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Effects of laterality and sex on cognitive strategy in a water maze place learning task and modification by nicotine and nitric oxide synthase inhibition in rats

L. Kanit[a,b], E.O. Koylu[a,b], O. Erdogan[a], S. Pogun[a,b,*]

[a] *Ege University, Center for Brain Research, Bornova, Izmir, Turkey*
[b] *Ege University, School of Medicine, Department of Physiology, Bornova, 35100 Izmir, Turkey*

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Abstract

The aim of the present study was to investigate sex differences in learning strategies and to elucidate the mechanisms, which may underlie these differences. In two separate experiments, rats were presented with different strategies that could be employed to learn the position of a platform in a water maze (WM); furthermore, rats received treatments that could influence these strategies. In the first experiment, we demonstrated that the response-learning paradigm can be applied to the WM and can be compared with visually cued learning and reversal learning. Naïve rats of either sex could acquire this protocol relatively easily. On the probe trial, where the rats are presented with a choice between using response versus visually cued learning, initially response learning was preferred, however, during these experiments, laterality emerged as a significant factor and rats trained to turn right had difficulty in reversing the learned pattern to find the platform. The second part of our study evaluated the effects of nicotine and nitric oxide synthase (NOS) inhibition on the aforementioned parameters. Drug treatments impaired acquisition compared to saline treatments and the effect was more pronounced with NOS inhibition. During the probe trial, while NOS inhibition enhanced the right-side bias in both sexes, nicotine treatment had the same effect only in males. In conclusion, naïve rats can acquire place learning using visible cues or reponse learning; however, there is a right side bias in both sexes and the laterality effect is more pronounced in male rats. In drug-treated animals, while NOS inhibition enhances laterality (right bias) in both sexes similarly, nicotine modifies the cognitive strategy in a sexually dimorphic manner by augmenting the right bias only in male rats.

Keywords: Place learning; Sex differences; NO; Response learning; Reversal learning

1. Introduction

Place learning in a water maze (WM) is a widely used cognitive test in rodents [33] and can be modified to discriminate between different strategies employed to solve the problem. Rats can learn to find the platform in a circular tank using different strategies such as using visual cues, navigational cues, response learning, thigmotaxis, etc. The WM task can be designed to force the animal to use only one strategy. For example, if the platform is visible, then the animal can use direct visual cues, but if it is hidden below the water, then the "visual cue" strategy will become ineffective and the animal will need to find another approach, such as using using spatial cues (i.e., extramaze cues) to navigate. The WM problem can be designed to allow for the use of a single strategy and performance of the rats can be assumed to reflect ability. In this case, preventive measures should be taken to differentiate between cognitive vs. motor capacity of the subjects and to rule out motivational differences. The place learning task can also be designed to present more than one option (i.e., a choice) for the animal to solve the problem, thereby allowing the experimenter to test if there is a cognitive strategy preference. Cognitive strategy is influenced by various factors including sex of the subject, pharmacological manipulations, age or hormones [38].

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[*] Corresponding author. Tel.: +90 232 388 2868; fax: +90 232 374 6597.
E-mail address: pogun@med.ege.edu.tr (S. Pogun).

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We have used this approach to study sex differences in cognitive strategy in rats and to analyze various factors, which may impact this difference [21,24–26]. The rat's ability to find the platform [escape latency (EL)] was assessed for 12 days, with the platform, always in the same place, but visible on days 1–3, 5–7, and 9–11, and hidden on the remaining days. The visible condition requires a "look-out" strategy for learning, whereas the hidden condition requires a spatial, "navigational" cognitive style. In these studies, different groups of rats acquired both the visible and hidden tasks similarly confirming that there were no cognitive ability differences between the groups. After acquisition, on Day 13, the rats were challenged by a new cognitive task (platform position shifted and the platform made visible). Although only the platform in the new place provided escape, initially the rats did not have that information and therefore the first trial of the shift task can be assumed to present a choice for the rats. If rats swam directly to the newly positioned visible platform, which was also closer, this was interpreted as preference for using direct visual cues; if they continued to search for the platform where it used to be during acquisition, this was interpreted to reflect preference for spatial, navigational cognitive style. Females had a very distinct preference for using visual cues in place learning which appeared only after puberty (the final critical period of brain development when the surge of gonadal hormones have substantial organizational effects) [24] and was not affected substantially by gonadal hormonal manipulations as adults [26], was eliminated by nicotine [23], correlated with cortical nitric oxide (NO) levels [26] and was sensitive to nitric oxide synthase (NOS) inhibition [21]. There are various factors that may additionally influence the rat's choice such as looking around to see the newly positioned visible platform, calculating the distance to the platforms, and motivation to try "novelty".

Rats can also find place by using their bodies as a reference; for example, they can find a platform by always turning to their right or left (i.e., response learning). In the present study, we have modified the WM place learning task to force the rats to locate the platform using response learning. Differences in cerebral lateralization patterns play an important role in sex differences in humans [19,27,28,45,47,56] and in rodents [18,44,48,57]. Sex differences depicted in rodents involve spatial learning (favoring males) and the hippocampal formation; male brain is more lateralized with a right bias. Starting with pioneer studies of Diamond et al. [12] sex differences in spatial learning have been reported (e.g., [16,51,52]) and attributed to changes in the hippocampus (e.g., [53–55]. Tabibnia et al. [48] confirmed previously reported findings of sex differences in the volume of the granule cell layer (GCL) of the dentate gyrus (i.e., larger in males than females) and also reported that GCL is asymmetrical in a sexually dimorphic manner; males have greater GCL volume in the right than in the left hemisphere whereas the asymmetry is not observed in females. Consequently, employing response strategies in place learning also accommodates lateralized responses and can be expected to be sexually dimorphic.

Nicotinic receptors are present in brain regions critical for cognitive function and addiction. Although the prevalence of tobacco smoking has declined over the past 30 years, the decline has been less pronounced in women than in men. The cognitive effects of nicotine may be a critical factor in the decision to smoke and sex differences in the central actions of nicotine may be instrumental in understanding and coping with tobacco addiction [38]. Nicotine eliminates the sex differences observed in strategies involved in a place learning task in the WM; nicotine-treated females behave like males [23].

Nitric oxide (NO) is a gaseous biological messenger molecule in the central nervous system that has been proposed to mediate in the neurotoxic actions of glutamate and transsynaptic regulation, as well as in learning and memory processes [39]. There are sex differences in the regional distribution of NO metabolites and also in the cognitive effects of NO [38]. Kanit et al. have shown that cortical NO is negatively correlated with the female-type strategy employed in a WM place learning task [26].

The aim of the present study was to develop a new experimental design to study response strategy in place learning in the WM, to compare response learning with visual cued learning and to examine the impact of sex and laterality. Considering the effects of nicotine and NO on cognitive strategy employed in solving place learning tasks and also the sex differences observed in these effects, nicotine and NOS inhibition were included in the study.

2. Materials and methods

2.1. Laboratory animals

Male and female Sprague–Dawley rats (3–4 months old, 220 ± 20 g) kept under standard colony conditions (three to four per cage, 20–22 °C, 12-h light/12-h dark cycle) with ad lib food and water, were used in experiments. Each animal was handled for about 2–3 min daily for three days before the experiments began. There were 10 rats in each experimental group, i.e., a total of 200 rats (40 for experiment 1 and 160 for experiment 2) were used for the study.

The animals were treated under the prescriptions for animal care and experimentation of the pertinent European Communities Council Directive (86/609/EEC), and all the procedures were approved by the Institutional Animal Ethics Committee of Ege University.

2.2. The water maze apparatus

A circular pool (130 cm Ø and 75 cm high) was filled to a depth of 45 cm with water at 22 °C, made opaque and dark yellow by a non-toxic, water soluble powder dye. The visible platform was constructed of wood (12 cm × 12 cm) and protruded 2.5 cm above the surface of the water. The hidden platform was metal (12 cm × 12 cm), painted yellow, and