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Running head: Olfactory discrimination in a prosimian primate

Keywords: Aging; Olfactory discrimination; Primate; *Microcebus murinus*

Abstract

In order to characterize age-related cognitive changes, olfactory discrimination was assessed in *Microcebus murinus*, a prosimian primate. We compared young (n=10) and old (n=8) animals for individual performance on three olfactory tasks. Animals had to perform a detection, a transfer, and a reversal learning task using a go, no go conditioning procedure. No differences were observed between the two groups, indicating that aging is not inevitably associated with a decline in cognitive function. We did, however, observe two aged animals showing altered behavior. One animal displayed impairments in the reversal learning task, and the other showed impairments in both the transfer and reversal tasks. Transfer impairment may be due to an hippocampal alteration, whereas the perseverative tendency noted in the reversal task may be associated with frontal lobe dysfunction. Because some aged *M. murinus* display lesions that are pathognomonic of Alzheimer's disease, our observations highlight its potential utility as a primate model for studying cognitive deficits in relation to age and associated pathologies.

Keywords: Ageing; Olfactory discrimination; Primate; *Microcebus murinus*

1. Introduction

A desire to understand the cognitive decline that accompanies age-associated neurodegenerative disorders has driven investigators to develop animal models and to assess cognitive function decline through different tasks. We have recently demonstrated that the gray mouse lemur (*Microcebus murinus*), a primitive non-human primate, is a good model for assessing cognitive function through olfactory two-odor discrimination tasks [13]. Indeed, olfaction is an extremely well-developed sensory modality in this primate, which uses olfactory cues (urine-marking behavior) to communicate with its congeners [19].

The mean life span of mouse lemurs is 5 years old [18]. As a consequence, animals of more than 6 years old are considered to be senescent and display anatomy, behavior, memory capabilities changes [4, 7, 20, 21]. In addition, a fraction of older animals spontaneously develop lesions pathognomonic of Alzheimer's disease [4]. As patients affected by Alzheimer's disease (AD) have impaired odor identification relative to normal elderly individuals [11], we were prompted to use *Microcebus murinus* as an animal model for better understanding age-related decline in sensory or cognitive function. We studied the effects of age on olfactory ability in the gray mouse lemur. The performance of young and old individuals was compared in three different two-odor discrimination tasks: detection, transfer and reversal learning.

2. Materials and Methods

2.1.Animals

Young adult (n=10; 3-4 years old) and aged adult females (n=8; 6-14 years old) were the subjects of the experiment. They were all born in captivity and belonged to our laboratory breeding colony. They had no previous experience with cognitive testing, and were totally

naive to the methods described below. Subjects were maintained under a 14h:10h light:dark cycle and housed individually during the experiment. They were fed daily with one piece of fruit (apple or banana) after testing, and they were allowed to drink during the test. Weight was monitored weekly and maintained between 60 and 70 g. Because these animals are nocturnal, testing was performed during the dark phase under a red light. The experiments were approved by the local ethics committee (Comité d'Ethique Régional en Expérimentation Animale, Languedoc-Roussillon, CE-LR-0303).

2.2. Conditioning procedure

The method employed was the same as that described previously [13]. Briefly, we used an eight channel liquid dilution olfactometer (Knosys Olfactometers; http://www.chemsenses.com). The unit consists of an operant chamber equipped with a glass odor-sampling tube and an eight-channel odor generator. Odorants (strawberry: GA512084 ; pear: GA512424) were nature-like aromas from Givaudan (Dubendorf, Switzerland). Odor concentrations used were those indicated by the manufacturer to elaborate fruity drinks.

Go, no go procedures (see [23] for details) were used for initial training and discrimination training. Initial training required that the lemur inserted its snout in the odor delivery port and responded by licking on a reinforcement tube when an odor was detected. Ethyl acetate diluted to 2% in water was used as the training stimulus. Once the animal responded reliably, it was trained on a second odor detection task in which strawberry (0.015%) served as the S+ stimulus and an odorless solvent (water) served as the S- stimulus. S+ and S- stimuli were presented in a modified random order in each session. Responding (*i.e.* licking at the reinforcement tube) in the presence of the S+ odor was rewarded with a drop of about 0.05 mL of apple juice and scored as a correct response (hit). Responding in the presence of S- stimulus was not rewarded and was scored as an error (false alarm). Not

responding in the presence of the S+ stimulus was scored as an error (miss), while not responding in the presence of the S- stimulus was scored as a correct response (correct rejection). Training on this task was carried out for 40 trial sessions, until the subject reached the criterion performance of 82.5% ($p<0.0001$), *i.e.* 33 correct responses. The task was terminated when the criterion was achieved in two consecutive sessions. Next, each lemur was tested on two other tasks: A transfer task in which the S+ stimulus was strawberry (0.015%) and the S- stimulus was pear (0.05%), and then, when the animals had achieved the criterion performance for this task, they had to perform a reversal task in which the S+ and S- stimuli assignments were reversed (the pear odor served as S+ and the strawberry odor served as S-).

2.3. Data analysis

Results are expressed in mean number of sessions ± SEM. First, to assess cognitive flexibility, we compared performances between tasks using a Wilcoxon signed rank test. On one hand, we compared performances on the detection and transfer tasks, and on the other we compared performances on the transfer and reversal tasks. Second, we compared performances between young and old animals on each task with a Mann-Whitney test.

3. Results

The performances recorded on the detection and transfer tasks differed significantly for both the young ($W=45$; $p=0.039$) and old ($W=34$; $p=0.0156$) individuals (Fig. 1). The transfer task was more easily achieved than the detection task. Similarly, success on the reversal task was more difficult to achieve than it was on the transfer task, for both the young ($W=-55$; $p=0.002$) and old ($W=-28$; $p=0.0156$) individuals (Fig. 1). No differences were seen between the two groups on the detection ($U=20$; $p=0.0831$), transfer ($U=31.5$; $p=0.4739$) or reversal

tasks (U=33; p=0.5629). Although no group differences were observed between young and old individuals, we did note inter-individual variation (Fig. 2). Although young animals and most of the old animals displayed similar performances on the three tasks (Fig. 2a, b), two aged subjects showed difficulties on either the reversal task or on both the transfer and reversal tasks (Fig. 2c, d). Specifically, one of them succeeded on the transfer task but took 20 sessions to successfully perform the reversal task (Fig. 2c), in comparison to the other aged females who achieved success in an average of only 9.33 sessions. The other animal failed on the transfer task and also displayed the poorest results observed on the reversal task (Fig. 2d). Indeed, it took 20 sessions to meet the criterion performance on the detection task, and 23 on the transfer task. On the reversal learning task, it attained a 50% correct response level (chance level) only at the 10^{th} session. Interestingly, these two subjects displayed perseverative behavior and continued to respond to the previous S- stimulus. Furthermore, at the beginning of the task, we observed distress calls and behaviors indicating irritation (hitting the test chamber wall with its tail), which had never been observed in previous sessions.They only began to inhibit their behavior at the 15th and 21st sessions respectively.

4. Discussion

Our study led to the conclusion that olfactory memory functions are preserved during aging in *Microcebus murinus*. Most of the aged subjects were able to detect, to transfer and to reverse well. To our knowledge, this is the first study evaluating the influence of age on olfactory abilities in non-human primates. There is however studies that also showed no differences on a discrimination task between young and old individuals rats [14], although others studies noticed a lower performance in old individuals during the reversal learning [25]. Conversely, a decrease of olfactory sensitivity in aged animals seems to be shared by

several species. For instance, such changes in olfactory sensitivity have been observed in the gray mouse lemur [2], in humans [27], but also in aged rats [1]. In the latter case, changes in sensitivity were related to changes in receptor density with maximal sensitivity occurring at approximately 200 days.

There seems to have no correlation between errors and absolute age. For instance, the oldest animal (14 years old) obtained similar results as young individuals: 11, 4, and 11 sessions in detection, transfer and reversal tasks respectively, compared to 13.7, 3.4, and 10.4 sessions for the young individuals. In addition, the youngest animal (7 years old) of the aged group displayed impaired performances (20, 23, and 27 sessions respectively). In humans, a recent follow-up study concluded that only about 9% of the elderly show a progressive cognitive decline over a given three year period [5]. Taken together, these results show that cognitive decline during aging is not inevitable.

We did observe, however, that transfer and reversal tasks were impaired for two aged subjects (7 and 8 years old): one female was almost unable to transfer the task, and the other was able to neither transfer nor reverse the tasks. We ruled out a motivational problem because these animals, when placed into the test box, quickly finished the 40 trials in less than 10 minutes. We also ruled out a loss of olfactory receptor cells, as it has been observed in senescence-accelerated mice [17], because the concentrations used in our experiments were easily detected by a human nose that is supposed to be less or equally sensitive to monkeys (see [10]). Moreover, if sensitivity was altered, animal that failed during both transfer and reversal tasks would not have displayed only 10 % correct responses in the first session of the reversal task. Indeed, this means that stimuli were discriminated. Our observations are thus more consistent with a central alteration than with a peripheral one, *i.e.* an alteration of the cortical structures rather than a defect of the olfactory bulb. In our case, all animals perceived and discriminated between two odors well, but failed in tasks for which greater cognitive

treatment was needed. The transfer task relies on the ability to shift. In humans, generalization impairments involving novel combinations of familiar stimuli have been associated with the hippocampal system [15, 16]. Therefore, the deficit patterns observed on the transfer task in *M. murinus* could result from hippocampal dysfunction. Such age-related deficits have been observed for performance on hippocampus-dependent tasks such as the Morris water task (for review, see [9]). In addition, in studies using magnetic resonance imaging and proton magnetic resonance spectroscopy in humans, such impairment patterns have been correlated with a decrease in hippocampal volume, implying neuronal loss and/or a decrease in neuronal density [8]. On the reversal task, animals must unlearn what they have learned in a previous task, and then shift. This behavior is considered as an executive function, and failure to reverse results in perseveration. Perseveration is associated with an impairment in inhibiting responses to previously rewarded stimuli. The perseverative nature of behavioral deficits has been associated with frontal lobe ablation in the case of reversal of visuospatial tasks in marmosets [22], and with frontal lobe neurotoxic lesions in rhesus monkeys [3]. Other recent studies of reversal learning deficits following damage to the ventral frontostriatal circuitry in rodents and nonhuman primates also support this observation [6]. In humans, fronto-temporal dementia is associated with stereotyped behavior and perseveration. Together, these data suggest that an inability to reverse a task is associated with frontal alterations.

Few studies have reported age-related impairments in olfactory ability in animals. Slotnick et al. [26], however, observed that rats with mediodorsal thalamic nucleus lesions had no deficits in retention, but moderate deficits in the acquisition of novel odor discrimination, and a severe deficit in reversal learning. Interestingly, in age-associated human pathologies, olfactory dysfunction has been well documented (for review, see [12]). For instance, in Alzheimer's disease (AD), olfactory disorders may represent an early feature of the disease, and smell sense impairments have been more often associated with central than

with peripheral levels, although there is a lack of large studies in this regard. For instance, in Alzheimer's disease (AD), olfactory disorders may represent an early feature of the disease, and smell sense impairments have been more often associated with central than with peripheral levels, although there is a lack of large studies in this regard. Shiffman and colleagues [24], however, evaluated the possibility that chemosensory measures, including smell, could be a sign heralding the onset of Alzheimer's disease in genetically at-risk individuals. They showed that the at-risk group displayed lower smell memory.

Further investigation is required to identify which brain structures are altered in our impaired animals. Overall, these data support the use of *Microcebus murinus* as a model for studying cognitive deficits in relation to age as a long term goal, as well as in association with pathologies such as neurodegenerative disorders.

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Figure legends

Fig.1. Comparison of performances between young and old individuals during the three tasks. Task-1: fruity odor *vs* pure air discrimination; Task-2: two fruity-odor discrimination; Task-3: reversal learning. Mean sessions ± SEM. * $p<0.05$, ** $p<0.005$.

Fig.2. Typical individual performances on the three discrimination tasks. Performances of ***a)*** a young individual, ***b)*** a "normal" aged individual, ***c)*** an aged individual with impairments on the reversal task and, ***d)*** an aged individual with impairments on both the transfer and reversal tasks. Chance level (dashed line) and criterion level (continuous line) are indicated. One session corresponds to 40 consecutive trials.

Figure 1

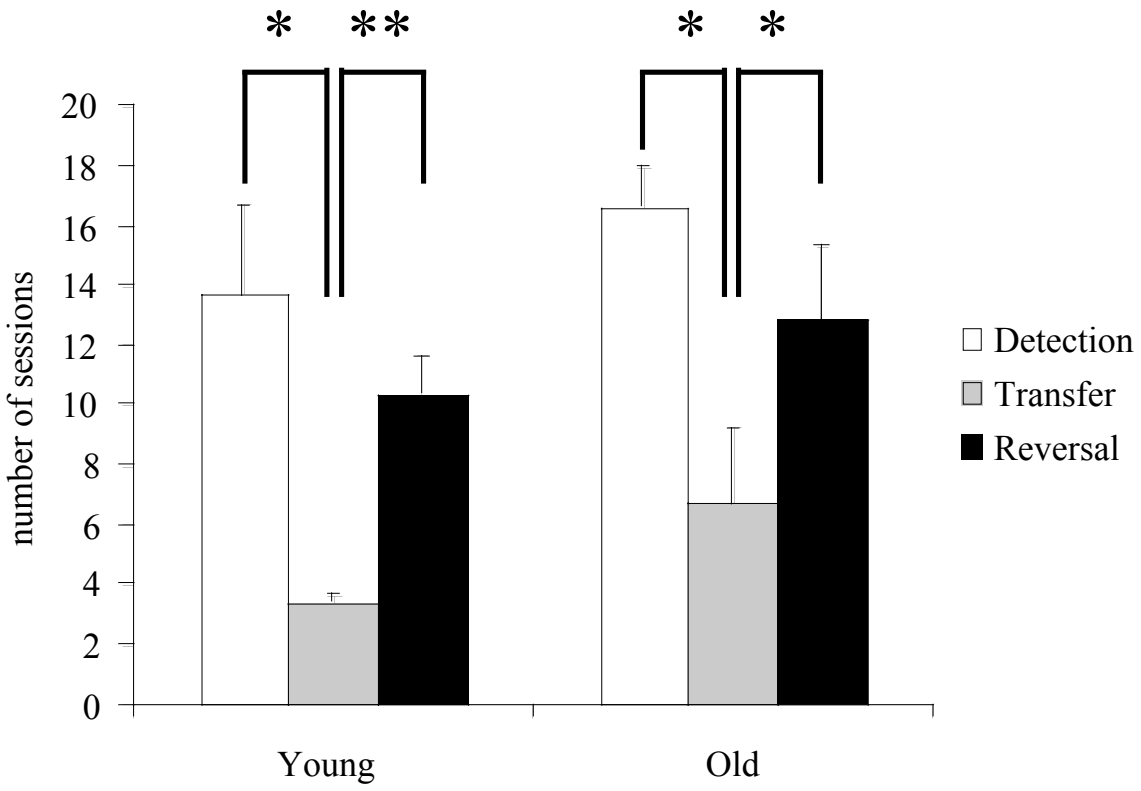

Figure 2