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The three panel runway maze adapted to *Microcebus murinus* reveals age-related differences in memory and perseverance performances

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Abstract

Microcebus murinus, a mouse lemur primate appears to be a valuable model for cerebral aging study and for Alzheimer's disease model since they can develop β -amyloid plaques with age. Although the biological and biochemical analyses of cerebral aging are well documented, the cognitive abilities of this primate have not been thoroughly characterized. In this study, we adapted a spatial working memory procedure described in rodents, the sequential choice task in the three-panel runway, to mouse lemurs. We analyzed the age-related differences in a procedural memory task in the absence or presence of visual cues. Sixty percent of young adult and 48% of aged lemurs completed the exploratory, choice habituation and testing phases at the beginning of the procedure. Young adult lemurs showed a higher level of perseverative errors compared with aged animals, particularly in the presence of visual stimuli. Over trials, old animals made more reference errors compared to young ones that improved quickly their performances under random level. No significant improvement was observed in young adults and old ones over sessions. This study showed that behavioural performances of *Microcebus murinus* assessed on the sequential choice task in the three-panel runway markedly differ from the previously reported abilities of rodents. The behavioural response of young adult lemurs was influenced by novelty-related anxiety that contributed to their performance in terms of perseverative errors. Conversely, aged lemurs showed less perseverative errors, a rapid habituation to the three-panel runway maze, but made more memory errors. Overall, these findings demonstrate the feasibility to use the three panel runway task in assessing memory performance, particularly in aged mouse lemurs.

Keywords

primates; working memory; three-panel runway test; aging

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Introduction

Microcebus murinus or grey mouse lemur is the smallest primate lemur identified in the world. It is considered to be a relevant model for the study of normal cerebral aging and Alzheimer's disease (AD) (Bons, Rieger, Prudhomme, Fisher & Krause, 2006). Along with age, some animals spontaneously accumulate within the brain deposits of amyloid- β peptides (A β) (Mestre-Frances, Keller, Calenda, Barelli, Checler & Bons 2000), and abnormally phosphorylated Tau protein (Bons, Jallageas, Silhol, Mestre-Frances, Petter & Delacourte 1995; Delacourte, Sautiere, Watez, Mourton-Gilles, Petter & Bons 1995), sometimes associated with cerebral atrophy (Dhenain, Michot, Privat, Picq, Boller, Duyckaerts & Volk 2000) and neuronal loss (Mestre & Bons, 1993). These features are highly reminiscent of the pathological changes observed in the brain of AD patients. These small nocturnal primates, with a body weight in the 70-120 g range, live about 8 to 14 years in captivity. They reach sexual maturity in less than one year and remain fertile until old age. Their reproductive activity is seasonal, under photoperiodic control (Perret, 1997). The modification of daytime duration can be used to shorten the annual cycle to 8 months, in order to shorten their reproduction cycle, leading also to a precocious aging with early signs of physiological changes (Aujard, Dkhissi-Benyahya, Fournier, Claustrat, Schilling, Cooper & Perret, 2001). Bred animals of over 5 yr-old are usually considered as aged (Perret, 1997).

Although the biological and biochemical analyses of cerebral aging in *Microcebus murinus* are well documented, behavioural studies are more limited (Gilissen, Dhenain, & Allman, 2001; Joly, Deputte & Verdier, 2006; Joly, Michel, Deputte & Verdier, 2004; Nemoz-Bertholet & Aujard, 2003; Picq, 1993, 2007). Most of them concern age-related cognitive impairments. Learning and memory abilities of *Microcebus murinus* have been tested to identify age-related deficits, one of the main criterion for AD in humans. The procedures involved different types of discriminative stimuli, including odor (Joly et al., 2006; Joly et al., 2004), spatial context (Picq, 1993, 2007; Picq & Dhenain, 1998), or visual cues (Ritchie, Silhol & Bons, 1997). Although no significative difference was observed between young adults and aged animals in procedures involving odor (Joly et al., 2006) or visual discrimination (Ritchie et al., 1997), some deficits were observed in a spatial rule-guided discrimination task (Picq, 2007). In general, discrimination tests using olfactory or visual systems are very time-consuming in lemur primates. Moreover, previous studies on age-related memory impairment have revealed a greater variability in aged animals compared to young requiring a large number of animals to be enrolled. The mouse lemur is a more practical model than larger primates in terms of cost of housing, life expectancy and ease of breeding. However, it is necessary to develop a sensitive and reliable procedure assessing its memory that will require a limited number of animals.

Following these needs, we adapted a classical spatial working memory procedure in rodents, the three-panel runway task (Furuya, Yamamoto, Yatsugi & Ueki, 1988), to *Microcebus murinus*. Animals had to pass through a series of gated panels, with two of the three gates locked and to memorize the sequence of the open gates that changed daily. Two groups of animals were tested: young adults (2-3 yr-old) and aged (6-12 yr-old) lemurs. The exploratory time, number of reference memory errors, number of repetitive errors and perseverative errors were determined over trials and over sessions. Half of the animals were also tested in presence of a visual stimulus represented by specific symbols on open and closed gates.

Material and Methods

Animals

A total of 98 grey mouse lemurs, *Microcebus murinus*, were included in the study. They were all born in captivity in our laboratory breeding (CECEMA, University of Montpellier 2). Animal care was in accordance with institutional guidelines and animal protocol was approved

by the local ethic committee (authorization #CE-LR-0713). The lemurs were individually caged in the same colony room, where the day/night light cycle was reversed to allow testing during their active period. Food was available every other day and water was provided *ad libitum*. Weight was monitored weekly and food regimen adapted to maintain body weight between 70 and 100 g. During behavioural testing, animals were fed after the experimental period. The ‘young adults’ group consisted of 22 animals tested without symbols and 28 tested with symbols, with age ranging from 2 to 3 yrs and the ‘aged adults’ group consisted of 22 animals tested without symbols and 26 tested with symbols, with age ranging from 6 to 12 yrs.

The behavioural testing was performed in an experimental room separately from the regular housing area, under dim red light, and during the dark phase of the lemur cycle. To avoid handling stress due to capture and transport, animals were previously trained to enter the wooden box in which they were carried to the runway apparatus and back to their home cage.

Apparatus

Working memory was assessed with a three panel runway maze (Matsumoto, Yamaguchi, Watanabe, & Yamamoto, 2004). The apparatus was made in grey polyvinylchloride and composed of a departure box, a goal box and long alley with four consecutive choice panels in between (Fig. 1A). Each choice panel consisted of three sliding gates that could only open in the forward directions because of front stoppers. During testing, rear stoppers were used to block the opening of two of the three gates in each panel. Before testing, animals had first to learn the maze configuration (exploratory stage) and then to understand how to navigate the maze by exiting through the open gates (habituation stage).

Exploratory stage

During the first entry of the animal into the maze, one gate per panel, randomly chosen, was removed and the other two were closed but not locked by rear stoppers (Fig. 1A). When animals reached the goal box, they received a reward consisting of a drop of pear juice. In this stage, a small amount of pear juice was also placed in the centre of each compartment to facilitate their exploration. Animals that reached the goal box were then taken back to their home cage. The lemurs were made to run the task repeatedly until the time elapsed from leaving the start box to reaching the goal box was consistently below 10 min.

Choice habituation stage

During the first choice habituation session, the gate of panel 4 was put back in place, but was not blocked by rear stoppers (Fig. 1B). The animal therefore learned that it had to push and go through one sliding gate of the panel to receive its reward. On the following session, all gates from the last two panels (3 and 4) were closed but not blocked. Then, based on lemur performance, the same protocol was applied to the last three panels. If the choice habituation stage was completed in less than 15 sessions, the lemur entered the testing phase. If no improvement was observed, the animals were not tested further.

Testing phase and procedure

We used the procedure described for studies in rat (Yamamoto, Yatsugi, Ohno, Furuya, Kitajima & Ueki 1990) for the testing phase. In this stage, animals could only open one gate per panel, with the two others being locked by rear stoppers (Fig. 1C). The lemurs had to perform six consecutive trials per daily session. At each choice panel we consider various types of errors. The number of times the animal pushed consecutively the same incorrect panel-gate was counted as perseverative errors. A reference error corresponded to the first attempt to open an incorrect gate. For reference errors, the random performance level is therefore expected to be 4 errors per trial (Yamamoto et al; 1990). A repetitive error corresponding to the re-attempt

to open an incorrect gate previously touched, typically depicts a working memory impairment. Indeed, and contrarily to rodents, lemurs showed a strong tendency to go back towards closed gates even if they had already tested them. For example, at one choice panel, in the following sequence **AAABBB**ABC (open gate: C), the bold letters are counted as reference errors, letters in italics as perseverative errors and underlined letters as repetitive errors. The sequence AAABBBAAABC therefore resulted in 2 reference errors, 5 perseverative errors and 2 repetitive errors. In the interpretation of the data, we mainly focused on the reference and the repetitive errors, all accounting for the total memory errors. A random sequence of open gates was chosen each day, e.g., CBCA (Fig. 1C, D). During this phase, half of the animals were tested with specific symbols drawn above the panels (Fig. 1C): a star corresponded to an open gate and a circle corresponded to a locked gate. Trials were run at 2-min intervals and the maze was cleaned with a 50% alcohol solution after each trial to eliminate interfering odours. The location of the correct gate within each panel was held constant within a session, but was changed between sessions. Therefore, the first trial served as a sample trial, to learn the new combination of open gates, and was included for data analysis in comparison to the following trials. The time spent to complete each trial and errors made during the subsequent five trials allowed assessment of the short term memory of the animal. During each trial, a maximum duration of 20 min was allowed per animal to go through all panels and enter into the goal box for the reward. Within a trial, the lemurs could either explore the maze and reach the goal box within 20 min, or fail to explore or to reach the goal box within that time period. Only lemurs that completed the trial were tested again after 2 min in a subsequent trial for a maximum of 6 trials per day (one session). When an animal completed 5 consecutive sessions, it was considered to have reached the inclusion criterion. For those animals, several parameters were analyzed during these five sessions: (1) number of session-to-criterion; (2) total exploratory time, or time to obtain the reward; (3) number of reference errors (first time pushing a closed gate), 4) number of repetitive errors (pushing a closed gate already touch), and 5) perseverative errors (consecutively pushing the same closed gate) were determined over trials and over sessions. Animals that failed to reach the inclusion criterion after 15 sessions were not tested further.

Data analysis

Individual repartition of animals according to the different stages and number of session-to-criterion were analyzed between experimental groups, young adults and aged adults, tested with or without symbols. The duration of each session and departure latency, were analyzed for sessions 1 to 5. Exploratory time, reference errors, repetitive errors and perseverative errors were analyzed for sessions 1 to 5 and over trials 1 to 6. Data were analyzed using repeated measure ANOVA, with group and session or trial as independent factors, followed by the Newman-Keuls' group comparison test. Averaged values were analysed using a Student's *t*-test. The level of statistical significance was $p < 0.05$.

Results

Group distribution among the different phases of the test

The initial cohorts included 22 young and 22 aged adults tested without symbols, and 28 young and 26 aged adults tested with symbols. Sixty percent of young adults and 48% of old lemurs succeeded in the training phase and reached the testing phase (Fig. 2A), with a tendency for young adult lemurs to improve their performance in the presence of symbols ($p=0.085$, Fisher's exact test). The lemurs that did not reach the testing phase were withdrawn. In the testing phase, no significant difference was observed with or without symbols in young adults ($p=0.086$, Fisher's exact test) whereas the presence of symbols seemed to disturb old animals. The cohort that had succeeded the test consisted of 7 or 8 individuals per group. These young and aged animals that navigated the maze with or without symbols did not differ in terms of number of session-to-criterion as shown in Fig. 2B.

General exploration over the 5 consecutive completed sessions

Total exploratory time was analyzed for young and aged adult lemurs in the absence or presence of symbols. The time to explore the maze did not decrease over sessions but decrease over trials ($p < 0.001$). However young adult lemurs significantly spent more time than old ones to explore the maze when tested without (Fig. 3A) or with symbols (Fig. 3B) ($p < 0.01$)

Memory evaluation over the 5 consecutive completed sessions

Memory was evaluated by analyzing the number of errors during the completed sessions. The repeated measure ANOVA showed that no significant improvement in the number of errors was observed over sessions when performances of the lemurs were analyzed in the absence or presence of symbols. However the aged animals made more errors than young ones over sessions without reaching significance (data not shown).

Evaluation of memory over trials

A surprising tendency for young adult lemurs to show more perseverative errors, *i.e.* consecutively pushing the same closed gate, than aged lemurs was observed in the absence of symbols (Fig. 4A), that became very significant ($p < 0.001$) for animals tested in the presence of symbols (Fig. 4B).

Reference memory was evaluated by analyzing the number of reference errors over trials (Fig. 4). No significant effect of age was observed in the absence of symbols or in the presence of symbols as shown respectively in Fig. 4C and Fig. 4D. However the number of errors decreased significantly over trials ($F_{(5,65)} = 17.30$, $p < 0.0001$ in absence of symbol and $F_{(5,60)} = 10.90$, $p < 0.0001$ in presence of symbols). Aged animals made more errors than young ones and their performance remain above the chance level excepted in the last trial. Conversely, young lemurs showed a gradual improvement without or with visual cues and after the third trial, their performances were clearly better than chance level, reaching significance after the trial 6 ($p < 0.05$).

At each choice panel, the lemur might show a peculiar behaviour: after pushing a wrong gate (for exemple A), the animal can touch a new wrong gate (gate B) and go back to the first one (gate A). These repetitive errors were considered as an impairment in working memory. Young and old lemurs improved significantly their performance from trial 2 to trial 6 concerning these working memory errors in the absence of symbols ($F_{(5,65)} = 18.96$, $p < 0.0001$; Fig 4E), and in the presence of symbols ($F_{(5,60)} = 9.99$, $p < 0.0001$; Fig. 4F) but the number of errors showed a tendency to be higher in old lemurs.

Discussion

The goal of the present study was to determine whether the spatial procedure based on exploration of the three-panel runway maze, largely used in rodents, could be adapted to the primate *Microcebus murinus*. This task allowed a rapid assessment (one month of testing) of a large number of lemurs whereas previously employed task are more time consuming. For example, a minimum of three months are required for odor discrimination in 18 animals (Joly et al., 2006) and over 9 months for visual discrimination in a couple of animals (Ritchie et al., 1997).). Our results showed that the three-panel runway task resulted in clear differences between young adults and aged animals: the young lemurs elicited significantly more perseverative errors whereas the old lemurs showed a tendency to display more reference and working memory impairments.

Previous reports have indicated that the three-panel runway maze allows a rapid evaluation of working memory performances in rats, using a repeated acquisition procedure and being

sufficiently sensitive to reach statistical significance with a low number of animals (Furuya et al., 1988; Matsumoto et al., 2004; Ohno & Watanabe, 1995; Yamamoto et al., 1990). Correct solution of the task requires a win-stay strategy more than a win-shift strategy as used in the radial-arm maze (Yamamoto et al., 1990). The animal should acquire a new and variable information available only within the session, in a series of repeated trials. This spatial discrimination task requires rapid procedural memory acquisition within each session. The different sessions can be considered as an equivalent of reversal tasks. When this procedure is used for rodents, only two days are necessary for the habituation to the apparatus probably because rats display exploratory-based behaviour and spontaneously explore the maze, going through the sliding gates to obtain the reward (Lacroix, White, & Feldon, 2002). However in rodents, about 20 to 30 sessions were required during the training phase for rats to reach the criterion (Ohno, Yamamoto, Kitajima, & Ueki, 1990). In contrast, all mouse lemurs tested showed, surprisingly, a lack of spontaneous exploration and two preliminary stages were necessary for habituation to the maze. The first stage allowed the lemur to understand the goal-directed strategy necessary to get the reward. The second stage tested its motivation to go through the gates. These preliminary stages in the three-panel runway procedure were marked by a high level of attrition, since 40% of young lemurs and 52% of old animals did not reach the testing phase. The first stages of exposure to the test are likely to induce anxiety in animals would subside over time after repeated exposures while learning takes place. The large number of animals excluded before cognitive assessment could be related to the emotionality of animals in a exogenous environment. The presence of symbols of different shapes on the sliding gate seemed effective to improve the access of the lemurs to the testing phase but was ineffective to succeed during the five consecutive sessions. In the future, a training similar to rodents (almost 30 sessions) and a food deprivation schedule could enhance the motivation and may improve the performance of young lemurs. It is well known that mouse lemurs instinctively exhibit a high level of anxiety and distress when placed in a novel environment. This high level of anxiety is likely to be responsible for the high level of perseverative errors. In a report describing the adaptation of a procedural cognitive test battery from the marmoset to the *Microcebus murinus*, Ritchie et al. (1997) outlined that the cage size and surrounding noise were of a major importance. Moreover, during the course of the tests, the authors noted that reversal learning was strongly disliked by the lemurs, which resulted in signs of aggressivity (Ritchie et al., 1997). When *Microcebus murinus* were observed in an open-field, the latency before the first movement, an index of reaction to novelty, was higher in young adult than in aged lemurs (Nemoz-Bertholet & Aujard, 2003). Similar differences in response to novelty have also been reported in memory tests (Picq & Dhenain, 1998). To summarize, these studies and our findings indicate that *Microcebus* are very sensitive emotionally and this could affect their performance in learning and memory as it has been observed in human where cognitive impairment was related to anxiety symptoms (Mantella, Butters, Dew, Mulsant, Begley, Tracey, Shear, Reynolds & Lenze, 2007, Beaudreau & O'Hara, 2009).

The number of session-to-criterion failed to discriminate between young adult and aged lemurs, when animals were tested in the absence or presence of symbols for visual guidance. Both young adults and aged animals learned the task procedure and completed successfully five successive sessions with a similar level of performance. No major cognitive deficit was noted in aged animals. This observation is in agreement with previous reports showing that behavioural procedures relying on simple visual stimulus-reward associations, and thus involving procedural memory, are preserved in aged *Microcebus murinus* (Picq, 2007). For instance, this author developed a procedure to assess executive functions, procedural and declarative memory. Picq (2007) proposed a series of tasks that determined go-no go and visual successive discrimination, set shifting (including extra-dimensional shift and reversal discrimination) and a spatial rule-guided discrimination. In the go-no go and visual discrimination tasks, no difference was detected between young adults and aged animals. Most of the aged animals perform inside the range score for the younger group (Picq, 2007). Our

findings led to similar conclusions, as compared to young adult animals, the aged were not suffering from lack of motivation, disability due to sensorimotor decline or general deterioration of behaviour.

After reaching the criterion, the animals failed to improve over sessions in their ability to perform the task, in terms of exploratory time and perseverative or memory errors. The numbers of memory errors were variable from one session to another. This might be related to the difficulty of the pattern sequence of correct gate locations. The working memory component of the mnesic process, involved in solving gate at each choice panel, overlapped the short term memory reference errors over trials and long term memory component involved in solving the procedure to get the reward. Interestingly mouse lemurs did not adapt their strategy during the five consecutive sessions as rodents do (Furuya et al., 1988; Ohno et al., 1990). Since they could improve their performance over trials, it is conceivable that each new session, based on a new randomized gate sequence, was a new challenge and therefore improvement over sessions could be detected only if location of the correct panel-gates were kept constant within consecutive sessions. This hypothesis will need to be verified in future experiments. Interestingly, a marked difference in exploratory time was detected between young and aged animals: aged animals performed the task faster. This effect was certainly related to the high number of perseverative errors of young adults. Lemurs compared to rats made more than two errors at each choice point (Ohno & Watanabe, 1995; Yamamoto et al., 1990). Aged lemurs made more reference errors than young ones with number of errors higher than the random performance whereas young adults improved the performance under the chance level after the third trial. The aged animals showed also a tendency to perform more repetitive errors. The inability to control repetitive responses to locked gates could be construed as a learning impairment.

These findings indicate that the three-panel runway test can be used to compare memory between young adults and aged lemurs. However, care needs to be taken to measure separately perseverative errors that otherwise would lead to erroneous conclusions. One of the characteristics of the lemur is a tendency to prefer a particular gate. This peculiar behaviour is likely the consequence of the highly emotional state of the young adult lemur, already mentioned. It is well established that anxiety and amygdalar stimulation induce or exacerbate compulsive behaviour (McGrath, Campbell, Veldman, & Burton, 1999). The high emotional state of the mouse lemurs could be related to the brain anatomy of the species. Compared to rat, the *Microcebus murinus* brain is about the same size but with more developed frontal and temporal lobes (Bons, Silhol, Barbie, Mestre-Frances, & Albe-Fessard, 1998; Le Gros Clarke, 1931), providing a greater extent of orbitofrontal cortex, the basal ganglia, amygdala nuclei and hippocampal formation. Such anatomical differences could sustain the observation that mouse lemur appear far more emotive than rodents. In order to attenuate emotional bias, a more ethological environment will be considered in future experiments.

Perseveration performances have been investigated in simians, particularly by object discrimination task (Nakamura, 2001), executive function task (Moore, Killiany, Herndon, Rosene, & Moss, 2006) or with intradimensional/extradimensional (ID/ED) set-shifting task (Weed, Bryant, & Perry, 2008). A perseverative error was recorded when a monkey made an error by choosing an incorrect stimulus that would have been correct under the previous response contingency. Moore (2003, 2006) generally observed that, in contrast to our finding in lemurs, that middle-aged and aged monkeys demonstrated a greater tendency toward perseverative responding than did young adult monkeys. Conversely, Weed et al. (2008) showed that, compared to adults, juvenile rhesus monkey were impaired on reversal of simple discrimination, intradimensional shift, reversal of intradimensional shift, and extradimensional shift stages of the task. These results indicated that juveniles committed more perseverative errors and more errors on the set-formation and set-shifting components of the ID/ED task. In

extradimensional shift and reversal discrimination in mouse lemurs, Picq (2007) reported that the difference in perseverative errors between young adult and old animals was not significant although some aged subjects showed great difficulties in disengaging from the previous rule, resulting in a large number of perseverative errors. The same conclusion was made by Joly et al (2006) with olfactory test where some aged mouse lemurs displayed perseverative behaviour whereas others performed like young animals. With regard to which brain regions may be involved in this behavioural pattern, the involvement of frontal cortical areas in perseverative errors has been suggested in monkeys (Moore, Killiany, Herndon, Rosene & Moss, 2003; Nakamura, 2001; Weed et al., 2008) but lesional studies are needed to confirm that the increase of perseverance errors is clearly due to prefrontal cortex. The prefrontal area could be involved in the inhibition of a previously learned strategy and the generation of new strategy. Several investigations have suggested that the prefrontal cortex, basal ganglia, thalamus and hippocampus, interacting in network, together, or putatively in parallel, are needed for acquisition of new information and attentional set-shifting (Burgess, Maguire, Spiers & O'Keefe, 2001; Murray, Bussey & Wise, 2000). We therefore propose that age-related changes in the frontal and temporal areas of the lemurs blunt their emotional responsiveness which results in less perseverative behavior. Further anatomical, histological and neurochemical analysis of the brains of behaviourally tested animals may help to confirm this hypothesis.

Finally, the three-panel runway procedure could be used to quickly test a large number of mouse lemurs. It will be necessary to improve the procedure but considering its relative ease of use, the three-panel runway test may be useful in longitudinal follow-up related to ageing and/or immunotherapy experiments in Alzheimer's disease. Not all aged subjects appeared equally affected by aging. These age-related and inter-individual variations should be related to cerebral anatomical changes and further investigations, particularly focusing on frontal and temporal areas, will be necessary to correlate age-related behavioural decline to neuropathological findings.

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Figure 1.

Whole behavioral procedure in the three-panel runway maze for lemurs. (A) Exploratory stage: two sliding gates are closed and one left open in each panel to allow the animals to exit the start box, explore the maze and retrieve the pear juice reward in the goal box. (B) Choice habituation: after successful exploration, all three sliding gates of the last panel are closed, i.e., in panel 4, then in panel 3 and 4, etc... (C) Testing phase: when animals successfully learned to push the sliding gates to pass through the next compartment for all 4 panels, specific pathways were randomly selected each day, like CBCA, and the two other gates in each panel blocked with front stoppers. Two procedures were followed with or without specific symbol drawn on the gates and associated with open (*) or closed (o) states. (D) Procedure: each session consisted in 6 trials per day, with inter-trial time interval of 2 min. The first trial was a sample trial in which animals learned the new combination of open gates each day and memory errors

were recorded during the 5 last trials. Animals succeeding in performing the 6 trials during 5 consecutive sessions reached the inclusion criterion.

Figure 2.

(A) Individual repartition of animals according to the different stages. Young and aged lemurs were tested in the procedure with or without symbols. The numbers in the pie charts indicated the numbers of animals reaching the different stages. Percentages over the whole population are shown within parentheses. No statistical significance was measured in repartitions for age and symbol ($p > 0.05$, Fisher's exact test). Note that $p = 0.08$ for symbols between the two young groups. (B) Number of sessions to reach the inclusion criterion. The number of animals is indicated within the columns. ANOVA, $F < 1$ for age, $F_{(1,12)} = 1.99$, $p > 0.05$ for symbol, and $F < 1$ for the age x symbol interaction.

Figure 3.

Exploratory time over trials in young and aged lemurs submitted to the three-panel runway test without symbols (A) or with symbols (B). Av: average of the data over the 5 test trials; * $p < 0.05$, ** $p < 0.01$ vs the first trial, Dunnett's test; # $p < 0.05$, ## $p < 0.01$ vs young group in the same trial (Newman-Keuls' test).

Figure 4. Evolution of perseverative errors (A, B), reference errors (C, D) and repetitive errors (E, F) over trials in young and aged lemurs submitted to the three-panel runway test. (A, C, E) procedure without symbols; (B, D, F) procedure with symbols. Av: average of the data over the 5 test trials. Repeated measure ANOVA; * $p < 0.05$, ** $p < 0.01$ vs the first trial, Dunnett's test). # $p < 0.05$, ## $p < 0.01$, ### $p < 0.001$ vs young group in the same trial (Newman-Keuls' test).