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Spatial learning in the genetically heterogeneous NIH-HS rat stock and RLA-I/RHA-I rats: Revisiting the relationship with unconditioned and conditioned anxiety

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HIGHLIGHTS

- We show the first characterization of the NIH-HS rat in spatial learning/memory.
- We examine the association between anxiety/fear and spatial learning/memory.
- Spatial learning and/or memory efficiency appear to be dependent on anxiety.

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ABSTRACT

To characterize learning/memory profiles for the first time in the genetically heterogeneous NIH-HS rat stock, and to examine whether these are associated with anxiety, we evaluated NIH-HS rats for spatial learning/memory in the Morris water maze (MWM) and in the following anxiety/fear tests: the elevated zero-maze (ZM; unconditioned anxiety), a context-conditioned fear test and the acquisition of two-way active avoidance (conditioned anxiety). NIH-HS rats were compared with the Roman High- (RHA-I) and Low-Avoidance (RLA-I) rat strains, given the well-known differences between the Roman strains/lines in anxiety-related behavior and in spatial learning/memory. The results show that: (i) As expected, RLA-I rats were more anxious in the ZM test, displayed more frequent context-conditioned freezing episodes and fewer avoidances than RHA-I rats. (ii) Scores of NIH-HS rats in these tests/tasks mostly fell in between those of the Roman rat strains, and were usually closer to the values of the RLA-I strain. (iii) Pigmented NIH-HS (only a small part of NIH-HS rats were albino) rats were the best spatial learners and displayed better spatial memory than the other three (RHA-I, RLA-I and NIH-HS albino) groups. (iv) Albino NIH-HS and RLA-I rats also showed better learning/memory than the RHA-I strain. (v) Within the NIH-HS stock, the most anxious rats in the ZM test presented the best learning and/or memory efficiency (regardless of pigmentation). In summary, NIH-HS rats display a high performance in spatial learning/memory tasks and a passive coping strategy when facing conditioned conflict situations. In addition, unconditioned anxiety in NIH-HS rats predicts better spatial learning/memory.

Abbreviations: NIH-HS, National Institutes of Health-Heterogeneous rat stock; MWM, Morris water maze; ZM, Elevated zero-maze; RHA-I, Roman High-Avoidance inbred rat; RLA-I, Roman Low-Avoidance inbred rat; WKY, Wistar-Kyoto rat; Time, Time spent in the open sections of the ZM; #entries, Number of entries in the open sections of the ZM; #SAP, Number of stretch-attend postures in the ZM; #HD, Number of head dips through the border of the open sections of the ZM; MDI, Mean directionality index in the MWM; SH, shuttle box; FR, context-conditioned freezing in the SH; CS, Conditioned stimulus in the SH (light + tone); US, Unconditioned stimulus in the SH (electric shock); ITI, Intertrial interval; #AV, Number of avoidances during the SH acquisition session; SD, Standard deviation; "High-", Used to define the superior subgroup of a selected variable (Mean + SD); "Low-", Used to define the inferior subgroup of a selected variable (Mean − SD).

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1. Introduction

The use of inbred stocks of laboratory rodents in the last three decades has promoted a very important progress in neuroscience and neurogenetic research. On the other hand, the use of outbred rodent stocks has been and continues to be a reliable source of knowledge in the study of the behavioral traits and of the mechanisms underlying psychobiological processes. For example, a strong case for the use of outbred rodent stocks comes from the demonstration that they are a unique resource for the identification and fine mapping of quantitative trait – genetic – loci (i.e. QTL) influencing many different complex traits, either behavioral, biological or disease-related, and for the identification of quantitative trait genes [1–4].

The genetically heterogeneous stock of rats was created in the 1980s in the National Institutes of Health (NIH) from eight inbred progenitors. These parental strains were: (i) the MR/N, WN/N and WKY/N, three strains that trace their ancestry to the original Wistar stock; (ii) the M520/N and F344/N (both established in the 1920s, but of unknown origin); (iii) the ACI/N, a hybrid between the August and Copenhagen strains; (iv) the BN/SsN, derived from a color mutant from a stock of wild rats kept at the Wistar Institute, and (v) the BUF/N strain [5,6]. The first characterization of the NIH-HS rats consisted in the study of 16 phenotypes related to the consumption of alcohol and sensitivity to ethanol [6]. That study demonstrated that the procedure used to generate the genetically heterogeneous stock had been successful, since for most traits related to consumption/effects of ethanol, NIH-HS rats fell approximately in the middle of the high and low values of the eight inbred progenitor strains [5,6]. The neurobehavioral characterization of NIH-HS rats carried out so far in our laboratory includes traits related to unconditioned and conditioned anxiety/fear, exploratory behavior and coping style, as well as hormonal sensitivity to stress and central gene expression [7–18]. These studies have led to the conclusion that the genetically heterogeneous rat stock displays relatively elevated anxiety/fear (as shown by unlearned and learned anxiety tests/tasks) and enhanced hormonal responses to stress, as well as a predominantly reactive (i.e. passive) coping style (e.g. [7,8,13,14]). However, the NIH-HS rat stock has not yet been characterized with respect to spatial learning and memory traits.

Accordingly, the main aim of the present study was to characterize the spatial learning and memory profiles of the heterogeneous rat stock. At present, it is impossible to obtain the 8 NIH-HS ancestor strains: two of them no longer exist, and some of the others are not the original ones. Therefore, in order to obtain initial evidence regarding the relative spatial learning ability of the heterogeneous rat stock, we compared the learning/memory performance of NIH-HS rats with that of the inbred Roman High-Avoidance (RHA-I, relatively low anxious and poor learner) and Roman Low-Avoidance (RLA-I, relatively high anxious and good learner) rat strains (e.g. [8,19–26]). Notably, like the Wistar derived Roman strains, three of the eight ancestor strains of the NIH-HS rat stock were Wistar substrains (e.g., [23,27]).

Several previous studies on the relationships between anxiety/fear and learning in rats have been based on comparisons among different rat strains, either unselected or selected for a particular trait (e.g., [21, 24,28–32]). The results of these studies, however, are inconsistent: some do not support any hypothesis on the relationship between anxiety and learning [28–30] whereas others indicate that selectively bred high-anxious rats, such as the RLA line, perform better in some learning tasks than their low-anxious counterparts (i.e., the RHA line), suggesting that increased anxiety or stress responses may favor learning (for a review, see [21]). In a different set of studies, anxiety/learning associations were investigated in outbred rats from a given strain. Thus, using subsamples of outbred Wistar rats, Herrero et al. [33] and Salehi et al. [34] have shown that anxiety is negatively related to spatial learning and memory [33–35]. In contrast, using conditioned and aversively-motivated passive/active avoidance tasks to measure memory, Ribeiro et al. [36] showed that higher anxiety is associated with better long-term retention (i.e. memory) in Wistar rats.

In view of the contrasting findings described above, it was considered of interest to investigate the associations between spatial learning/memory performance (i.e. place learning/memory in the Morris water maze — MWM) and unconditioned or conditioned fear/anxiety in NIH-HS rats. To this aim, we tested NIH-HS rats in the elevated zero-maze (ZM) test to obtain behavioral measures of unconditioned fear/anxiety and recorded two additional behavioral measures in the two-way active avoidance paradigm: (i) freezing (FR) time across the first 5 intertrial intervals as an index of context-conditioned fear, and (ii) the total number of avoidances as a parameter of conditioned anxiety; finally, we compared the performance in the above mentioned tests of NIH-HS rats to that of RHA-I/RLA-I rats that were used as reference groups.

2. Materials and methods

2.1. Subjects

Subjects used in this study were 86 NIH-HS male rats (from 40 different litters), 14 RHA-I and 17 RLA-I male rats which were 3 months old at the beginning of the experiments (weight: 340–400 g). RHA-I and RLA-I rats came from at least 10 different litters/strain. The inbred Roman strains are from the breeding colony maintained at our laboratory since 1997 [23]. We obtained a colony of the genetically heterogeneous rat stock from Dr. Eva Redei (Northwestern University, Chicago, US) in 2004 [7,10]. Animals were housed in pairs in makrolon cages (50 cm × 25 cm × 14 cm) and maintained with food and tap water available ad lib, under conditions of controlled temperature (22 ± 2 °C) and a 12 h light–dark cycle (lights on at 08:00 h). Experiments were performed during the light cycle, between 09:00 h and 19:00 h in accordance with the Spanish legislation on "Protection of Animals Used for Experimental and Other Scientific Purposes" and the European Communities Council Directive (86/609/EEC) on this subject.

2.2. Procedure and apparatus

Several behavioral tests were administered, with approximately 1-week interval elapsing between consecutive tests unless otherwise indicated.

2.2.1. Elevated "zero-maze" (ZM)

The maze, similar to that described by Shepherd et al. [37], comprised an annular platform (105 cm diameter; 10 cm width) made of black plywood and elevated 65 cm above the ground level. It had two equally-sized open sections (quadrants) and two enclosed ones with 40 cm high walls. The rat was placed in an enclosed section facing the wall. The apparatus was situated in a black-painted testing room, dimly illuminated with red fluorescent light, and behavior was videotaped and measured outside the testing room. Time spent in the open sections (time), number of entries in the open sections (#entries), number of stretch-attend postures (#SAP) and number of head dips (through the border of the open sections of the maze, #HD) were measured for 5 min [37,38].

2.2.2. Morris water maze (MWM)

The testing apparatus consisted of a circular pool (diameter: 150 cm, height: 60 cm), filled to a depth of 30 cm with 24 °C water. There were no local signals available within the swimming pool. Four points equally spaced around the perimeter of the tank were arbitrarily designed to serve as starting locations (N, S, E, and W). On this basis, the tank was divided into four equal quadrants. Located in the center of one of these quadrants was a circular platform (diameter: 15 cm, height: 27 cm) whose upper surface was 2 cm below the water level and equidistant from the sidewall and the center of the pool. The behavior of the animal (latency, distance and speed) was monitored by a video camera mounted on the ceiling above the center of the pool and using a computerized tracking system (Smart v.2.5.14; PANLAB, Barcelona, Spain). Four