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Effects of continuous vs. cycling estrogen replacement on the acquisition, retention and expression of place- and response-learning in the open-field tower maze

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ABSTRACT

Estrogen has been shown to either enhance or impair learning and memory in female rats. The use of different experimental paradigms or estrogen treatment regimens may contribute to these disparate findings. In order to assess the effect of different estradiol (E2) treatments on several aspects of cognition, we trained ovariectomized female rats with either continuous, cycling, or vehicle E2-replacement, in an open-field tower maze task (OFTM) designed to test reference memory in a low-stress environment. In addition, in order to compare two distinct learning and memory systems, rats were trained to use either a dorsolateral striatum-based response type learning or a hippocampal-based place type learning to solve the maze. Results showed that cyclic, but not continuous, E2-replacement facilitated the acquisition of spatial memory in place-learners. Neither E2 regimen affected acquisition in response-learners. Additionally, when all experimental groups were performing at asymptote, rats were evaluated for performance stability by changing the location of their start position in the OFTM. Both regimens of E2 disrupted the expression of spatial memory in place-learners following the novel start position. However, E2-replacement protected ovariectomized female rats from the disruption of memory expression following a start position change in response-learners. Additionally all experimental groups performed equally well when tested following a 21-day period during which rats were absent from the maze. These results suggest that E2 fluctuation is particularly important in the acquisition of hippocampal-mediated spatial learning, and that hippocampal-based memory may be subject to disruption following environmental change, while striatum-based memory is subject to protection.

1. Introduction

Estrogen is known to modulate several brain regions essential for learning and memory (Becker, Breedlove, Crews, & McCarthy, 2002), and estrogen-induced neuroplasticity in these brain regions has been empirically linked to cognitive function (Barha & Galea, 2010; Brinton, 2009; Woolley, 1998). However, due to a wealth of contradictory data, whether or not estrogen actually benefits or hinders cognition remains the subject of considerable debate. Sources of inconsistent data within both human and non-human literature likely include: differences in the estrogenic compounds used, variable routes of administration, timing of treatment relative to training, different estrogen treatment regimens, estrogen with the simultaneous use of a progestin, and the fact that a variety of behavioral paradigms are utilized (Frick, 2009; Gibbs & Gabor, 2003; Lacreuse, 2006; Sherwin, 2006). The duration of treatment (e.g., acute vs. chronic) as well as whether the treatment produces hormone fluctuations similar to the natural cycle may also be responsible for producing some of the differential outcomes in measures of cognitive function (Frick, 2009; Sherwin & Henry, 2008). Moreover, although the same apparatus is often used for different behavioral procedures, these are usually done by different investigators and hence, on different experimental groups. For instance, the T-maze has been used by two different groups to determine estrogen's effect on basic spatial learning and on delayed matching to position (Gibbs, 2000; Marriott & Korol, 2003). Thus, investigator-specific differences in handling, housing, estrogen regimen, and cohort make comparisons difficult even when investigators are using the same behavioral apparatus. Consequently, although the use of cycling vs. continuous estrogen administration is thought to be an important factor in producing some of the disparate data on estrogen and learning, only a limited number of studies incorporate the use of more than one estrogen

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regimen within a single experimental design. Therefore, one aim of this present study is to determine the effect of two different long-term 17β-estradiol (E2)-replacement regimens on learning within a single experimental paradigm.

The other factor that may help explain the conflicting data on estrogen and cognition is the use of cognitive tasks that may inherently involve more than one learning and memory system (Daniel & Lee, 2004). This is important because it has been demonstrated that multiple central learning and memory systems exist, and the utilization of different systems by female animals is dependent on estrogen. Specifically, several studies have explored the dissociation between neural pathways underlying hippocampal-dependent vs. non-hippocampal-dependent memory systems (see Poldrack & Packard, 2003 for review). In these studies, inactivation of the hippocampus – but not dorsal striatum – disrupts acquisition of the spatial location in a water maze (Packard & McGaugh, 1992) and place-learning (approach a particular place) in a plus-maze (Packard & McGaugh, 1996). On the other hand, inactivation of the dorsal striatum and not the hippocampus disrupts visual discrimination on the water maze (Packard & McGaugh, 1992) and response-learning (make a left or right turn) in a plus-maze (Packard & McGaugh, 1996). Hence, place and response learning and memory are sub-served by two distinct brain systems. The role of estrogen in these two systems has been characterized in detail by Korol and colleagues. Following their initial findings that during high levels of E2 (both naturally present and induced) female rats prefer to use a place strategy to solve a T-maze task rather than a response strategy (Korol & Kolo, 2002; Korol, Malin, Borden, Busby, & Couper-Leo, 2004), they demonstrated that infusion of E2 into the hippocampus of ovariectomized female rats improves place-learning but does not affect response-learning, while infusion of E2 into the dorsolateral striatum impairs response-learning and does not affect place-learning (Zurkovsky, Brown, Boyd, Fell, & Korol, 2007). These findings suggest that the effects of E2 are dependent upon the brain system utilized by the animal during learning and memory, and that targeting the specific type of learning used to solve a task would provide a more concrete understanding of E2's role in modulating cognition within specific brain systems. Therefore, a second aim of this current study is to investigate the influence of E2-replacement on hippocampal-mediated place-learning compared to dorsolateral striatal-mediated response-learning within the same task.

To accomplish these goals, we utilized an open-field tower maze (OFTM) (adopted from (Cole, Clipperton, & Walt, 2007)). This paradigm was designed to measure precisely hippocampal-dependent place learning, un-confounded by direction learning. Place learning is thus more clearly defined by the use of both intra- and extra-maze cues than a T-maze or a radial-arm maze. However, the same apparatus can be used to examine dorsolateral striatal-dependent response memory, in which the rat has to follow a specific direction and disregard spatial cues. In addition, the OFTM is specifically designed to lack aversive features, in that although the OFTM is equivalent in complexity to the Morris Water Maze, it does not require the animal to swim, therefore removing the stressful and anxiety-provoking features inherent to the water maze. Moreover, there are a number of additional strengths in this particular methodology. For example, the probability of making the correct choice based on chance alone is 25% lower in the OFTM than in tests of spatial learning utilizing a T-maze. Also, rather than being forced to make one choice from a limited number of predetermined directions (as is required by a T-maze and a radial-arm maze), the path a subject takes in the OFTM is not restricted, while maintaining a clear measure of acquisition using percent of first choice correct response. In addition, proficient performance in this maze relies on reference memory acquired over several days rather than working memory.

Previously, we reported that continuous E2-replacement, maintained with the use of subcutaneous E2 pellets, does not affect the acquisition of place-learning in the OFTM in female rats, but it does preserve maze performance over a 21-day retention period (Lipatova & Toufexis, 2013). In that study we also evaluated whether a change in the test conditions, induced by a switch to a new start position in the maze, would disrupt peak memory performance in our ovariectomized and continuous E2-replaced rats. We found that continuous E2-replaced rats were impaired following the switch, while ovariectomized female rats were not (Lipatova & Toufexis, 2013). In order to extend this study and to address the additional objectives described above, we: (1) assessed the effects of continuous vs. cycling E2-replacement regimens within a single experiment using the OFTM; (2) differentiated the effects of two E2-replacement regimens on the acquisition of response vs. place learning in the OFTM; (3) tested the stability of maze memory performance acquired through place vs. response learning in the two E2-replacement regimens under novel test conditions in the OFTM.

2. Materials and methods

2.1. Subjects

The subjects were 48 naive, ovariectomized, female Sprague-Dawley rats obtained from Charles River Laboratories, St. Constant, Quebec. To avoid several confounds that may contribute to the conflicting data on estrogen and cognition, the female rats used in this study were all the same age (3 months old at the time of hormone replacement) and from the same cohort, and all experienced identical handling and housing (double-housed with ad-lib water and rat chow). The cage room was maintained at 23 °C with a 12:12-h light-on:light-off cycle. The experiment was carried out during the light-on phase. All rats were on restricted feeding throughout the experiment. This was accomplished using a daily assessment of their body weight to calculate the appropriate amount of food that needed to be administered in order to keep them at 85% of their free-feeding weight.

2.2. Apparatus

As shown in Fig. 1, the apparatus consisted of four food towers set 60 cm apart in the center of a circular arena 2 meters in diameter. The boundary wall was constructed from white laminate, and was 40 cm high. The inside of the walls was painted with different patterns, in order to provide the animals with additional cues to navigate in the maze. Specifically, one quarter of the wall was painted with black and white stripes, one quarter was painted solid black, one quarter was painted white with black circles, and the last quarter was painted solid white. The floor of the maze was beige vinyl used for floor surfaces. The food towers – indicated by letters A, B, C, and D in Fig. 1 – were 8 × 8 × 20 cm wooden blocks, coated with polyurethane. A plastic food cup, 2.5 cm in diameter and 2 cm deep, was created from a 35 mm film canister and was attached to the center of the top of each tower with a small screw. Due to the height of the towers, a rat could not investigate the contents of a food cup without rearing up on the tower on which the cup was mounted. This action was unmistakable for the purpose of scoring a response. Three covers, each containing three small holes, were made from the lids of the 35-mm film canisters. Pressing one of these tightly fitting covers onto a food cup made it possible to place an inaccessible reward (fruit loop) into the food cup on top of any one of the towers. Thus, to control for odor cues, all four towers contained a fruit loop on every trial, but the reward was accessible only on one, "correct" tower. The

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