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Object recognition is not impaired in old rats

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Abstract. Twenty two months old and eight months old rats were used. They were submitted to object recognition test with 1 min interval between two successive presentations of the same object or two different objects. Initial exploration of the object was lower in aged than in young rats. However, both groups showed comparable habituation of exploratory responses in the presence of the familiar object. The changed object did not produce the decrease of exploration. These findings provide further evidence that memory involved in object recognition is not affected by aging.

Key words: object recognition, aging, rat

INTRODUCTION

A growing body of evidence points to a distinction between the spatial and nonspatial type of working memory. This distinction is particularly evident in the effects of damages to the hippocampal region and aging. In rats and monkeys hippocampal lesions produced severe deficits in spatial tasks whereas tasks using visual stimuli, in particular - objects were spared (Parkinson et al. 1988, Xavier et al. 1990, M'Harzi et al. 1991, Rothblat and Kromer 1991, Jackson-Smith et al. 1993). Age related declines in learning and memory of old subjects has received a great deal of attention. One of the most consistent findings is that aged animals and humans are impaired in tasks demanding processing of spatial informations. Memory dysfunction in the aged rats was found in studies using the delayed non-matching T-maze test (Ando and Ohashi 1991), radial arm maze (Ingram et al. 1981, Barnes and McNaughton 1985) and Morris water maze (Rapp et al. 1987). Several studies indicated the dissociation between the spatial and nonspatial tasks. Aggleton et al. (1989) demonstrated an impairment in the performance of aged rats on the spatial delayed non-matching-to-sample, whereas nonspatial delayed non-matching-to-sample was normal. Cavoy and Delacour (1993) found that spatial but not object recognition was impaired by aging in rats. In the study by Willig et al. (1987) old rats discriminated between novel and familiar objects as accurately as young rats.

As stressed by Ennaceur and Delacour (1988), the tests used in most studies on working memory in animals involve also a component of reference memory since they are based on the learning of a rule, e. g. matching or non-matching to sample, the choice of the arm previously closed (delayed alternation in rats) or avoiding the arms that were already visited in the trial (radial arm maze). In addition, stimulus-reward or response- reward associations during a pretrening stage may pose a difficulty on an interpretation of results.

Tests have been recently developed for rats based on their spontaneous exploratory behaviour and preference for novelty using the principle of discrimination between familiar and novel objects (Ennaceur and Delacour 1988, Myhrer 1989, Thinus-Blanc et al. 1991, Łukaszewska 1993a). For spatial memory, discrimination between familiar and novel places or detection of change in the spatial rearrangement of familiar objects were used (Łukaszewska and Dławichowska 1985, Thinus-Blanc et al. 1991, Ennaceur and Meliani 1992, Thinus-Blanc and Foreman 1993). These recognition tests may be considered as measuring forms of working memory since they are based on the match-to-sample principle. The additional advantage of these test consists in not involving the reinforcement such as food or electric shock. The features of the recognition tests allow for clearer dissociation between object and spatial working memory in research on brain pathology or aging.

The aim of the present paper was to compare recognition of the familiar or novel objects by young (8 months old) and aged (22 months old) rats. These experiments could further contribute to validation of the statement that object recognition memory is not affected by aging.

METHOD

Subjects

The subjects were two groups of male Wistar rats. One group consisted of 14 rats 22 months of age, the other group consisted of 20 rats 8 months old. They were born in the Animal Breeding Laboratory of the Nencki Institute. Rats were housed in groups of 6 in semi - transparent plastic cages in the colony room with natural lighting conditions. Food pellets and water were available *ad lib.* Animals were not naive. Prior to the start of the present experiment rats from the old group were submitted to several different tests involving object recognition. The last test was performed 8 months before the present experiment. Rats from the young group were used in three similar tests - the last one preceded the present experiment by 1 month.

Apparatus

The apparatus was circular arena 75 cm in diameter with the wall 38 cm in height, painted uniformly in light grey. A camera above the apparatus was connected to a video recorder and TV screen. Thus, the experimenter could observe the animals not disturbing their behaviour.

The object used were metal can and plastic or glass bottles never used before in the preceding tests. The objects existed in duplicate. Their weight was such that they could not be displaced by rats so the objects could be classified as non manipulable.

Procedure

Preceding the testing session rats were individually given a 2 min exploration of an empty arena for 1-2 days. Animal did not show emotional reactions during exploration, specifically, they did not defecate and urinate. Testing session consisted of two 3 min trials separated by 1 min (old rats) or 5 min (young rats) retention interval. Rats spent the intertrial intervals placed singly in the plastic buckets.

During the Trial I (exposure trial) an object was placed in the middle of arena. During the Trial II either the duplicate of the same object or different object was presented. To reduce object preference effect, for half of the group object " X " was presented either on the Trial I or on the Trial II. For the other half of group object " Y " was presented in the same way. So the appearence of the two objects was counterbalanced.

Old rats were subjected to two tests: in the first test the group was confronted with the same object on both trials, in the second test, performed two weeks later, the group was confronted with different objects on Trial I and Trial II.

Young rats were submitted only to one test: half of the group had familiar object on Trial II, the other half - the new object. Rats were put into the apparatus always in the same place. The apparatus was cleaned between subjects to eliminate scent marks.

Data collection

The data collected from the videotape consisted of the number of contacts with object and their duration. These data were used for calculation of the overall exploration time. The locomotor activity in the arena was also scored. For estimation of this measure the picture of arena was divided on 9 equal sectors (Fig. 1). The drawing was performed on translucent sheet of foil and placed on TV screen. The number of crossings of the arena sectors provided the index of locomotor activity.

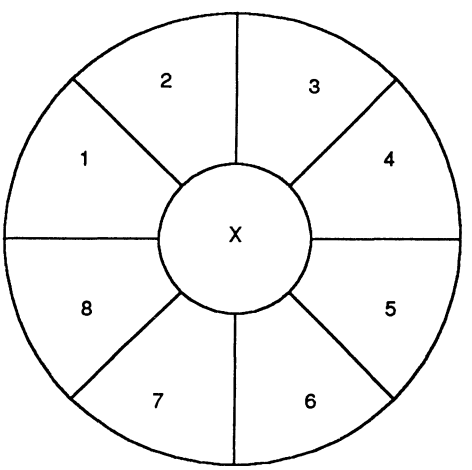

Fig 1. Division of the arena in nine sectors. X, the place of the object location.

RESULTS

Exploration of a familiar object

OVERALL EXPLORATION TIME

Figure 2A shows the time course of exploration of an object never seen before (Trial I) and exploration of the same object presented again on Trial II, in the two age-groups.

Three dimensional analysis of variance was conducted on exploration time with the age, within-trial habituation (successive minutes) and between-trial habituation (successive trials) as the main factors. No significant difference was found between the

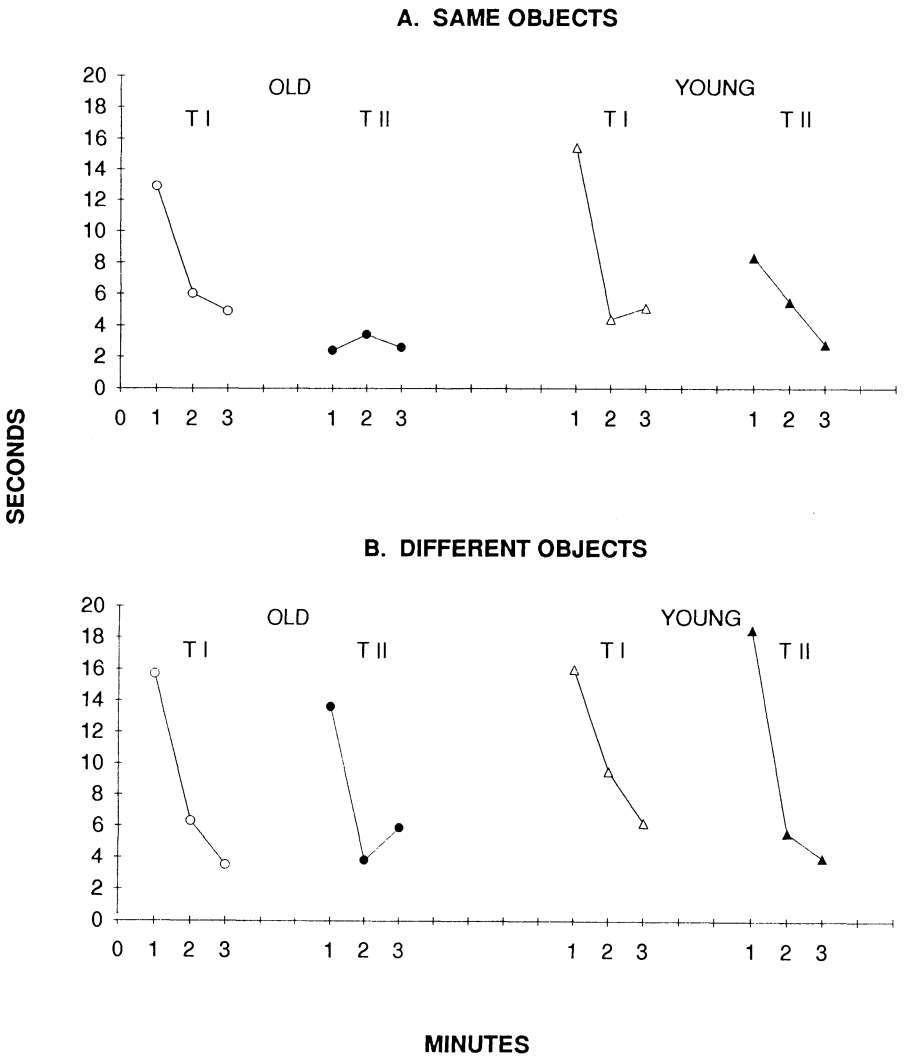

Fig 2. Overall time of exploration in successive minutes of Trial I (T I) and Trial II (T II). A, the same object in Trial I and Trial II; B, different objects in Trial I and Trial II. Circles, old rats, 22 months old. Triangles, young rats, 8 months old. Open symbols, Trial I. Filled symbols, Trials II.

old and the young group. The within-trial habituation appeared significant $F(2,22)=14.680$, $P<0.001$ as well as between-trial habituation $F(2,44)=20.150$, $P<0.001$. Both age groups showed similar habituation as evidenced by nonsignificant interactions between age and habituation factors. The significant interaction of within-trial x between-trial habituation $F(2,44)=13.03$, $P<0.001$ indicates different pattern of exploration decrease in Trial I and Trial II. As is evident in Fig. 2 some small but significant ($P<0.05$, Wilcoxon test) dishabituation between the last minute of Trial I and the first minute of Trial II occurred in the young group.

NUMBER OF CONTACTS AND MEAN CONTACT DURATION

The mean number of contacts with the object was lower in Trial II than in Trial I (Table I). Two dimensional ANOVA indicated that the decrease was significant $F(1,22)=18.110$, $P<0.001$. The age groups did not differ in this respect as shown by both nonsignificant age effect and age x trial interaction. The mean duration of contacts did not contribute to between- trial habituation of exploration time. Two dimensional ANOVA did not reveal significant effect of trials. The age effect and age x trial interaction were also nonsignificant. The duration

TABLE I

Measures of object exploration							
Age		22 months			8 months		
Measures		Number of contacts	Mean contact duration (s)	Duration of I-th contact (s)	Number of contacts	Mean contact duration (s)	Duration of I-th contact (s)
Familiar	Trial I	9.6	2.4	3.4	9.9	2.4	3.4
	Trial II	4.3*	2.1	2.5	6.7*	2.4	3.9
Novel	Trial I	10.6	2.3	3.3	11.6	2.7	2.1
	Trial II	7.3***	3.1*	4.7**	8.6***	3.2*	9.4**

*P<0.01, **P<0.02, ***P<0.001

of the very first contact with the object in Trial I and Trial II did not differ either in the old or in the young group (ANOVA).

Exploration of a changed object

OVERALL EXPLORATION TIME

The time course of object exploration in Trial I and Trial II is presented in Fig. 2B. In both trials the objects were new for the rats, therefore the overall exploration time in Trial I was not significantly different from that in Trial II either in the old group or in the young group. The within-trial habituation occurred in both groups $F(2,40)=38.610$, $P<0.001$. Old rats explored less than young rats. The difference was small and barelly attained the low level of significance $F(1,20)=4.400$, $P=0.046$. No interaction appeared significant.

NUMBER OF CONTACTS AND MEAN CONTACT DURATION

Although the difference between Trial II in the overall exploration was insignificant in either age group, the number of contacts with the object decreased from Trial I to Trial II $F(1,20)=26.360$, $P<0.001$. Nonsignificant age effect and age x trial interaction indicate similar decrease in both age groups.

In contrast to the number of contacts the mean contact duration increased significantly between Trial I and Trial II $F(1,20)=9.19$, $P=0.006$ in both groups (nonsignificant age factor and age x trial interaction). The duration of the very first contact with the object in Trial I and Trial II differ significantly $F(1,20)=7.040$, $P<0.02$. This means that rats immediately perceived the difference between the object presented in Trial I and that in Trial II and increased the duration of the contact already on the first encounter. There were not significant age factor and age x trial interaction.

Locomotor activity

Old rats displayed slightly lower level of locomotor activity than young rats did (Fig. 3). Three dimensional analyses of variance (with age, trials and successive minutes as the main factors) were performed separately for two kinds of tests i. e. when the object was changed in Trial II or when it remained unchanged in this trial. Both analyses indicated a significant age effect $F=(1,22)=4.710$, $P=0.039$ for the familiar object test and $F(1,20)=4.290$, $P=0.045$ for the novel object test.

Similarly to the object exploration, the locomotor activity decreased within each trial $F(2,44)=55.210$, $P<0.001$ for familiar object test and $F(2,40)=25.250$, $P<0.001$ for the changed object test. However, in contrast to the object explor-

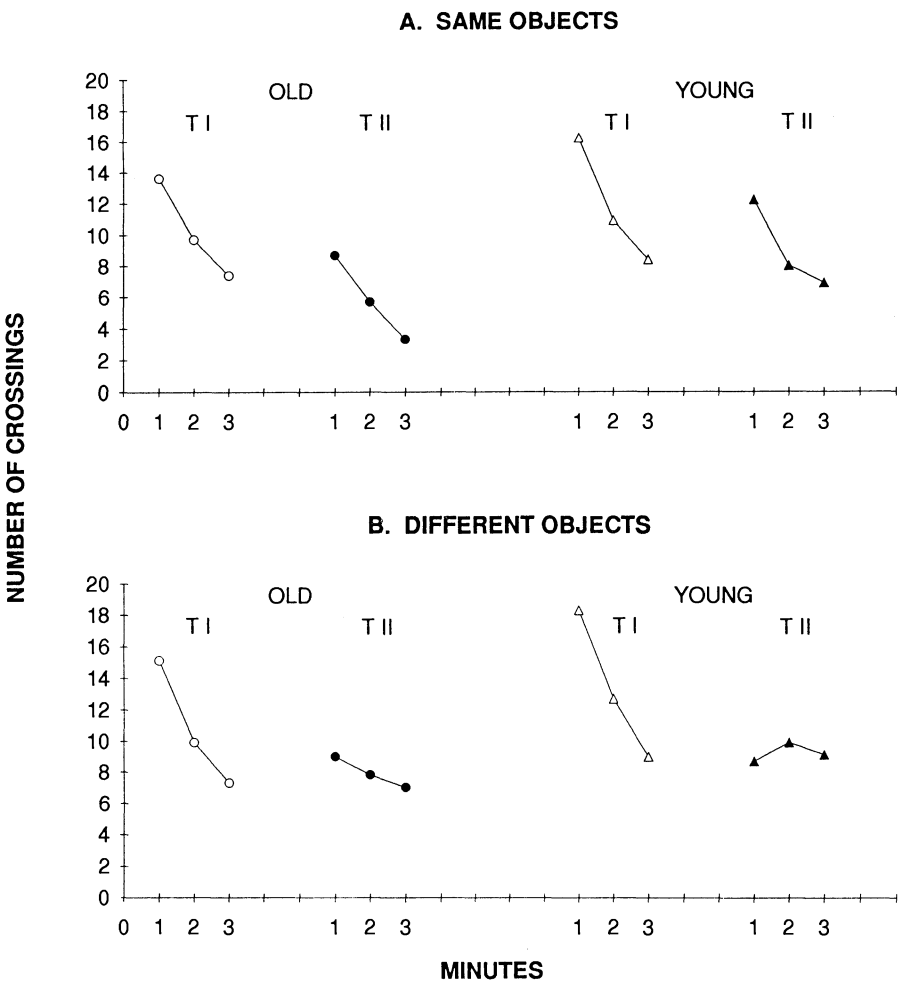

Fig 3. Locomotor activity in successive minutes of Trial I (T I) and Trial II (T II). Denotations as in Fig 2.

ation, locomotor activity in Trial II was lower than that in Trial I in both tests, irrespectively if the change of object occurred $F(1,22)=28.260$, $P<0.001$ for the familiar object test and $F(1,20)=33.460$, $P<0.001$ for the novel object test. Nonsignificant interactions age x minutes and age x trials point to similar decrease of locomotion in old and young rats in successive minutes of the trial and in successive trials.

DISCUSSION

The present experiment provides additional evidence to the findings of several earlier studies (see Introduction) suggesting that in rats working memory involved in object recognition is not affected by aging. The similarity of our results to those reported in the previous experiments is of importance because the tests differed from our test in several metodological aspects. Particularly, in Cavoy and Delacour study (1993) rats were exposed to two identical objects in Trial I, then in trial II rats were exposed again to two objects, one of them was the same as in Trial I (familiar object), the other one was different (novel object). In our experiment in both trials only one object was presented, in Trial II either the same as in Trial I (familiar object) or different one (novel object). The shape and size of the apparatus were also different in these two studies. However, in spite of the differences in methods, the main result was the same: old rats were able to recognize the familiar or novel object with the same efficiency as young mature (8 months old) rats.

Similarity of habituation processes in old and young group was evidenced by no significant interactions between age and successive minutes or trial factors. Both groups reacted to familiar or novel object with the decrease or increase of the same elements of exploration (number of contacts or mean contact duration). It should be stressed that both old and young rats could immediately recognize a novel object as showed by the increased duration of the very first contact with the object.

The lower locomotor activity in old group was probably caused by greater fatigue. Rats were well familiarized with arena so this was not reason for neophobia. It seems that locomotion reflected species-specific "patrolling" activity (Shillito 1963), with the decrement between Trial I and Trial II that would be expected in a stable environment (arena). Lower locomotor activity in Trial II, irrespectively of the presence of familiar or novel object, points to the different processes operating in object exploration and locomotion aimed at patrolling of environment. Again, no difference was found in behaviour of old and young group.

The present results jointly with our previous findings on the effects of scopolamine on visual and spatial tests (Łukaszewska and Dławichowska 1985, Łukaszewska 1993a) support the idea of dissociation of working memory into two subtypes, one involved in object and the other in spatial recognition. Scopolamine (1.0 mg/kg) decreased the level of object exploration (Łukaszewska 1993a) which is in accordance with other studies (Cheal 1981, Willig et al. 1987) but had no effect on object recognition. This is evidenced by a significant habituation of exploratory responses even when long (20 min) retention interval was applied. In contrast, the test which required the spatial localization of visual change following short (1 min) retention interval was affected by scopolamine (1.0 mg/kg) if the rats acquired the relevant information by distal observation (the passive test). However, similar test based on the same principle, but allowing the rats for free exploration (the active test), was not affected by scopolamine when 1 min retention interval was applied (Łukaszewska and Dławichowska 1985). This apparent inconsistency was resolved by results of the active test utilizing longer (20 min) retention interval. In this case scopolamine affected performance (Łukaszewska 1993b). It is noteworthy that the untreated rats can retain the information about spatial localization of visual cues for longer time (45 min, unpublished observation) in active version of the test than in the passive one (25 min, Łukaszewska and Dławichowska 1982). Actively acquired information may involve the greater number of associations and therefore it is more resistant to disturbing factors like passage of time or drug influence. Similarly, encoding of an object may be based on abundant information with regard to its various features like size, colour, temperature etc. This is in line with the suggestion of Cavoy and Delacour (1993) that object and spatial recognition tests could differ not only by the nature of the information but also by the difficulty because discrimination between objects could be based on several dimensions.

The task difficulty might contribute to the dissociation of spatial and object recognition, however, considerable body of data indicates the existence of separate processing of the informations relevant either to object perception such as shape, colour and texture or to movement and spatial location (Livingstone and Hubel 1988). There is evidence in non-human primate that these streams remain differentiable throughout the visual system (see DeYoe and Van Essen 1988). Experimental and clinical lesion studies on monkeys and humans provided compatible results. Damage to posterior parietal cortex impairs spatial but not object information processing whereas damage to the inferotemporal areas produced deficits in object but not spatial information processing (Newcombe et al. 1987). A functional dissociation of the spatial and object visual systems was revealed by selective interference in intact young adults (Tresh et al. 1993). Huxby et al. (1991) found that in people performing a spatial task increases in blood flow occur in posterior parietal areas, whereas increases in flow in inferior temporal areas occur when people are engaged in an object or colour task.

These data are in favour of the idea of Cavoy and Delacour (1993) suggesting the existence of two dissociable neural systems respectively involved in object and spatial recognition.

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