated by a house-light located on the ceiling. The curved rear wall comprised nine contiguous 23 mm square holes (i.e., nose-poke holes), 14 mm deep, and 22 mm above the grid floor. Each hole was equipped with an infrared photocell beam to detect nose-pokes and could be illuminated for an adjustable duration and intensity by a lamp located at the hole’s
rear. The presentation of a light in one of the holes constituted the stimulus to which rats had to respond. Food pellets (45 mg, Bioserv) were delivered automatically into a magazine located at the opposite side of the chamber and equidistant from each hole. The rat collected the delivered food pellet by pushing a Perspex panel covering the magazine.

Each chamber was automatically controlled by Packwin software (Panlab S.P., Cornella, Barcelona, Spain) and data were collected via a microcomputer.

2.2.3.2. Training procedures. Rats were initially given access to food pellets in their home cage (10 pellets per day during 5 consecutive days) to familiarize them with the reinforcer. Each rat was always placed in the same chamber throughout the experiment. In a first training phase (one session), rats were placed in the chambers for 15 min with the house-light off; the panel of the magazine was blocked in order to maintain the food magazine open. This magazine was filled with 15 food pellets to familiarize rats to eat the reinforcer in the magazine. In a second phase, rats received two food magazine training sessions (20 min per session) in which 20 food pellets were delivered in the magazine according to a variable time schedule (mean = 60 s). The house-light was turned on during this phase. On the first session, the panel was blocked in order to maintain the food magazine open. For all other sessions, rats had to push away the panel in front of the food magazine to retrieve the food pellet. During these two phases, each hole was blocked by a metal cover. In a third phase, the central hole (hole 5) was accessible and illuminated for the whole duration of a 30-min session. Each time a rat introduced its nose (nose-poke) into the illuminated hole, a food pellet was delivered in the magazine. This training was continued until the rats made at least 50 nose-pokes during the session.

Then, rats were trained to respond to a brief visual stimulus presented randomly in one of the five spatial locations (holes 1, 3, 5, 7, 9), as previously described (Barbelivien et al., 2001; Harati et al., 2008). At the beginning of each test session, the house-light was turned on and a single food pellet was delivered to the magazine without requiring a nose-poke. The first trial was initiated when the rat had pushed the panel to collect this pellet. After a fixed delay (inter-trial interval, ITI = 5 s), one hole was illuminated for a given stimulus duration (see below). A response into the illuminated hole or a response in that particular hole during a fixed period of time after the stimulus offset (the limited hole hold period = 5 s) was rewarded with the delivery of a food pellet; in that case, a correct response was recorded. Additional responses in any hole after a correct response were recorded as perseverative responses. The next trial was initiated when the rat pushed the panel to collect the food pellet. Responses in a non-illuminated hole during the signal period (i.e., the stimulus duration + the limited hole hold period; incorrect response) and failures to respond within the limited hole hold period (omission) were punished with a period of darkness (time-out = 5 s). Any nose-poke made during the time-out period restarted the time-out for a further 5-s period. Responses in the holes during the ITI were recorded as premature responses and were followed by a time-out period. After a time-out period, the next trial was initiated when the rat pushed the panel giving access to the (empty) magazine. A daily session consisted of 100 trials or was terminated after 30 min of testing. During a 100-trial session, the visual stimulus was presented an equal number of times in each of the holes, in a random order. During the first training sessions, the stimulus duration was set at 10 s for Y and MA rats. During the following sessions, the stimulus duration was progressively reduced to 5, 4, 3, 2, 1 and finally 0.5 s. For each rat, the reduction of the stimulus duration to the immediately inferior one occurred when the percent of correct responses was higher than 80%, the percent of omissions was lower than 20% and the rat completed more than 35 trials per session. As AG rats were initially unable to perform the task when the stimulus duration was of 10 s, the first training sessions were started with a stimulus duration of 45 s, which was progressively reduced to 30, 15, 10, 5, 4, 3, 2, 1, and finally 0.5 s. For each AG rat, the stimulus duration was reduced when the percent of correct responses was higher than 70%, the percent of omissions was lower than 40% and the rat completed more than 35 trials per session.

Then the rats were trained with the 0.5 s stimulus duration until stable performance was achieved (i.e., three consecutive sessions with no significant between-session difference).

For each session, the following variables were analyzed: the number of trials completed (number of correct responses + incorrect responses), the percentage of correct responses ([number of correct responses/number of trials completed] × 100), the percentage of omissions ([omissions/trials started that were not terminated by a premature response] × 100), the percentage of premature responses ([prematures/trials started] × 100), the percentage of perseverative responses ([perseverative responses/correct responses] × 100), the response latency for correct responses (correct latency) and the latency to collect a food pellet (magazine latency).

As reviewed by Robbins (2002), the percentage of correct responses, also termed response accuracy in other studies, reflects errors of commission without including errors of omission and is one of two variables best accounting for attentional performance. The percentage of omissions (no response after stimulus presentation) is the second variable accounting for attention; it reflects detection failures. The number of premature responses is an index of impulsivity. The number of perseverative responses corresponds to another form of inhibitory deficit related to compulsive behavior. The last two variables were the correct latency, i.e., the time between the stimulus onset and the correct choice, and the magazine latency, i.e., the time between a correct choice and food collection. The correct latency is considered to depend on the speed of decision making, and the magazine latency on motivation; both variables can also be affected by
motor dysfunctions. It is noteworthy, however, that an exact interpretation of each of these variables can only be made in the light of the other ones, as emphasized by Robbins (2002). Ideally, an alteration of attentional functions should be reflected in reduced response accuracy and/or increased omissions, with the four other variables being unaffected (Robbins, 2002).

2.2.3. Behavioral challenges. After reaching stable performances, the rats were exposed to various parametric challenges aiming at evaluating their performances (i) under different stimulus durations (0.5, 2 or 0.2 s, and in this order), (ii) when the stimulus intensity (SI) was decreased from that used for training, i.e., SI9 = 100% to SI2 = 22% maximal light intensity, and (iii) when the session’s length was set at 60 min (or 250 ITI) under standard stimulus conditions (maximal intensity and 0.5 s duration). Each testing condition was presented until stable performances were reached and the data used for the analyses were calculated across two consecutive sessions. A new challenge was initiated when stable performances were again reached under standard conditions over two consecutive sessions.

2.3. Immunohistochemical evaluations in aged rats

2.3.1. Perfusion and preparation of tissue sections

Histological determinations were performed only in aged rats as these rats were the only ones to exhibit significant effects of enriched environment on both memory and attention performances. One day after completion of behavioral testing, all AG rats (now aged of 29.5 months) were injected with an overdose of sodium pentobarbital (180 mg/kg, i.p.) and transcardially perfused with 60 ml of phosphate-buffered 4% paraformaldehyde (pH 7.4, 4 ◦C). The brains were then extracted, post-fixed for 4 h in the same fixative (4 ◦C solution for about 36–40 h (4 ◦C) and transferred into a 0.1 M phosphate-buffered 30% sucrose (−22 ◦C) and 40 ◦C). All brains were frozen using isopentane (−40 ◦C), and subsequently kept at −80 ◦C until sectioning. Coronal sections, 40 μm thick, were cut on a freezing microtome (−20 ◦C). Depending on the region of interest, sections were collected according to a schedule in which four successive sections were discarded before one was collected (approximatively from Bregma +1.7 mm to Bregma +1 mm for the striatum) or serially (approximatively from Bregma +1 mm to Bregma −1.6 mm for the other brain structures). The sections were collected into a cryopreservative medium, where they were kept at −80 ◦C until staining.

2.3.2. Immunohistochemistry

Anti-p75NTR, anti-choline acetyltransferase (ChAT) and anti-parvalbumine (Parv) immunostainings were used to visualize the effect of enrichment on low-affinity neurtrophin receptor (p75NTR), cholinergic neurons and a subpopulation of GABAAergic neurons, respectively, in the striatum (except for anti-p75NTR, adult rats having no such receptors on cholinergic interneurons in this structure), the medial septum (MS), the diagonal band of Broca (DBB) and the nucleus basalis magnocellularis (NBM).

The sections were rinsed three times for 10 min in PBS (0.1 M, pH 7.4) containing 0.02% mercaptoh (PBS + M) before being soaked for 1 h in 5% normal donkey serum (BioWest, Nuaillé, France) in PBS + M containing 0.5% Triton X-100. The sections were then transferred without rinsing into the primary antibody solution, a goat polyclonal antibody directed against ChAT (1:500; Chemicon International, AB 144 P, Temecula, CA), a mouse monoclonal antibody directed against p75NTR (1:200; Chemicon MAB 365), or a mouse monoclonal antibody directed against Parv (1:4000; Sigma–Aldrich, P 3088, St. Louis, MO). Incubations for 18 h with the primary antibodies were followed by three PBS rinses. Then, all sections preincubated with the anti-ChAT primary antibodies were soaked for 1 h in a buffer solution containing biotinylated donkey anti-goat antibody (1:500; Vector Laboratories International, AP 180 B, Burlingame, CA), and those preincubated with the anti-Parv primary antibody and anti-p75NTR primary antibody were soaked in a buffer solution containing biotinylated horse anti-mouse antibody (1:500; Vector Laboratories International, BA 2001, Burlingame, CA). For the primary and the secondary antibody, the sections were incubated at room temperature (22 ◦C).

After three PBS + M washes, the sections were exposed for 1 h in a standard avidin–biotin–peroxidase complex (Vector-tain Elite ABC, Vector Laboratories, Burlingame, CA). The slices were then rinsed twice in PBS + M and once in 0.6% Tris-buffer (pH 7.6) and subsequently exposed to a Vector Peroxidase substrate kit DAB (SK-4100, Vector Laboratories). Finally, after three PBS + M rinses, the sections were mounted onto gelatine-coated slides, dried at room temperature, dehydrated and cover-slipped.

2.3.3. Cell counting

For ChAT-, Parv- and p75NTR-immunostaining, cell counting was performed as previously described (Harati et al., 2008). Briefly, anatomical landmarks were used to select the location of counting frames of a set size in the whole striatum, the MS, the vertical limb of the DBB (vDBB) and the NBM. The MS was defined dorsally and laterally by the distribution of stained neurons, and ventrally by a virtual line joining the most dorsal level of the left and right parts of the anterior commissures. The vDBB was limited dorsally by this line, and ventrally by a line located 1 mm below. Immunostained neurons were counted in the left and right hemisphere under a microscope (Olympus: Vanox—AHBT3), using a calibrated eyepiece grid to facilitate counting (Olympus: WH 10X2-H). At least two sections chosen such as to be separated by a 40 μm gap were selected from each region at about the same levels of anteriority in each rat. The cell bodies being largely smaller than the inter-section gap, the risk of double counting was thereby discarded. Only cells that were clearly distinguishable from the background (i.e., clear-cut contrast and well-delineated borders) were counted.
2.4. Statistical analysis

Most data were subjected to analysis of variance (ANOVA) with “Age” and “Housing condition” as between-subject factors. When necessary, an additional within-subject factor was considered (i.e., “Hour” for locomotor activity during the habituation period, “Day” for acquisition performances in the water maze, “Trial” for the visible platform procedure, “Stimulus intensity” and “block” for 5-CSRT performances). These analyses were completed by post hoc comparisons using the Newman–Keuls (NK) multiple range test, even in the absence of a relevant interaction, as advocated by Howell (1997), when the figure suggested possible differences at a particular age. In aged rats, correlations between cell counts (ChAT- and p75NTR-positive neurons) in the different structures and cognitive performances in the water maze (distance to reach the platform averaged over all acquisition days and time spent in the target quadrant during the probe trial) and the 5-CSRT (percent correct responses and omissions) were assessed using Pearson’s parametric regression method. The threshold for rejecting the null hypothesis was 0.05 throughout. All the tests were performed using the software Statistica (Statsoft, Inc., Tulsa, OK).

3. Results

3.1. Subjects

Two rats (1 MA standard and 1 AG enriched) died before the completion of the experiment. Eight rats were excluded (2 Y, 3 MA and 1 AG standard, and 2 AG enriched) because they did not reach the criterion in the 5-CSRT task. Finally, nine AG rats which had started the testing sessions (3 enriched and 6 standard rats) were euthanized during water-maze performance or 5-CSRT evaluations due to health problems (e.g., respiratory infection or tumour development, mainly mammary). As we know by experience that aged rats showing evidence for such sickness usually enter into declining, euthanasia was an ethically acceptable issue to deal with this problem. Thus, the final sample sizes for the analyses were: Y standard (n = 10), Y enriched (n = 13), MA standard (n = 14), MA enriched (n = 14), AG standard (n = 9) and AG enriched (n = 6) rats.

3.2. Body weight

Data are shown in Table 1. ANOVA revealed significant effects of “Age” (F 2/59 = 72.96, p < 0.005), “Housing condition” (F 1/60 = 10.07, p < 0.005) and “Age × Housing condition” interaction (F 2/60 = 3.93, p < 0.05). Post hoc comparisons showed significant body weight differences between each age group in the standard rats (p < 0.05, at least). In enriched rats, MA and AG rats, although significantly heavier than their Y counterparts, did not significantly differ from each other. AG enriched rats were significantly lighter than their standard counterparts (NK, p < 0.05), but there was no significant effect of the enriched environment in the Y or the MA rats. In AG rats, the difference between enriched and standard rats was of about 70 g in average.

3.3. Locomotor activity in the home cage

Inspection of Fig. 1 shows that the activity scores decreased over the 3 h of the habituation phase, whatever the age and housing condition. During the first hour, these scores appeared lower in the enriched vs. standard rats regardless of age, and seemed higher in the Y, intermediate in the MA and lower in the AG rats. The ANOVA showed significant effects of “Age” (F 2/59 = 15.17, p < 0.001), “Housing condition” (F 1/59 = 9.69, p < 0.005) and “Hour” (F 2/118 = 63.81, p < 0.001), as well as “Age × Hour” (F 4/118 = 3.77, p < 0.01) and “Housing condition × Hour” (F 2/118 = 3.29, p < 0.05) interactions. In fact, overall scores of the AG rats were significantly lower than in the MA and the Y rats (NK, p < 0.05), and the scores of the MA rats were significantly below those

![Activity scores during the habituation period](image)

Table 1: Body weight (g; mean ± S.E.M.) in the different group of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Standard</th>
<th>Enriched</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young</td>
<td>236.0 ± 4.6</td>
<td>229.4 ± 2.5</td>
</tr>
<tr>
<td>Middle-aged</td>
<td>317.7 ± 11.4*</td>
<td>305.4 ± 7.8*</td>
</tr>
<tr>
<td>Aged</td>
<td>413.1 ± 21.0* &amp;</td>
<td>342.7 ± 18.5* &amp;</td>
</tr>
</tbody>
</table>

* Statistics: significantly different from standard rats at the same age, p < 0.05.

* Statistics: significantly different from Young rats in the same housing condition, p < 0.05.

& Statistics: significantly different from MA rats in the same housing condition, p < 0.05.
of the Y rats (NK, \( p < 0.005 \)); these effects were mainly due to significant differences during the first hour (NK, \( p < 0.05 \), at least). Post hoc comparisons also showed that the activity scores of enriched rats were significantly below those found in standard rats only during the first hour (\( p < 0.001 \)). Finally, in the standard rats, the activity decrease was significant between the first and the second hour (NK, \( p < 0.001 \)), as well as between the second and the third one (NK, \( p = 0.05 \)), whereas in the enriched ones, the decrease was significant only between the first 2 h (NK, \( p < 0.001 \)).

From Fig. 2, for both diurnal and nocturnal activity periods, it appears that, once the rats were habituated to the testing environment, the activity scores were no longer influenced by the housing conditions; they were, however, affected by the age. ANOVA showed a significant “Age” effect (\( F_{2/59} = 16.45, p < 0.001 \); \( F_{2/59} = 31.45, p < 0.001 \) for diurnal and nocturnal activity, respectively), but neither a significant “Housing condition” effect (\( F_{1/59} = 0.01 \) and 0.17, ns for diurnal and nocturnal activity, respectively), nor a significant interaction between these two factors (\( F_{2/59} = 1.16 \) and 0.1, ns for diurnal and nocturnal activity, respectively). The overall “Age” effect can be explained by scores that were highest in the Y rats, lowest in the AG rats and intermediate in the MA ones, all inter-group differences being significant (NK, \( p < 0.05 \), at least).

### 3.4. Water-maze performance

#### 3.4.1. Swimming distances

From Fig. 3A, it appears that the average distance to reach the platform decreased across the 5 days in all groups of rats except in the AG standard rats. The distance swum was always lower in the enriched rats, whatever the age. The ANOVA showed significant effects of “Age” (\( F_{2/60} = 9.09, p < 0.001 \), “Housing condition” (\( F_{1/60} = 36.37, p < 0.001 \)), “Day” (\( F_{4/240} = 86.60, p < 0.001 \)), and a significant “Age \( \times \) Day” interaction (\( F_{8/240} = 5.57, p < 0.001 \)). This interaction was due to lower performances in AG rats as compared to their Y and MA counterparts, but only on days 3, 4 and 5 (NK, \( p < 0.01 \), at least).
3.4.2. Thigmotaxis

Inspection of Fig. 3B shows that the % thigmotaxis decreased more rapidly in the enriched as compared to the standard rats, whatever the age. The % thigmotaxis in the AG standard rats was higher than in the Y and MA ones, whereas in the AG enriched rats it was at about the same level as in their MA and Y counterparts from the third day onwards.

The ANOVA showed significant effects of “Age” (F 2/60 = 30.63, p < 0.001), “Housing condition” (F 1/60 = 72.75, p < 0.001), “Day” (F 4/240 = 223.71, p < 0.001), as well as of the following interactions: “Age × Housing condition” (F 2/60 = 5.07, p < 0.01), “Age × Day” (F 8/240 = 2.12, p < 0.05), “Housing condition × Day” (F 4/240 = 4.353, p < 0.005) and “Age × Housing condition × Day” (F 8/240 = 4.51, p < 0.001). Post hoc comparisons revealed that the MA rats never differed from the Y rats, with respect to their housing condition. The % thigmotaxis was significantly higher in the AG standard vs. the Y and the MA standard rats for each day (NK, p < 0.005, at least). In the AG enriched rats, this % was higher than in the MA and the Y enriched rats, but only on the first 2 days (NK, p < 0.05). The enriched rats exhibited significantly lower % thigmotaxis than the standard rats, but only on the second day in the Y rats (NK, p < 0.05), only on the first 2 days in the MA (NK, p < 0.05, at least) ones, and over the 5 days of acquisition in the AG rats (NK, p < 0.005, at least).

3.4.3. Swim velocity

Swim speed was slower in the AG than in the MA and Y rats and this effect of age was less marked in enriched rats (data not illustrated). The ANOVA showed significant effects of “Age” (F 2/60 = 31.03, p < 0.001), “Housing condition” (F 1/60 = 15, p < 0.001), “Day” (F 4/240 = 17.05, p < 0.001), as well as of the “Age × Housing condition” (F 2/60 = 2.96, p = 0.06) and “Age × Day” (F 8/240 = 4.12, p < 0.001) interactions. Post hoc analysis indicated that the swim speed was significantly lower in AG rats as compared to MA and Y rats, regardless of the housing conditions (at least, p < 0.005). Furthermore, the AG enriched rats had a significantly higher swim speed than their AG standard counterparts (NK, p < 0.005).

3.4.4. Probe trial

Inspection of Fig. 4 shows that the time spent in the target quadrant was higher in enriched vs. standard rats, but only in the MA and AG rats. The ANOVA revealed a significant “Housing condition” effect (F 1/60 = 6.18, p < 0.05), but no effect of “Age” and no interaction between both factors. The comparison between the probe trial scores of standard and enriched rats at each age showed no significant effects of the enrichment, whatever the age populations; a tendency was noticed in AG rats (NK, p = 0.10). Student's t-tests, which were performed to compare the average time spent in the target quadrant to 7.5 s (corresponding to chance), however, indicated that in the MA and the Y rats, whatever the housing condition, this time was significantly above chance (p < 0.05, at least). In the AG enriched rats, this time was also significantly above chance (p < 0.05). In the AG standard rats, however, it did not differ from chance.

The number of annulus crossings was decreased by the age, but this effect was less pronounced in the enriched rats (data not illustrated). ANOVA showed significant “Age” (F 2/60 = 8.10, p < 0.001) and “Housing condition” (F 1/60 = 10.24, p < 0.005) effects, and a significant interaction between both factors (F 2/60 = 4.33, p < 0.05). Post hoc comparisons revealed that the number of annulus crossings was lower in the AG standard rats as compared to their Y and MA counterparts (p < 0.01, at least), whereas in the AG enriched rats the number of annulus crossings was not different from that found in Y and MA enriched rats, but was significantly higher than in the AG standard rats (p < 0.005).

3.4.5. Visible platform

On the first day (see Fig S1-A in Supplementary material), the evolution across trials of the distances to the platform was different among groups. In Y rats, regardless of the housing conditions, no clear-cut modification was observed over trials, whereas the distance to reach the platform decreased across trials in MA and AG rats. Moreover, whereas no difference between enriched and standard rats was observed in Y and MA rats, the distance to reach the platform was globally higher in AG standard rats than in AG enriched rats. The ANOVA showed significant effects of “Age” (F 2/60 = 25.43, p < 0.001), “Housing condition” (F 1/60 = 6.06, p < 0.05), “Trial” (F 3/180 = 16.17, p < 0.001), as well as of the “Age × Housing condition” (F 2/60 = 6.46, p < 0.005) and “Age × Trial” (F 6/180 = 2.81, p < 0.05) interactions. Post hoc comparisons revealed that the overall distance swum was significantly higher in the MA and AG rats when compared to the Y rats, whatever the housing conditions (p < 0.05, at least).
least), and was lower in AG enriched rats as compared to AG standard rats ($p<0.005$). These comparisons did not show significant between-trial fluctuations in Y rats, but pointed to a significant difference between the first and the three subsequent trials in the MA rats ($p<0.001$), and between the first and last trials ($p<0.005$) in the AG rats.

On the second day (see Fig S1-B in Supplementary material), there was no obvious between-trial modification of performance in Y and MA rats housed in standard and enriched conditions; this was also the case in AG enriched rats. In contrast, in AG standard rats, the distance swum exhibited a strong decrease from the first to the second trial. Moreover, in AG rats and for both housing conditions, the distance swum was globally higher than in their Y and MA counterparts. The ANOVA showed significant effects of “Age” ($F(2/60)=144.87$, $p<0.001$), “Housing condition” ($F(1/60)=19.49$, $p<0.001$), “Trial” ($F(3/180)=15.74$, $p<0.001$), as well as of the following interactions: “Age × Housing condition” ($F(2/60)=7.85$, $p<0.001$), “Age × Trial” ($F(6/180)=5.55$, $p<0.001$), “Housing condition × Trial” ($F(3/180)=5.18$, $p<0.005$) and “Age × Housing condition × Trial” ($F(6/180)=2.8$, $p<0.05$). Post hoc comparisons revealed that the distance swum by the AG rats from both housing conditions was significantly higher than in the MA and the Y rats, especially in the first two trials ($p<0.05$, at least). In the first trial, the distance swum by the AG standard rats was higher than in the AG enriched rats ($p<0.001$). Finally, such comparisons showed that differences between the first and the subsequent trials were only observed in AG standard rats ($p<0.001$, at least).

### 3.5. Attention performance in the five-choice serial reaction time task

#### 3.5.1. Performances under standard conditions (stimulus duration of 0.5 s, maximal light intensity)

Data are shown in Fig. 5A and B. Inspection of Fig. 5A suggests that the % of correct responses decreased only in AG standard rats, but the ANOVA failed to reveal significant effects. ANOVA of the % of omissions (Fig. 5B) showed a significant “Age” effect ($F(2/60)=45.17$, $p<0.001$). Post hoc comparisons revealed that the % of omissions was significantly higher in MA and AG rats than in the Y rats ($p<0.001$, at least).

The complementary behavioral variables are shown in Table S2 in Supplementary material. ANOVA of the correct latencies only showed a significant “Age” effect ($F(2/60)=6.39$, $p<0.005$). Post hoc comparisons revealed that the correct latency was significantly longer in the AG rats than in Y and MA rats ($p<0.05$, at least). ANOVA of the % of perseverative responses showed a significant “Age” effect ($F(2/60)=6.0$, $p<0.005$) and a significant “Age × Housing condition” interaction ($F(2/60)=3.26$, $p<0.05$). Post hoc comparisons revealed that the % of perseverative responses was significantly higher in AG standard rats when compared to their Y and MA counterparts ($p<0.01$, at least), and also when compared to AG enriched rats ($p<0.05$). No effect of age was observed in enriched rats. The other variables were affected neither by the age nor by the housing condition.

#### 3.5.2. Performances at the 2-s stimulus duration

Data are shown in Fig. 5C and D. ANOVA of the % of correct responses (Fig. 5C) did not show any significant effect. ANOVA of the % of omissions (Fig. 5D) showed only a significant “Age” effect ($F(2/60)=23.12$, $p<0.005$). Post hoc comparisons revealed that the AG rats exhibited a higher % of omissions than their MA and Y counterparts ($p<0.05$, at least), and that MA rats produced more omissions than the Y rats ($p<0.005$).

The other variables are shown in Table S2 in Supplementary material. ANOVA of the correct latencies showed a significant “Age” effect ($F(2/60)=5.11$, $p<0.01$); the “Age × Housing condition” interaction was close to significance ($F(2/60)=2.86$, $p=0.064$). Post hoc comparisons revealed that the correct latency was significantly longer in the AG standard vs. their Y and MA counterparts ($p<0.05$, at least). The AG enriched rats, in contrast to the AG standard rats, performed as well as the Y and MA enriched rats. ANOVA of the magazine latency showed significant effects of “Age” ($F(2/60)=6.73$, $p<0.005$), “Housing condition” ($F(1/60)=5.99$, $p<0.05$) and “Age × Housing condition” interaction ($F(2/60)=4.86$, $p<0.05$). Post hoc comparisons revealed that the magazine latency was significantly longer in the AG standard rats than in their Y and MA counterparts, but also than in the AG enriched rats ($p<0.001$, at least). ANOVA of the % of perseverative responses showed significant effects of “Age” ($F(2/60)=6.48$, $p<0.005$), “Housing condition” ($F(1/60)=5.91$, $p<0.05$), and “Age × Housing condition” interaction ($F(2/60)=7.51$, $p<0.005$). Post hoc comparisons indicated that the AG standard rats exhibited a significantly higher % of perseverative response than their Y and MA counterparts, and also than AG enriched rats ($p<0.001$, at least). The % of premature responses was affected neither by the age nor by the housing condition.

#### 3.5.3. Performances at the 0.2-s stimulus duration

Data are shown in Fig. 5E and F. Inspection of Fig. 5E suggests that the % of correct responses decreased only in AG standard rats. ANOVA of the % of correct responses (Fig. 5E) revealed only a significant “Housing condition” effect ($F(1/60)=7.43$, $p<0.01$). The comparison between standard and enriched rats at each age indicated that the % of correct responses was higher in enriched rats when compared to standard rats, but only in AG rats (NK, $p<0.05$). ANOVA of the % of omissions (Fig. 5F) showed a significant “Age” effect ($F(2/60)=16.21$, $p<0.001$). Post hoc comparisons indicated a significantly larger % of omissions in AG vs. MA and Y rats ($p<0.05$, at least), and also in MA vs. Y rats ($p<0.001$).

The other variables are shown in Table S2 in Supplementary material. ANOVA of the correct latency showed only a significant “Age” effect ($F(2/60)=3.78$, $p<0.05$), which, as shown by post hoc comparisons, was due to cor-
Fig. 5. Performances (mean ± S.E.M.) in the 5-CSRT task at different stimulus durations: 0.5 s (A and B); 2 s (C and D) and 0.2 s (E and F). Statistics: * significantly different from standard rats at the same age, $p < 0.05$. Group abbreviations as in Fig. 1.

Rect latencies that were significantly longer in AG vs. MA ($p = 0.05$) or Y ($p < 0.05$) rats. ANOVA of the magazine latency showed significant effects of “Age” ($F_{2/60} = 31.12, p < 0.001$), “Housing condition” ($F_{1/60} = 4.64, p < 0.05$) and “Age × Housing condition” interaction ($F_{2/60} = 10.03, p < 0.001$). Post hoc comparisons revealed that the magazine latency was significantly longer in the AG rats as compared to their MA and Y counterparts, regardless of the housing
conditions \((p < 0.05, \text{ at least})\). Magazine latencies were also significantly shorter in AG enriched vs. AG standard rats \((p < 0.001)\). ANOVA of the % of perseverative responses showed a significant “Age” effect \((F_{2/60} = 8.1, p < 0.001)\). Post hoc comparisons indicated that the % of perseverative responses was significantly higher in AG vs. MA and Y rats \((p < 0.001, \text{ at least})\). ANOVA of the % of premature responses showed only a significant “Age” effect \((F_{1/60} = 6.81, p < 0.05)\). Post hoc comparisons revealed a significantly weaker percentage of premature responses in AG and MA rats vs. Y rats \((p < 0.01)\).

3.5.4. Decrease of stimulus intensity from SI-9 (100%) to SI-2 (22%)

Decreasing the stimulus intensity (data not shown) only slightly affected performance. This effect was not influenced by the age or the housing conditions. Globally the effects of “Age” and “Housing condition” were similar to those previously described under standard testing conditions. Thus, to facilitate the description of the results, only the effect of “Stimulus intensity” and its interaction with the other factors will be described. ANOVAs showed that a significant “Stimulus intensity” effect \((F_{1/60} = 4.18, p < 0.05)\) was observed only for the % of correct responses. This effect was due to a weak decrease of overall % of correct responses at the lowest vs. maximal intensity, whatever the age or housing conditions.

3.5.5. Performances at 60-min session’s length

Data are shown in Fig. 6. This figure indicates an age-related decrease of performance, but no effect of the housing conditions. For the % of correct responses (Fig. 6A), statistical analysis revealed only significant “Age” effects \((F_{2/60} = 7.35, p < 0.005)\) and a nearly significant “Age × Housing condition” interaction \((F_{2/60} = 3.37, p = 0.054)\). Post hoc comparisons showed that the AG rats exhibited an overall lower % of correct responses than the MA and Y rats \((p < 0.05, \text{ at least})\). ANOVA of the % of omissions (Fig. 6B) showed a significant “Age” effect \((F_{2/60} = 4.01, p < 0.05)\) for % of correct responses. Post hoc comparisons showed that the AG rats exhibited an overall lower % of correct responses than the MA and Y rats \((p < 0.05, \text{ at least})\). ANOVA of the % of omissions (Fig. 6B) showed a significant “Age” effect \((F_{2/60} = 7.35, p < 0.005)\) and a nearly significant “Age × Housing condition” interaction \((F_{2/60} = 3.37, p = 0.054)\). Post hoc comparisons revealed an increase of omissions in AG standard rats as compared to their Y and MA counterparts \((p < 0.05, \text{ at least})\), but not in AG enriched rats. Moreover, Y enriched rats produced higher % of omissions than their standard counterparts \((p < 0.05)\).

The other variables are shown in Table S3 in Supplementary material. ANOVA of the correct latencies showed

<table>
<thead>
<tr>
<th>Table 2</th>
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<tr>
<td>Average ± S.E.M. number of ChAT- , p75NTR- and Parv-positive neurons in the nucleus basalis magnocellularis, the medial septum and the diagonal band of Broca in the aged standard (AG-S) and the aged enriched (AG-E) rats.</td>
</tr>
<tr>
<td>Number of</td>
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<td>ChAT-positive neurons</td>
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<td>p75NTR-positive neurons</td>
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<td>Parv-positive neurons</td>
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In parentheses, the % change vs. standard.

* Statistics: significantly different from standard, \(p < 0.05\).

** Statistics: significantly different from standard, \(p < 0.01\).
a significant “Age” effect \( (F_{2/60} = 4.87, p < 0.05) \), which was due to significantly longer correct latency in AG vs. MA and Y rats (NK, \( p < 0.01 \), at least). ANOVA of the magazine latency showed significant “Age” \( (F_{2/60} = 15.16, p < 0.001) \) and “Housing condition” \( (F_{1/60} = 5.6, p < 0.05) \) effects and a significant “Housing condition \( \times \) Age” interaction \( (F_{2/60} = 7.02, p < 0.005) \). Post hoc comparisons revealed that the magazine latency was significantly longer in the AG standard rats when compared to their Y and MA counterparts \( (p < 0.001) \), but also when compared to AG enriched rats \( (p < 0.001) \). ANOVA of the \% of perseverative responses showed a significant “Age” effect \( (F_{2/60} = 8.46, p < 0.001) \) which was due to higher \% of perseverative responses in the AG vs. the MA and Y rats (NK, \( p < 0.001 \), at least). ANOVA of the \% of premature responses did not show any significant effect.

To assess vigilance decrement during the 60-min session, the \% of correct responses and the \% of omissions were analyzed in two blocks of 30 min (Fig. 7). ANOVA of the \% of correct responses (Fig. 7A) showed significant effects of “Age” \( (F_{2/60} = 5.71, p < 0.01) \), “Block” \( (F_{2/60} = 6.71, p < 0.005) \) and “Age \( \times \) Block” interaction \( (F_{2/60} = 3.16, p < 0.05) \). Post hoc comparisons showed that the \% of correct responses in AG rats was lower than in the Y and the MA rats in the second block \( (p < 0.01) \), but not in the first one. Furthermore, only AG rats exhibited a significant decrease of the \% of correct responses from the first to the second block which was significant \( (p < 0.01) \). ANOVA of the \% of omissions (Fig. 7B) showed significant effects of “Age” \( (F_{2/60} = 5.22, p < 0.01) \), “Housing condition” \( (F_{1/60} = 4.07, p < 0.05) \) and “Block” \( (F_{1/60} = 9.15, p < 0.005) \). Post hoc comparisons showed that the \% of omissions was higher in the second block as compared to the first one, but only in Y rats \( (p < 0.01) \).

### 3.6. Immunohistochemical evaluations in aged rats

Typical examples of ChAT-, p75NTR-, and Parv-positive immunostaining in the NBM are illustrated in Fig. 8. The results of our quantitative evaluations are presented in Table 2. In all the structures studied, the number of ChAT- and p75NTR-positive neurons was higher in AG enriched rats than in AG standard rats, whereas the number of Parv-positive neurons did not significantly differ between both groups.

Statistical analyses of the number of ChAT-immunoreactive neurons showed a significant “Housing condition” effect in the NBM \( (F_{1/13} = 7.27, p < 0.05) \), the MS \( (F_{1/13} = 9.36, p < 0.05) \), the vDBB \( (F_{1/13} = 16.34, p < 0.005) \) and the striatum \( (F_{1/13} = 18.81, p < 0.05) \). In the enriched rats, the number of ChAT-positive neurons exceeded that found in standard rats by 30 (striatum) to 64\% (NBM). For the number of p75NTR-positive neurons, there was a significant “Housing condition” effect in the NBM \( (F_{1/13} = 7.43, p < 0.05) \), the MS \( (F_{1/13} = 8.66, p < 0.05) \) and the vDBB \( (F_{1/13} = 5.66, p < 0.05) \). In the enriched rats, the number of p75NTR-positive neurons exceeded that found in standard rats by about 35 (NBM) to 44\% (MS). As regards the number of Parv-positive neurons, there was no significant “Housing condition” effect, whatever the counting region (largest \( F_{1/13} < 1.0 \) in all cases, ns).

### 3.7. Regression analyses

Considering the histological and behavioral data from our aged rats (standard and enriched collapsed), we performed a series of regression analyses. Among the relevant ones, we noticed an almost significant negative correlation between average distance in the water maze during the acquisition task and the number of ChAT-positive neurons in the NBM \( (r = -0.5, p = 0.053) \). The number of ChAT- and p75NTR-positive neurons in the NBM was also positively correlated to probe trial performance, but this correlation was significant only for p75NTR-positive neurons \( (r = 0.51, p < 0.05); \) for ChAT, \( r = 0.49, p = 0.06 \). The largest correlation coefficient was between thigmotaxis in the water maze (average over five acquisition days) and the number of ChAT-positive interneurons in the striatum \( (r = -0.64, p < 0.01) \). There was also a significant positive correlation between the number of p75NTR-positive neurons in the NBM and the \% of correct responses in the 5-CSRT task with a stimulus duration of 0.2 s.
(r = 0.62, p = 0.014) or 0.5 s (r = 0.53, p = 0.04), but not when the duration was of 2 s (r = 0.30, ns). Regarding the number of ChAT-positive neurons, the correlation coefficients were also positive, but they failed to achieve significance.

4. Discussion

Our results indicate that, in comparison with standard housing conditions, the environmental enrichment (i) reduced the body weight gain found over lifespan, (ii) had no effect on the age-related decrease of spontaneous locomotor activity in the home cage, (iii) reduced the age-related alterations of performance in a spatial learning/memory task, (iv) prevented some age-related alterations of performance in a visuo-spatial attentional task, but had no effect on the age-related vigilance decrement and did not affect performance in young or middle-aged rats, and (v) in aged rats, resulted in more ChAT- and p75NTR-positive neurons in the basal forebrain, and more ChAT-positive neurons in the striatum.

4.1. Female rats and cyclic hormonal status

No individual determination of the oestrous cycle stage has been carried out in our rats. Concerning aged female rats, the question of the oestrous cycle does not seem crucial. Indeed, according to the literature, female rats are in a permanent dioestrous phase after the age of 17 months (LeFevre and McClintock, 1988). As all aged rats were over 25 months, their hormonal status was constant and comparable among subjects. The cyclicity of the hormonal status might be of concern, whether in terms of cognitive capabilities or effects of environment, in young and middle-aged rats. Regarding the first issue, it is worth mentioning that the influence of the ovarian cycle on spatial memory/learning is not clearly established in the rat; some authors do not find any alteration of spatial learning and memory performances, especially in the Long–Evans strain (Berry et al., 1997; Stackman et al., 1997), while other authors reported changes, which remain weak across the oestrous cycle (Warren and Juraska, 1997). Furthermore, unpublished
observations in the laboratory have shown that, under our housing conditions, the oestrous cycle of female rats does not synchronise (see also Schank, 2001). Therefore, considering the number of animals in each experimental group, oestrous cycle influence might have at best contributed to inter-individual variability, most probably not to an overall group effect (see also Cassel et al., 2007). Regarding the second issue (i.e., effects of environment), interactions between sex hormones and environmental enrichment have been investigated. In mice, Gresack et al. (2007) reported complex interactions between estrogen, enrichment and memory performance. In an earlier study, Gresack and Frick (2004) showed that an enriched environment prevented the effects of sex hormones on cognitive performance. If the same was true for rats, the cognitive benefits which we report in females would probably not reflect a main influence of an indirect cycle-related bias of performance. Finally, in female rats, it was shown that ovarian hormones do not interfere with the effects of enriched environment on radial maze and water-maze performance (Daniel et al., 1999). Thus, although we cannot exclude a contribution of the hormonal status as a potential bias of our observation, based on the aforementioned arguments, this bias was most probably minimal. Whatever may be, it seems prudent to consider that our results should not be generalized to male rats.

4.2. Body weight

The body weight of the rats reared in standard conditions increased over the lifespan. Such an increase was not observed in enriched rats between the ages of 13 and 25 months. As the impact of enriched environment on body weight has not been attributed to a decrease of food intake (Augustsson et al., 2002; Spangenberg et al., 2005), this preventive effect may be due to an increased physical activity promoted by the enriched housing conditions. Indeed, physical activity has been shown to limit the weight gain over the aging process in rats (Skalicky et al., 1996).

4.3. Locomotor activity

Confirming previous reports (Casadesus et al., 2001; Stemmelin et al., 1999), our data show that aged rats exhibit decreased spontaneous locomotion in their home cage. As it was also apparent in middle-aged rats, this decrease appears to be a progressive phenomenon. Enriched housing did not affect spontaneous locomotor activity in a familiar environment but it attenuated the locomotor reaction to novelty whatever the age of the rats. This attenuation is in line with data showing that rats reared in an enriched (vs. standard) environment show weaker locomotor activity in an unfamiliar environment whether young (Elliott and Grunberg, 2005; Schrijver et al., 2002) or aged (Segovia et al., 2008; Van Waas and Soffie, 1996). The difference of activity in the open-field was proposed to reflect a more efficient exploratory behavior and a faster habituation of enriched rats (Elliott and Grunberg, 2005; Schrijver et al., 2002; Zimmermann et al., 2001), which might correspond to a faster processing of contextual information (Barbelivien et al., 2006; Woodcock and Richardson, 2000). Altogether, our data demonstrate that an exposure to an enriched environment during the entire lifespan did not prevent the age-related decrease in locomotor activity in a familiar context.

4.4. Spatial memory

Young and middle-aged rats, whether reared in standard or enriched conditions, reached the same performance at the end of the acquisition phase. However, as illustrated in Fig. 3A, enriched rats were faster in learning the task. Our data also show that this effect of enrichment paralleled the decrease in thigmotaxis (i.e., the time spent swimming near the wall). Usually, thigmotactic behavior is prominent on the initial encounter of a novel space. It helps organisms to define the borders of an enclosed space and to identify escape routes from that space. As thigmotactic swimming has been proposed to be a manifestation of spatial exploration or information gathering (Topic et al., 2005), our results may suggest that rats housed in an enriched environment learned more quickly about the environment and thus shifted more rapidly to an appropriate search strategy. However, thigmotaxis may also be affected by fear/anxiety (Herrero et al., 2006). As environmental enrichment has been shown to decrease anxiety in adult rats (Benaroya-Milshtein et al., 2004; Galani et al., 2007), one cannot rule out the possibility that the decrease of thigmotaxis found in enriched rats relied on decreased anxiety.

In comparison with aged rats reared in standard conditions, aged enriched rats were able to learn the task. Furthermore, probe trial performance confirmed that aged enriched rats could retrieve the location of the platform. The longer distance swum to reach the platform was not the sole impairment found in those rats, which also exhibited reduced swim speed. The effect of enriched environment on the distance swum to reach the platform was associated with a reduction of this effect of age. As aging results also in motor and sensory-motor impairments (Ingram et al., 1994) and as environmental enrichment is able to ameliorate sensory-motor performances (Christie and Dalrymple-Alford, 1995), one may wonder to which extent the age-related impairment in the water maze could reflect motor or sensory-motor dysfunctions rather than cognitive disabilities, and thus whether the enrichment-induced improvement might be a manifestation of improved sensory-motor functions. Several points, however, argue against such an interpretation. First, we have analyzed the distances swum, which are considered to be less sensitive to sensory-motor biases than other variables such as the latency (Lindner, 1997). Second, aged rats exhibited better performances when trained with the visible platform, indicating that they were motivated to search for the tar-
get, and that they possessed the motor and visual abilities required for performing the task. Third, no difference was observed between aged enriched and aged standard rats during the last trials of the visible platform task. Regarding the effect of the enriched environment on the swim speed, in contrast to aged standard rats, enriched rats could learn the task and were therefore prompter to reach the target. It is also possible that the swim speed has been affected by the body weight, which was largest in the aged standard rats. The effect on water-maze acquisition of the enriched environment in aged rats may also be questioned in terms of thigmotactic behavior. In aged standard rats, such behavior was marked and persistent. This persistence can be regarded as a maladaptive strategy, as previously described (Schulz et al., 2004, 2007; Topic et al., 2005), a high level of thigmotaxis preventing the development of an efficient search strategy. Maintenance of high levels of thigmotactic swimming has also been linked to behavioral rigidity (Schulz et al., 2004) and to anxiety (Herrero et al., 2006). Topic et al. (2005), however, showed that enhanced thigmotaxis in aged rats does probably not reflect anxiety. Several studies have shown that aging is associated with a decline of cognitive flexibility, which may lead to perseverative behavior (Barense et al., 2002; Means and Holsten, 1992; Schoenbaum et al., 2002). Therefore, the maintenance of thigmotactic behavior in aged standard rats might be the consequence of a deficit in spatial integration as already postulated by Topic et al. (2005). As the maintenance of pronounced thigmotaxis exhibited by aged rats was much reduced by the enriched housing conditions, our results indicate that aged enriched rats have preserved a genuine capacity of spatial learning. Water-maze performance may depend on the integrity of both the septo-hippocampal system and the striatum (Devan et al., 1996; Mura and Feldon, 2003) that are both altered in aged rats (e.g., Baskerville et al., 2006; Stemmlein et al., 2000). Even if the regression analyses in aged rats did not clearly support a link between preserved neurons and performance, our data show that aged enriched rats exhibited a higher number of ChAT- and p75NTR-positive neurons in the septum and striatum than aged standard rats. This suggests that part of the enrichment-induced preservation of spatial learning/memory capabilities in aged rats could rely upon a preservation of (at least) cholinergic projection neurons and interneurons in the septum and striatum. Nevertheless, this interpretation could not be supported by the currently reported data, as we only kept histological material from our aged rats. In a parallel study, however, which addressed a different series of questions in Y, MA and AG rats (same strain, sex, ages, etc.), reared in standard or enriched environments for comparable durations, histological evidence from each age condition showed that the enriched environment actually prevented an age-related decline of ChAT-positive neurons in the basal forebrain (Harati et al., in preparation).

4.5. Attention

Our data indicate that age altered several performances in the 5-CSRT task and that some of these alterations were already detectable in middle-aged rats. Reducing the brightness of the stimulus resulted in an equivalent decrease in choice accuracy in all groups, indicating that the impairments observed in middle-aged and aged rats were not related to visual deficits. Among the different variables analyzed in the 5-CSRT task, the % of correct responses (or choice accuracy) is generally assumed to reflect attentional processes (Robbins, 2002). Our data suggest that choice accuracy tend to decrease in aged standard rats (stimulus duration of 0.5 or 0.2 s), as previously shown (Jones et al., 1995; Muir et al., 1999). The absence of choice accuracy deficits in aged standard rats at the longest stimulus duration (i.e., 2 s) was associated with a marked increase in correct latency. Thus, when the aged standard rats had the opportunity to respond while the stimulus was still present, they performed at the level of young rats, suggesting that the decrease in choice accuracy in aged standard rats reflected a true attentional deficit. This was confirmed by the performance when the session duration was lengthened, a condition under which aged rats made significantly fewer correct responses than young and middle-aged rats and were the only group to exhibit a decrease in choice accuracy over time, indicating a vigilance decrement as already demonstrated (Grottick and Higgins, 2002). In contrast to standard rats, aged enriched rats did not present a decrease of choice accuracy. They also produced more correct responses than aged standard rats, but only when the attentional load was high. When the session duration was lengthened, however, the beneficial effect of environmental enrichment was not observed, indicating that aged enriched rats, as their standard counterparts, were not able to sustain their attention over a long period of time. These data suggest that environmental enrichment prevented the age-related attention deficits, but was unable to compensate for the vigilance decrement observed in aged rats. Aged rats, whether enriched or standard, were also slower in making a correct response. As this increase in correct latency was not systematically associated with an increase in magazine latency, it may reflect a deficit in decisional processes (Robbins, 2002). However, the fact that correct latency was longer even when the choice accuracy was not altered (especially in enriched rats) suggests that aged rats had adopted a speed/error trade off strategy, as proposed by Muir et al. (1999).

One obvious effect of aging in the 5-CSRT task was the larger number of omissions, an effect found in both housing conditions. This increase emerged already in middle-aged rats, whether standard or enriched, and was not influenced by parametric manipulations of the task. As this increase was not systematically associated with a parallel increase in magazine latency, it is unlikely to reflect a reduced motivation towards the food reward (Robbins, 2002). Indeed, only aged standard rats exhibited increased magazine latencies, which
may be related to their high level of perseverative responses rather than to a reduced motivation. Therefore, a larger number of omissions may reveal detection failures, which may be interpreted in terms of altered attention, as in previous studies (e.g., Jones et al., 1995). It is noteworthy that this increased % of omissions in middle-aged and aged rats was still apparent under decreased attentional load (i.e., stimulus duration of 2 s). Moreover, in contrast to choice accuracy, which varied according to the stimulus duration in young, middle-aged and aged rats, the % of omissions was modified in middle-aged rats only when the stimulus duration was shortened. It was not altered by the stimulus duration in aged rats (see Fig. 5), suggesting that the higher % of omissions found in aged rats could reflect more than an attentional deficit. Indeed, in the 5-CSRT task, a trial is initiated when rats push the panel after a correct response (to collect the food) or after a time-out period. Rats had thus 5 s to turn around and to be ready to respond to the next stimulus. The middle-aged and aged rats were perhaps not able to maintain a sufficient activity level to respond, and by consequence have omitted more trials.

Aged standard rats were also characterized by a marked increase in the % of perseverative responses. Previous authors, who focused on 5-CSRT performance in aged rats, did not report such an increase (Jones et al., 1995; Muir et al., 1999). The fact that the perseverative responses were not punished by a time-out period in the present study, contrasting with previous protocols (Jones et al., 1995; Muir et al., 1999), may explain this difference. We did not punish a perseveration because aged rats required more time to learn the task (Jones et al., 1995) and punishment of perseverative responses makes learning more difficult (Bari et al., 2008). Nevertheless, this effect of age was not observed in enriched rats, at least when the demand of the task was not too high. In fact, aged enriched rats exhibited an increase in perseverative responses only when the stimulus duration was of 0.2 s or when the session duration was lengthened.

Altogether, our 5-CSRT task data indicate that a lifelong rearing in an enriched environment prevents sustained attention deficits manifested by aged rats. In young and middle-aged rats, however, performance in the 5-CSRT task was not sensitive to environmental enrichment, most probably because attention-based responses do not require plasticity-based processes comparable to those required for memory formation, and on which environmental enrichment usually exerts strong effects. It is noteworthy, however, that environmental enrichment in aged rats contributed to a larger number of ChAT-positive (+64%) and p75NTR-positive (+35%) neurons but not of Parv-positive neurons in the NBM. In addition, the attentional performances (% of correct responses) in aged rats were positively correlated with the number of p75NTR-positive neurons of the NBM only when the attentional load of the task was high (i.e., when stimulus duration was set at 0.5 or 0.2 s). As we recently demonstrated that selective immunotoxic cholinergic lesions in the NBM produced marked attention deficits (Harati et al., 2008), it can be suggested that part of the mechanism(s) underlying the better 5-CSRT performance in aged enriched rats might be an enrichment-driven preservation (or reduced loss) of these neurons. Most probably, this higher number of cholinergic and p75NTR receptor-bearing neurons might be the consequence of an enrichment-induced stimulation of neurotrophic activity (Mohammed et al., 2002; van Praag et al., 2000), which is known to decline over aging (Backman et al., 1996; Cooper et al., 1994).

5. Conclusion

While aging alters attention and memory functions, our current results show that environmental enrichment has positive effects on memory at all ages. As regards attention, such beneficial effects become evident only at an age at which attention performance has declined. This difference is probably not only due to the fact that memory and attention do not depend on the same subregions of the basal forebrain (e.g., Lehmann et al., 2003). Memory also crucially depends on neural plasticity, and neural plasticity is stimulated by enriched housing conditions at all ages (Mora et al., 2007). We may assume that this could be the reason why enriched rats showed better memory performances whether young, middle-aged or aged. Compared to memory functions, attention is probably less dependent on neuroplastic reorganizations (Nilsson et al., 1999; Williams et al., 2001), and may therefore exhibit a much weaker sensitivity towards environmental enrichment. This is probably the reason why performance in young and middle-aged rats was not affected by the enriched rearing conditions. In aged rats, however, in which the enriched (vs. standard) housing conditions resulted in a larger number of ChAT- and p75NTR-positive neurons in the basal forebrain, including the NBM, 5-CSRT performance was left unaltered by age, most probably as a result of an enrichment-driven preservation of such neurons.

Disclosure statement

We have no actual or potential conflicts of interest that might have influenced our work. None of our respective institutions has contracts relating to this research through which it may stand to gain financially, whether now or in the future, and we have no other agreement of authors or related institutions that could be seen as involving a financial interest in this work.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neurobiolaging.2009.03.012.

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