Assessment of learning strategy in young and aged rats in modified holeboard test: Prevention of memory deficits by ladostigil

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Aim

The aim of the study is to investigate learning strategy in young and old rats, to detect the leading modalities in formation of behaviour in holeboard test [1] (HB) and to assess the potential of ladostigil (TV-3326) - a novel drug with anti-inflammatory activity [2] to prevent aged-related cognitive decline.

Methods

The subjects were male Wistar rats, aged 3.5 months (Young, n=10) and 20 months (Aged, n=14; Aged + ladostigil, n=8). Chronic oral treatment with ladostigil (1 mg/kg) commenced 4 months before training and lasted till the end of testing. *Apparatus:* The holeboard is a box (70 * 70 * 45 cm) with dark brown transparent walls and lid. There are 16 holes (3.5cm diameter) in the black opaque floor. Each hole with a plastic cup (1.5 cm deep) could be illuminated from below. Sunflower seeds (45 mg) served as reinforcement. To minimize odour cues additional seeds (100g) were spread under the floor. The experiments were carried out under dim illumination.

Adaptation sessions began one day after the start of food-restriction. The animals received 85% of their usual ration till the end of the training. The seeds were scattered randomly on the floor and their number was reduced to four at the end of the sessions. Adaptation lasted 5 consecutive days and was considered complete when the rat found 4 seeds during 5 min.

Acquisition of discrimination lasted 9 days with 2 trials per day. Inter-trial interval was 30 min. The rats were trained to collect seeds from a fixed set of four illuminated holes. The trial was terminated when the rat found all the seeds or when 180 sec had elapsed. Visits to the holes were recorded by means of a computer program, which was used to identify nose pokes by light beam crossings. Two measures of training were analyzed:

- Reference memory (RM) = (number of food rewarded visits + revisits to the baited holes) / (number of visits + revisits to all holes)
- Serial numbers of visits to the baited holes (the optimum performance is when the fourth rewarded visit has the serial number of 4)

To assess the contribution of stimuli associated with reward four probe tests were conducted after completion of Acquisition stage. Test 1 aimed to reveal a role of spatial factors. All of 16 holes were illuminated. No food reward was given. The test was terminated when the rat visited all 4 formerly learned holes. Because light in this test has an incentive but not an informative value this probe was called ”no food / no light”. In Test 2 we investigated the role of (food + light) in directing learned behaviour, apart from its association with fixed location. Four randomly chosen holes were lit and baited with seeds. In Test 3 (“no food/ light”) we assessed the role of the learned light signal alone. Four randomly chosen holes were lit. No food reward was given. In Test 4 (“food/ no light”) the directing role of odour was investigated. Four randomly chosen holes were baited with seeds. Light was not presented. The last 3 tests were terminated when the rat has visited all 4 holes designated by light and/or odour of food, or when 180 sec had elapsed.

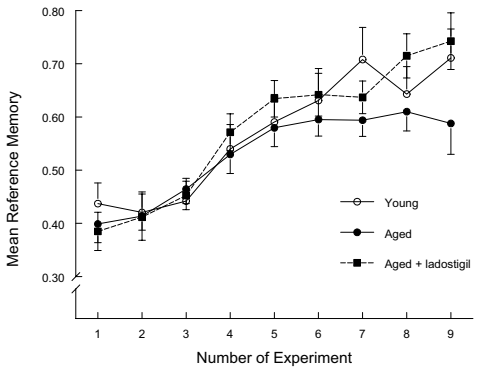

Figure 1. *Reference memory*

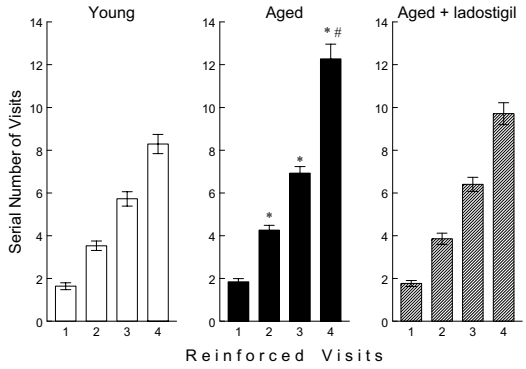

Figure 2. *Serial number of visits to the baited holes *Sig cf Young, $p<0.01$; # sig cf Aged + ladostigil, $p<0.01$.*

Results

As a result of food restriction the aged animals lost weight at the end of experiments, but no more than 7% (Young: from (392 ± 9) g to (399 ± 3) g; Aged: from (595 ± 11) g to (572 ± 11) g; Aged + ladostigil: from (635 ± 11) g to (591 ± 7) g. At the stage of Acquisition the rats demonstrated the same rate of initial learning, but in contrast to Young and Aged + ladostigil, Aged rats did not improve their level of memory (60%) until the end of the sessions (*Treatment*: $F_{2, 384} = 7.0$, $p < 0.001$), see Figure 1. Analysis of serial numbers of visits to the baited holes revealed that performance of Aged rats differed from those of Young and Aged + ladostigil, see Figure 2.

In the probe tests it was found, that in aged rats in the “no light” condition the presence of food did not improve RM, in contrast to Young rats. Aged rats were much more dependent on a conditional stimulus, and less able to remember the spatial position of baited holes than Young or Aged + ladostigil rats, see Figure 3.

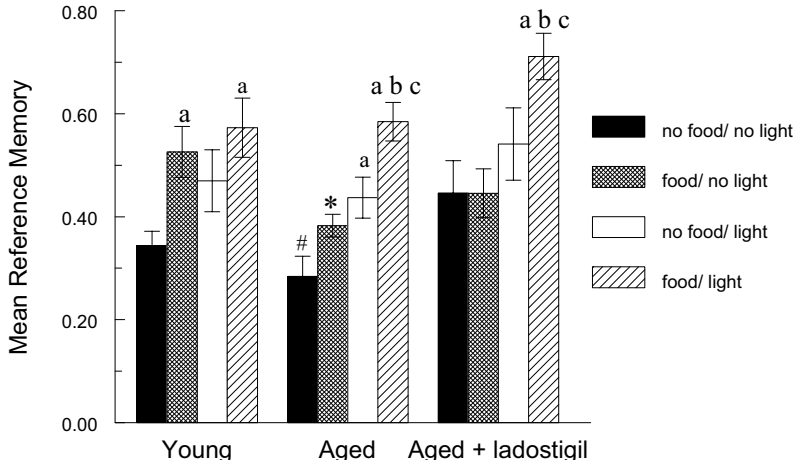

Figure 3. *Reference memory in 4 probe tests*
[a] Sig. cf "no food / no light", p<0.01; [b] sig. cf "food / no light", p<0.01 ; [c] sig. cf "no food / light", p<0.01.
**Sig diff from Young, p<0.05; [#]sig diff from Aged + ladostigil, p<0.05.*

Conclusions

The modification in the HB used in this study, the short duration and minimal food deprivation and harassment, made it sensitive enough to detect a differential contribution of natural and conditional stimuli in the expression of RM in young and older animals. Aged rats show different strategies in the performance of learning tasks characterized by a less flexibility than young rats. Aged rats have evidence of brain inflammation characterized by significant increases in activated astrocytes and microglia, which is prevented by a low dose of ladostigil (Weinstock and Shoham, unpublished observations). This may contribute towards the ability of the drug to prevent age-related cognitive deterioration.

References

1. van der Staay FJ, van Nies J, Raaijmakers W (1990). The effects of aging in rats on working and reference memory performance in a spatial holeboard discrimination task. *Behavioral and Neural Biology.* **53**, 356-370.
2. Shoham S, Bejar C, Kovalev E, Schorer-Apelbaum D, Weinstock M (2007). Ladostigil prevents gliosis, oxidative-nitrative stress and memory deficits induced by intracerebroventricular injection of streptozotocin in rats. *Neuropharmacology* **52**, 836-843.