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"Spatial Memory in Long Evans and *Rattus Norvegicus* rats"

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ABSTRACT

Rodents in search of food use visual environmental signals and complex spatial strategies and do not return to previously-visited locations, known as the win-shift strategy. The solution to the Olton Octagonal Maze (OOM) involves Working Memory (WM). A modified OOM was used that allows for measuring WM and Long Term Memory (LTM). The delayed spatial win-shift task consisted of a Training and Test phase separated by a delay. Prior to the Training phase, four arms were chosen at random and blocked, and food pellets were placed in the food cups of the four remaining open arms. Each rat was allowed to retrieve the pellets from the four open arms and then return to its home cage for the delay period (either 5 or 20 min). In the Test phase all 8 lanes were open, and the bait was placed in those blocked in the previous phase. Two experimental groups of rats, Long Evans and *Norvegicus,* and their corresponding control groups were trained. The experimental subjects performed Training-Delay-Test. The controls were only trained in the Test phase. Revisiting an arm previously explored in the 1st Phase was considered a LTM error. Revisiting an arm in the same trial constituted a WM error. It was concluded that the experimental groups do in fact possess LTM, with differences in favor of *Norvegicus*. There was no difference with respect to WM errors. The *Norvegicus* control group changes its strategy from allocentric to egocentric, which did not occur in the Long Evans control group.

Key terms: Long Term Memory, Working Memory, Olton 4X4, Long Evans Rats, *Rattus Norvegicus* Rats

INTRODUCTION

Memory of Place is highly relevant in biology because it is related to both individual and species survival. Therefore, spatial memory plays an important role in the foraging conduct of rodents (29), nest return in birds (18), and the ability of predators to recover prey buried in the past, etc. Rodents must find sufficient food in a minimum amount of time. What strategies could direct their foraging behavior? One alternative is to remain in the vicinity of the first food source found, known as a 'win-stay' strategy. Another strategy is to employ the 'win-shift' strategy and abandon the initial site of discovery in search of another source of food, which is most appropriate when the food supply is dispersed (30). Kamil (15, 16), on the other hand, has provided evidence that

honeycreeper birds collect nectar from flowers and do not return to those already visited until sufficient time has passed for more nectar to accumulate.

Authors such as Olton (27, 28, 30), Bennett (6), Benhamou and Poucet (4), and Chamizo (8) agree that the integration of signals from an heterogeneous and changing environment requires complex cognitive processing. This process involves Long Term Memory (LTM) and Working Memory (WM) (30). Baddeley (1) employed the term Working Memory because the memory trace must remain active until the task is completed. Spatial Memory has been of great interest in recent decades due to the design of new experimental situations such as the Olton Octagonal Maze (OOM) (30) and Morris' Water Maze (21, 22, 23). WM is involved in solving the OOM, while LTM or

Referential Memory is required for the water maze (10). These mazes have made it possible to discover important aspects of the structure of memory. The rats in both tasks use distal extramaze signals. Other species use egocentric signals (5, 13, 14).

At our laboratory, Saavedra *et al.* (37) recently conducted a study to evaluate WM in three strains of rats: Albino (Sprague-Dawley), Pigmented (*Norvegicus)* and Hooded (Long Evans), using the OOM. In this apparatus, the *Norvegicus* rats showed better WM than Long Evans or Albino.

In the present study we compared Long Evans and *Norvegicus* rats in the modified 4x4 Olton Maze (31), given that this makes it possible to evaluate both WM and LTM or Referential Memory. Our study investigates whether Long Evans and *Norvegicus* rats have LTM and evaluates their performance. We also determine whether the control groups of both strains, with no prior experience, use the same resolution strategies.

METHODS

Subject

Two different strains of male rats were used: Gray (*Norvegicus*) (n=10) and Hooded (Long Evans) (n=10), of approximately 3 months of age, obtained from the bioterio of the Physiology and Biophysics Program of the School of Medicine. The average weights of the Gray and Hooded rats were 183 gr and 203 gr, respectively. The Gray rats were deprived of food up to 85% of their original weight and the Hooded to 95% (see Discussion section). Two groups were formed from each strain: Experimental (Gray n=6 and Hooded n=5) and Control (Gray n=4 and Hooded n=5). The rats were maintained in the cage during the entire experiment with an inverted light-dark cycle with ligth-off at 0800.

Apparatus

The OOM was placed at a height of 92 cm above the floor. An octagonal platform, 34

cm in diameter, was placed in the center, connected to 8 arms of equal length (71 cm) and width (7.5 cm), distributed radially. The bait was placed in a circular receptacle at the far end of the arm, hidden from the view of the rat (Fig 1). The same octogonal maze was used with all groups. The Modified 4x4 OOM, with the same physical characteristics as the classic Octogonal Olton version, was introduced in 2 phases (Fig. 1A). The 4x4 modification refers to the design of the block of some lanes in the two phases.

Procedure

Adaptation: took place during a 2-3 day period in the classic OOM. The rat was placed in the central platform and allowed to explore the arms, obtaining grains of puffed rice placed in the arms of the maze. The experimentation room was equipped with artificial lighting and a variety of visual signals on the walls.

Training: the experimental groups were trained daily in the Modified 4x4 OOM in 18 video-taped sessions. The weight of each rat was controlled each day, and training only took place at the required weight. Each trial consisted of 2 phases: Training and Test. In the first phase, 4 arms of the maze were loaded with the bait, and the other 4 were blocked. The arms to be blocked were selected at random (see Fig. 1A). The rats were required to enter the 4 un-blocked arms and retrieve the bait in a period of no more than 5 min. There was a 5-minute delay between the Training and Test phases in the first 17 sessions, and a 20-minute delay (LTM) in the final session. In this phase the bait was placed in the arms that had been blocked during the previous phase (see Fig. 1A). The rat was expected to enter the arms that had **not** been visited in the first phase of training (win-shift strategy). Failure to do so was recorded as a LTM error. The procedure was the same for both experimental groups.

The control groups performed only the Test phase, without any prior experience (see Fig. 1B). They were introduced into the maze with all 8 lanes open, although the
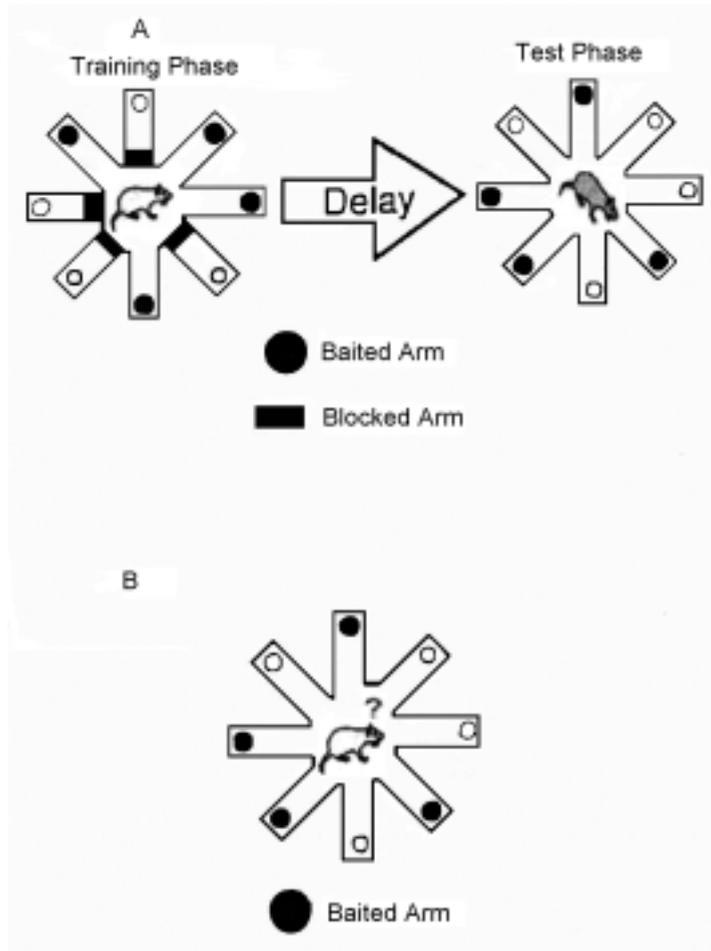
Figure 1
Diagram of the delayed spatial win-shift and random foraging eight-arm radial-maze tasks. **1A**. The delayed spatial win-shift task consisted of a Training and Test phase separated by a delay. Prior to the Training phase, four arms were chosen at random and blocked, and food pellets were placed in the food cups of the four remaining open arms. Each rat was allowed to retrieve the pellets from the four open arms and then return to its home cage for the delay period (either 5 or 20 min). During the Test phase of each daily trial, all arms were open, but only those that were previously blocked contained food. Errors were scored as entries into unbaited arms. **1B**. The random foraging task required rats to forage for pellets placed at random in food cups in 4 of the 8 arms. A different set of arms was baited each day according to those used in the Test trial.

bait was only located in the arms that corresponded to the Test phase of the experimental group. Only the LTM and WM errors made during the Test phase were recorded, and classified as either:

• LTM error: re-entry during the Test of an arm already visited during the training phase. This denomination was valid for the experimental groups (with prior experience) as well as the control groups (without experience), whose performance was considered a baseline random.

• WM Error: repetition of an arm during a trial. Valid for both groups.
Scores were submitted to the U-Mann Whitney Test, a non-parametric statistical test for small and independent groups, was required a level of significance of p=.05.

RESULTS

Using discreet methods, the average LTM and WM errors per session in each of the four groups were compared. Figure 2A shows the average number of LTM errors per session for the *Norvegicus* experimental group (n=6) and control group (n=4). The *Norvegicus* experimental group committed significantly fewer errors than its control group (U=0, p=.004). In Figure 2B, the Long Evans experimental group (n=5) and its control (n=5) also yielded highly significant differences in favor of the experimental group (U=0, p=.005). Thus both experimental groups demonstrated evidence of LTM. Figure 2C displays the curves of average LTM errors per session for the two experimental groups, showing the advantage of the *Norvegicus* (U=4, p=.026). In contrast, Figure 2D shows that there was no difference between the two groups in the average number of WM errors committed per session (U=10, p=.214). Figure 2E presents the average WM errors per session of the two control groups (with no previous experience), in which pronounced differences are seen between the *Norvegicus* and Long Evans rats. The former made virtually no WM errors during the 18 Training sessions, adopting an egocentric strategy in which they systematically choose the adjacent arm, whether to the right or left, and thereby avoided a repetition of previously-visited arms. The Long Evans rats, however, maintained a random allocentric strategy for choosing an arm (U=0, p=.008).

DISCUSSION

These studies confirm the ability of both strains to using previously-acquired information after a delay of 20 minutes, thus indicating they possess Projective Memory or LTM. In the Modified (4x4) Olton test, which evaluates both WM and LTM, the *Norvegicus* rats had a significantly better LTM performance than the Long Evans. Both experimental groups use the win-shift strategy and are guided by allocentric signals. With respect to the control groups (without experience), the strains differed from one another; and when faced with a new situation, the *Norvegicus* rat changes its strategy and explores the maze, selecting the adjacent arms in succession, thus avoiding WM errors and employs egocentric signals.

The differences in foraging behavior strategies can be analyzed at different levels:

a) Natural Habitat
b) Genetic Factors
c) Differing Memory Neural Circuits as it relates to Projective Memory (as is the case of the 4x4 maze with delay versus those in which there is not prior information).

a) Natural Habitat: *Rattus Novegicus* are able to survive in hostile human environments due to their complex social mechanisms that ensure community peacefulness, equal opportunity between males and females, and the ability to quickly learn vital information about the environment. Lore and Flannelly (20) have studied the behavior of the *Norvegicus* rat in a situation that simulates their habitat. The authors found that when the food supply is scarce, the male yields to the female. We had similar findings in our laboratory; in competitive situations with food-deprived male and female *Norvegicus*, the male ceded access to the food to the female. Under similar conditions with Hooded rats, however, the male was found to dominate (Pinto et al, not published). We were able to confirm that in other learning tasks the *Norvegicus* rat required greater levels of deprivation to resolve the tests (12, 32).

b) Genetic Factors: Behavioral and genetic differences were recently discovered between two strains of mice: the Montane vole (*Microtus montanus*) and the Prairie vole (*Microtus ochrogaster*) (19). The two strains differ in social conduct and in genotypic characteristics associated with peptidal hormonal receptors that regulate conduct. Vasopressin modulates affiliation, pair-
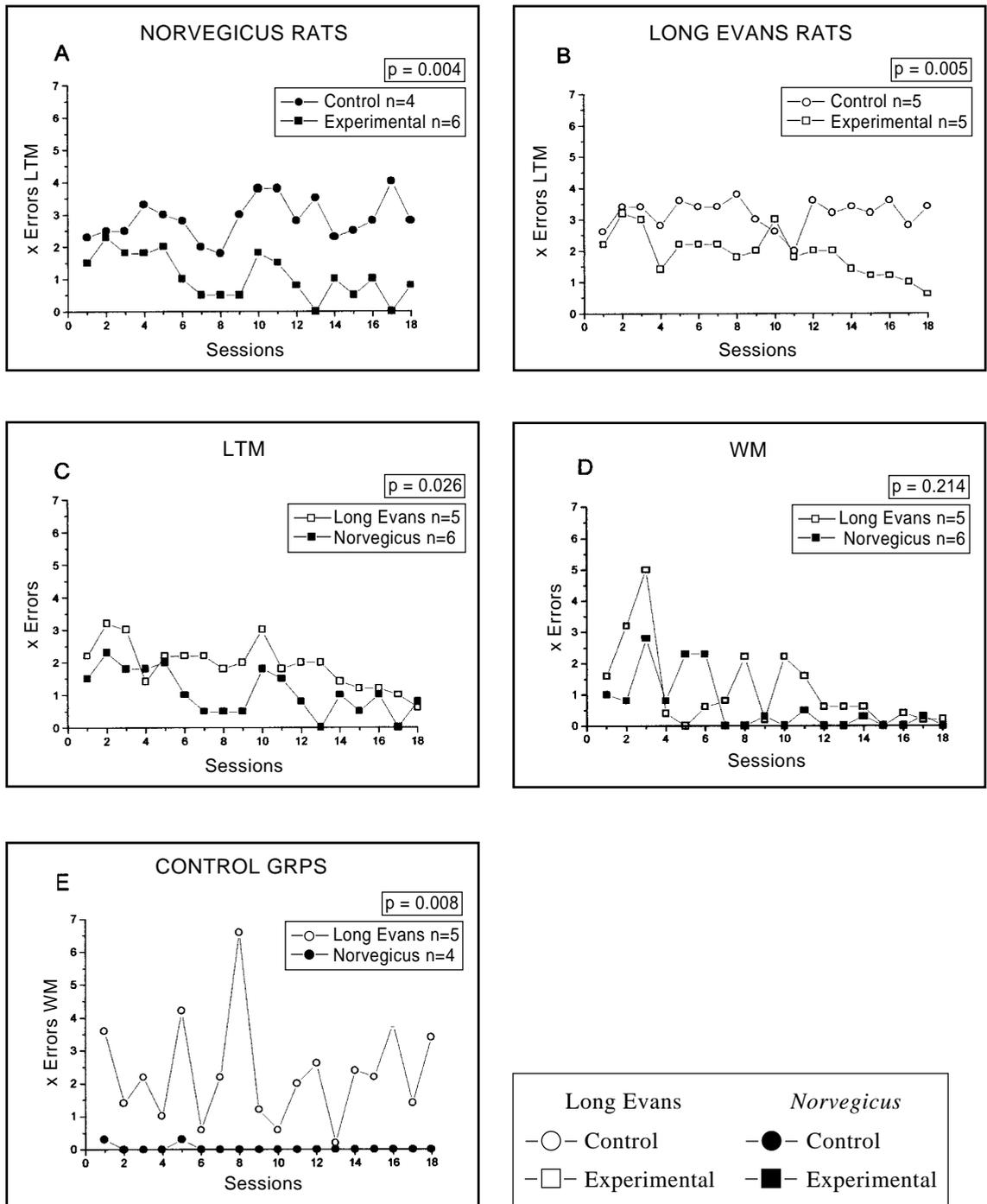
Figure 2
Figure 2A shows average LTM errors made by *Norvegicus* rats (U=0, p=0.004 during Experimental (-■-) and Control (-●-) sessions. Figure 2B depicts the average LTM errors of the Long Evans Experimental (-□-) and Control (-○-) groups (U=0, p=0.005). Figure 2C shows similar average LTM errors between Experimental Groups: Long Evans (-□-) and *Norvegicus* (-■-) (U=4, p=0.026). Figure 2D corresponds to the average WM errors between Long Evans (-□-) and *Norvegicus* (-■-) Experimental Groups (U=10, p=0.214). Figure 2E shows the average WM errors of *Norvegicus* (-●-) and Long Evans (-○-) Control Groups (U=0, p=0.008).

bonding, and paternal care in monogamous Prairie males, but not in non-monogamous Montane males (39,40). The pattern of expression of the vasopressin receptor in the brain presents large differences between the strains, probably resulting in a differential activation of neuronal circuits due to vasopressin release. The gene structure of the vasopressin receptor reveals molecular mechanisms that are useful in understanding strain-specific patterns of receptor expression. While the receptor's codifying region is nearly identical in both strains, Prairie voles have a vasopressin receptor transcription start site that is absent in the gene of the Montane voles (19).

From an ethological perspective, the Hooded rat is rather homologous to the Prairie vole in terms of behavioral characteristics, male-female competition, low aggression, and in male monogamous social systems. On the other hand, the Gray rat presents characteristics similar to the Montane vole. Both are aggressive, promiscuous, and in situations of scarcity, the males facilitate female access to food (2, 7). As is true of the Prairie vole, the habitat of the Hooded rat is rich in visual (allocentric) signals, while most *Norvegicus* rat activity occurs underground (20).

c) Neurological Circuits: The findings of O'Keefe and Dostrovsky (24) and O'Keefe and Nadel (25, 26) demonstrate that the hippocampus is a central structure in integrating multi-modal signals that allow for the configuration of a cognitive spatial map. Here we refer solely to the neurological bases underlying learning situations comparable to those used in our work. When a task requires previous experience, the neurological system becomes corticalized (33, 35, 36). Using the lidocaine (reversible lesion) technique, Seamans and Phillips (38) were able to identify this circuit: Ventral CA1/ Subiculum Region of the Hippocampus-Nucleus Accumbens and Prelimbic Region of the Prefrontal Cortex (11). In our laboratory (33), in the Modified (4x4) OOM, cortical injections of lidocaine into the Posterior Parietal Cortex of the rat produced a significant increase in the number of LTM and WM errors made. This effect did not occur with the classic Olton Maze, which only evaluates WM (3). When the rat is faced with a new situation, its strategy changes, and in this case, subcortical circuits are involved: Hippocampal - Nucleus Accumbens - Striatal, primarily Caudate Nucleus (9, 11, 17, 34, 36). In relation to the Caudate Nucleus, numerous authors (11) have demonstrated that it is involved in egocentric behavior and would be important in the Stimulus-Response (S-R) association.

In summary, we conclude that given previous information, both *Norvegicus* and Long Evans rats use the same strategies: win-shift and allocentric signals. However, when facing a new situation, the *Norvegicus* rat uses egocentric signals that involve the Caudate Nucleus, while the Long Evans rat does not.

The habitat of the *Norvegicus* rat is poor in the allocentric visual signals that would allow it to modify its behavior. As mentioned previously, the *Norvegicus* requires a greater degree of alimentary deprivation to resolve learning problems (12, 33). In this case, the degree of deprivation did not appear to be determinant, as in situations involving previous information (Projective Memory), both strains used the same win-shift strategy with allocentric signals.

A genetic study of the strains exploring the parallelism of behavioral features and their expression in their dependents would be of interest.

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