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Effects of long-term estrogen replacement on social investigation and social memory in ovariectomized C57BL/6 mice

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Abstract

Estrogen has been shown to play a role in modulating social recognition memory. However, the literature regarding the influence of estrogen on social memory is sparse and only covers two experimental manipulations: acute injections and receptor knockout. Long-term effects of estrogen replacement on social investigation and social recognition are unknown. Furthermore, existing social recognition protocols focus on memory of very short durations (<2 h). In the present study, we examined long-term effects of estrogen replacement on both short- (<30 min) and long-term (24 h) social recognition in ovariectomized female C57BL/6 mice by implanting 60-day time-release pellets containing physiological doses of estradiol (0, 0.18, or 0.72 mg of 17β-estradiol). After 55 days of treatment, evidence of social recognition memory, measured by 24-h habituation, was found only in mice receiving the 0.72-mg pellet. This result is remarkable as previous reports indicate that individually-housed untreated rats and mice do not show habituation beyond 2 h. Our study further revealed that estrogen also increased frequencies of baseline social investigation without affecting general activity levels and decreased delayed post-swim-stress serum corticosterone concentration. Thus, these results suggest that long-term estrogen replacement increased the interest in social interaction as well as decreased stress responses. It is likely that the 24-h habituation observed in the estrogen replacement group is mediated jointly by the non-mnemonic effects of estrogen on the behavior displayed during the stage of memory encoding as well as mnemonic effects during the stage of memory consolidation.

Keywords: Social recognition memory; Estrogen replacement; Mice; Female; Corticosterone; HPA axis

Introduction

Rodents are highly social animals (Barnett, 1958; Lore and Flannelly, 1977). Their social recognition memory can be inferred by a decrease in the frequency of social investigation after repeated exposures to a conspecific (Thor and Holloway, 1982). This habituation is said to reflect the presence of a memory for the specific individual; therefore, a need for further investigation is reduced. Dishabituation occurs when a previously habituated animal increases its investigation in response to a newly introduced conspecific, thus serving to rule out the possibility that the habituation reflects generalized social fatigue. This form of learning appears to be modulated by major neuromodulators, neuropeptides, and hormones, such as acetylcholine (Winslow and Camacho, 1995), norepinephrine (Guan et al., 1993), dopamine (Dluzen and Kreutzberg, 1993), vasopressin (Dantzer et al., 1987), oxytocin (Ferguson et al., 2002; Popik and Vetulani, 1991), corticosterone (Tang et al., 2003), and estrogen (Hlinak, 1993).

In contrast to the growing interest in the role of the neuropeptide oxytocin (OT) in social memory (Ferguson et al., 2002; Popik and van Ree, 1998; Popik and Vetulani,

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1991; Winslow and Insel, 2004), the literature on the role of the sex hormone estrogen is relatively sparse. While several studies provided the important findings that acute changes in circulating estrogen concentration (Hlinak, 1993) and the presence of estrogen receptors (ER) (Choleris et al., 2003; Imwalle et al., 2002) are important for social recognition, social memory in ovariectomized animals after prolonged treatment with estrogen replacement remains unknown. Furthermore, most social recognition studies measured habituation of social investigation over a period of no more than 2 h (Choleris et al., 2003; Ferguson et al., 2000, 2001), even though it is known that longer lasting social recognition memory can be achieved with social housing (Kogan et al., 2000), pharmacological manipulation (Popik and Van Ree, 1992), and neonatal stimulation (Tang et al., 2003). So far, the role of estrogen in long-term social recognition (more than a few hours) has not been explored. Therefore, the primary goal of this study is to investigate the effect of long-term estrogen replacement on both short- and long-term social recognition in ovariectomized (OVX) mice. Given that estrogen can have a significant modulatory effect on HPA function (Burgess and Handa, 1992; Ferrini et al., 1999; Redei et al., 1994; Viau and Meaney, 1991, 2004), which in turn has been shown to covary with social recognition (Tang et al., 2003), a secondary goal is to determine whether the same estrogen treatment also altered HPA function.

Materials and methods

Animals

Twenty-three female C57BL/6 mice obtained from Jackson Labs (Bar Harbor, ME) arrived at 4 weeks of age and were maintained on a 12 h light–dark schedule (lights off at 0900 h) with food and water ad lib. The room temperature was kept at 21 ± 2°C. Animals were housed individually. All procedures were carried out in accordance with the guidelines established by the NIH Guide for the Care and Use of Laboratory Animals. Mice were randomly assigned to three experimental groups: OVX with no estrogen (OVX), and OVX with two levels of estrogen replacement (OVX-E1; OVX-E2) (see next section for details). The order in which surgical procedures and behavioral testing were performed, and in which the individual cages were placed on the shelves, was all counter-balanced across the three groups to prevent the time of the testing or operation from confounding the experimental treatment. The experimenters who performed the surgeries, behavioral testing, and data coding from the videotapes were kept blind to the experimental conditions to avoid potential experimenter expectancy effects. All testing was performed between 1300–1800 h. The social recognition test and the surgeries were performed in two different rooms to avoid any priming effects from the surgical procedures. Animals remained in their housing room until immediately before testing.

Ovariectomy (OVX) and estrogen replacement

Six weeks after arrival, all animals were ovariectomized under ketamine and xylazine anesthesia (averages of 54.1 and 7.6 mg/kg, i.p., respectively) and then immediately implanted with a 60-day timed-release pellet (Innovative Research of America, Sarasota, FL) containing either 0.00 (placebo), 0.18, or 0.72 mg of 17β-estradiol for the OVX ($n = 8$), OVX-E1 ($n = 7$), and OVX-E2 groups ($n = 8$). The two halves of the placebo pellets were matched to the inactive ingredients contained in the 0.18 and 0.72 mg pellets, respectively. Pellets with 60-day continuous release were chosen to allow a sufficiently large time window for evaluating the long-term effects of estrogen replacement. These doses were used because previous pilot studies showed that these doses produce circulating estrogen levels indicative of an estrous or proestrous C57BL/6 female mouse (unpublished observation), and have been shown to improve non-spatial and spatial memory (Rissanen et al., 1999).

Social recognition memory test

To study the long-term effects of estrogen replacement, a social recognition memory test was carried out 55 days after surgery. The test consisted of an initial cage-habituation session (Hab) and several 5-min sessions of social exposure on 2 consecutive days (Day 1: Hab; S1–S3; Day 2: Hab; S1–S2; within-day inter-trial interval was 5 min). During the Hab, two mice were placed into the same testing cage separated by a cardboard partition and each was allowed to explore its own side of the cage. It was understood that olfactory cues available through the partition were not a factor that could confound the treatment effects since all mice became familiar with each other's olfactory cues throughout the weeks of being housed in the same room. To dissociate differences in social investigatory activity from differences in general activity levels, we measured frequency of non-social movements during the Hab session on Day 1. These include movements in the vertical (rearing) and horizontal (turns) planes. Each time an accumulation of 90° turn in a given direction was achieved, the frequency of turns in that direction was increased by one. Total general activity was measured as the total frequency of rearing and 90° turns.

S1 followed Hab immediately and commenced when the partition was removed to allow two mice to be exposed to each other. During Day 1 S1–S3 and Day 2 S1, all animals were paired with the same partner (SAME PARTNER) they experienced on Day 1 S1. To rule out generalized social fatigue as a potential confound for habituation to a specific individual, during Day 2 S2, all animals were exposed to a new partner (NEW PARTNER). This was achieved by testing two pairs of mice at the same time and swapping two mice between the two pairs on Day 2 S2. Testing cages with the same type of bedding and of the same size as the home cages were used during social recognition tests. The same