

## 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 17 $\beta$ -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.

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### 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  $21 \pm 2^\circ\text{C}$ . 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  $17\beta$ -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  $90^\circ$  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  $90^\circ$  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

cages were used within each testing day for each given pair of mice. Social investigatory behaviors were videotaped under room lighting for offline analysis. Experimenters left the testing room during each session of social exposure to avoid interfering with the animals' ongoing behavior.

Social investigatory behaviors were defined as being proximally oriented to a conspecific (the tip of the nose within approximately 1 cm) or in direct contact while sniffing, following, nosing, grooming, and generally inspecting any body surface (Thor and Holloway, 1982). The frequency of social investigation for each animal was measured in 5-s increments. If the behavior was present any time during a 5-s interval, an occurrence of one was counted. Short-term social recognition memory was assessed by a normalized habituation score, defined as  $STH = (D1S1 - D1S3) / D1S1 \times 100$ ; long-term social recognition memory (24 h ITI) was assessed by a second normalized habituation score, defined as  $LTH = (D1S1 - D2S1) / D1S1 \times 100$ , where  $S_i$  represents the frequency of social investigation during session  $i$ . Response to a new conspecific on Day 2 S2 was assessed by a dishabituation score  $(D2S2 - D2S1) / D2S1 \times 100$ . Each animal within a pair was identified by coloring the tails using either a red or black permanent marker, which was distinguishable on the videotapes.

Traditionally, social recognition was studied in an intruder paradigm using adult–juvenile pairing where juvenile is introduced into the adult animal's homecage. However, such pairing introduces strong within-pair social dominance. To minimize potential influence of social dominance on social recognition memory, we used a neutral cage for social exposures to minimize territorial behaviors (no aggression was observed in the present study). A salient factor that strongly influences social interaction is the within-pair weight difference, which can account for up to 50% of the variance in social dominance behavior (unpublished data). Unfortunately, it is not possible to simultaneously match the weights between the testing and stimulus mice and maintain the perfect equivalence between the stimulus mice used for the different treatment groups. For example, estrogen-treated mice had lower body weights than the OVX mice (Gureviciene et al., 2003). If we match the weight of every stimulus mouse's weight to a testing mouse, then the stimulus mice of the estrogen-treated mice would be inevitably higher than those for the OVX mice in their body weights. In principle, true stimulus equivalency cannot be achieved in any studies of social interactions involving pharmacological treatment that produce weight difference. Given this inevitability, we simply formed within-group pairs as this pairing minimized the number of animals needed for the present study.

#### *Blood collection and radioimmunoassay*

Blood and uteri tissue were collected 2 days after the social memory test. To determine whether estrogen treatment alters HPA function, we measured delayed cortico-

sterone stress response, evoked by four 1-min swimming trials during a 60-min period. Thirty minutes later, trunk blood was collected after cervical dislocation and decapitation. Blood samples were centrifuged and plasma was removed and stored at  $-70^{\circ}\text{C}$  until radioimmunoassay was performed. Plasma estrogen and corticosterone concentrations were measured in duplicate in a single assay using the Coat-a-Count Estradiol and Corticosterone Kits, respectively (Diagnostic Products, Los Angeles, CA). The lower limit of detectability of the estrogen and corticosterone assay was 21.63 and 20.03 pg/ml and the intra-assay coefficient of variation was 7.5 and 9.7%, respectively. Similar to findings from Morgan and Pfaff (2001), estrogen levels were not detectable due to technical difficulties with the pellets. We therefore used uterine weights to verify the effectiveness of the ovariectomy and estrogen pellets. In addition, using leftover pellets from the same order, we implanted 4 mice separately. The estrogen levels measured at comparable times were below the detection limit for the 0.18-mg pellets and 29.2 pg/ml for the 0.72-mg pellets.

#### *Data analysis*

Because both uterine weights and behavioral measures between OVX and OVX-E1 did not differ significantly, we combined OVX and OVX-E1 into a single group named OVX ( $n = 15$ ) and renamed the OVX-E2 group as OVX-E ( $n = 8$ ). When assumptions of normality and equal variance were met, ANOVA with repeated measure was used. Otherwise, various transformations were performed. If the transformed data failed to meet the assumptions required by parametric tests, non-parametric tests were used when available. If non-parametric tests were not available, parametric tests were used after removing outliers. One-tailed one-sample  $t$  tests and Wilcoxon signed ranks tests were performed to test for expected habituation and dishabituation in one particular group. Spearman's rank order correlation,  $R_s$ , was used because of heterogeneity of variance in the raw data. To determine whether a correlation was independent from the estrogen treatment effect, we also computed partial correlations and adjusted means using ANCOVA with one outlier removed. Outlier was defined as measure  $>1.5$  interquartile range above or below the top or bottom quartile (Agresti and Finlay, 1997). Effect sizes were expressed as Cowan's  $d$  for  $t$  tests and as  $\eta^2$  for ANOVA with repeated measure and ANCOVA. All reported statistics are 2-tailed unless otherwise noted.

## **Results**

### *Effect of estrogen replacement on uterine and body weights and corticosterone stress response*

The effectiveness of estrogen replacement was demonstrated by changes in the uterine weights as well as in

the body weights. Specifically, the uterine weights were significantly greater in the OVX-E group than in the OVX group (Mann–Whitney  $U$  test;  $n = 23$ ;  $Z = -2.979$ ;  $P = 0.002$ ; Fig. 1a). In contrast, body weights were significantly lighter in the OVX-E group than in the OVX group [ $t(21) = 4.137$ ;  $P < 0.001$ ;  $d = 1.81$ ; Fig. 1b]. Delayed post-swim-stress serum corticosterone concentration was significantly lower in OVX-E than in OVX mice (Mann–Whitney  $U$  test;  $n = 23$ ;  $Z = -1.807$ ;  $P = 0.038$ ; 1-tailed; Fig. 1c).

#### Effects of estrogen replacement on short- and long-term habituation

Repeated encounters to conspecifics significantly decreased frequencies of social investigation in both groups across the first 4 sessions [Session effect;  $F(1,20) = 10.999$ ;  $P = 0.003$ ,  $\eta^2 = 0.355$ ; Fig. 2a]. The rate of decreases in social investigation differed between the OVX and OVX-E groups, indicated by a significant Session by Group interaction effect [ $F(1,20) = 4.332$ ;  $P = 0.05$ ;  $\eta^2 = 0.178$ ]. Baseline social inves-

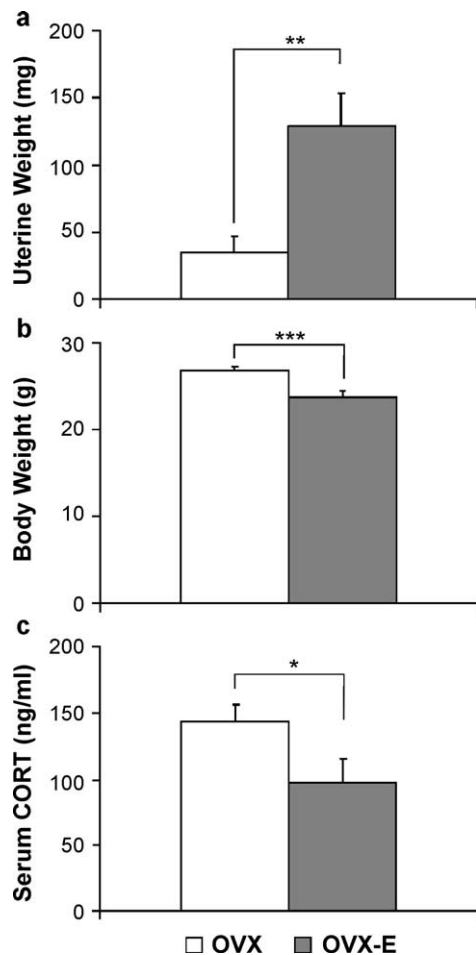


Fig. 1. Effects of estrogen replacement on (a) uterine weight; (b) body weight; (c) delayed post-swim-stress serum corticosterone concentration. Results in all figures are expressed as means  $\pm$  SEM. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ . OVX:  $n = 15$ ; OVX-E:  $n = 8$ .

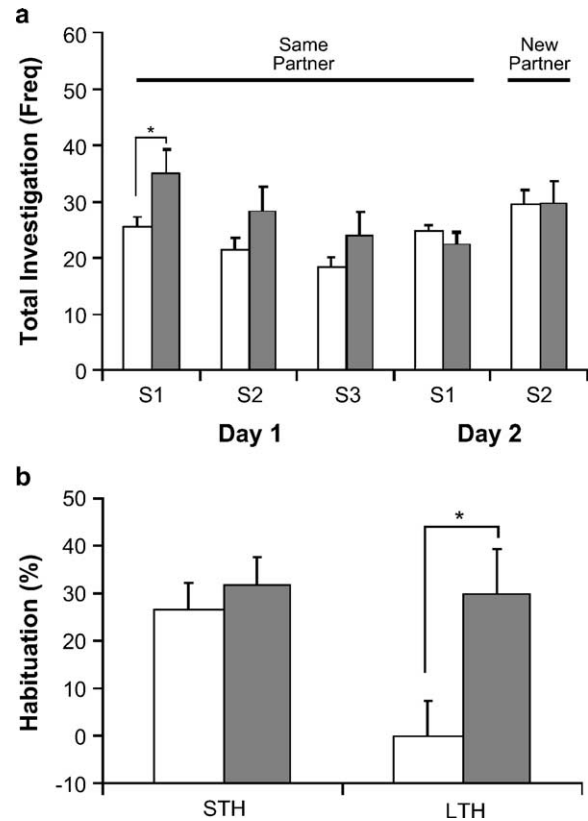


Fig. 2. Effect of estrogen replacement on habituation of social investigation. (a) Frequencies of social investigation as a function of sessions. Same partner was paired throughout all sessions except on Day 2 S2 during which a new conspecific was introduced. (b) Estrogen selectively affected long-term habituation [ $LTH = (D1S1 - D2S1) / D1S1 \times 100$ ] leaving short-term habituation [ $STH = (D1S1 - D1S3) / D1S1 \times 100$ ] unaffected. Shaded bar: OVX-E, open bar: OVX.

tigation (D1S1) was significantly higher in the OVX-E group than in the OVX group (Mann–Whitney  $U$ ;  $n = 23$ ;  $Z = -2.072$ ;  $P = 0.040$ ; Fig. 2a). The OVX and OVX-E mice did not differ significantly in their STH ( $P > 0.2$ , Fig. 2b—left). In contrast, they differed significantly in their LTH ( $t(21) = 2.433$ ;  $P = 0.023$ ;  $d = 1.06$ ; Fig. 2b—right). When each individual group was evaluated to determine whether 24-h habituation had occurred (testing for LTH score significantly  $> 0$ ), only the OVX-E group showed a significant LTH [one-sample  $t$  test;  $t(7) = 3.20$ ,  $P < 0.01$ ;  $d = 2.42$ ].

To rule out the possibility that the above measured 24-h long-term habituation was due to generalized social fatigue, a new conspecific was introduced during D2S2. We found that social investigation of a new conspecific on D2S2 was significantly greater than the investigation of a familiar conspecific on D2S1, thus showing dishabituation (Wilcoxon signed ranks test;  $n = 23$ ;  $Z = 2.366$ ;  $P = 0.009$ ; 1-tailed). Separate analyses of each treatment group revealed that both groups responded to a novel conspecific with increased social investigation ( $P < 0.05$ ). The OVX and OVX-E groups differed in neither dishabituation ( $P > 0.2$ ) nor general activity indexed by frequencies of turning

and rearing during the cage-habituation session ( $P = 0.15$ ; data not shown).

#### Association between LTH and baseline social investigation

The high baseline investigation in the OVX-E mice may allow them to acquire more information from conspecifics, thereby enhancing the acquisition of social memory. We thus computed the correlation between this baseline frequency of investigation (DIS1) and LTH, and found that mice with a greater initial social investigation also showed greater LTH ( $R_s = 0.713$ ;  $P < 0.001$ ;  $n = 23$ ; Fig. 3a). This correlation remained significant even after controlling for the effect of estrogen replacement (partial correlation:  $r = 0.637$ ;  $P = 0.001$ ;  $df = 20$ ). In contrast, the correlation between baseline frequency (DIS1) and short-term habituation (STH) was not significant ( $R_s = 0.289$ ;  $P = 0.182$ ;  $n = 23$ ; partial correlation:  $r = 0.264$ ;  $P > 0.2$ ;  $df = 20$ ). To determine whether estrogen replacement had significant effects on LTH after its effect on DIS1 had been removed, an ANCOVA was performed using DIS1 as a covariate. Although the difference between the adjusted LTH scores of the OVX and OVX-E mice was no longer significant, the adjusted LTH scores of the OVX-E (but not of the OVX) mice

remained significant [ $t(6) = 2.03$ ,  $P = 0.029$ ; 1-tailed;  $d = 1.66$ ; Fig. 3b].

#### Discussion

We found that in adult OVX mice, long-term estrogen replacement: (1) increased the delay at which habituation can be detected in mice reared in social isolation, from less than 2 h to at least 24 h; (2) selectively increased long-term (24-h) but not short-term (10-min) habituation of social investigatory behavior; (3) increased frequencies of baseline social investigation, which accounted for approximately 40% of the variance in long-term habituation; and (4) reduced the delayed corticosterone response to behavioral stress.

#### Estrogen replacement increased baseline frequency of social investigation

We found that the baseline social investigation in OVX-E mice observed during the initial social encounter was higher than the OVX mice. This difference could be explained by a reduction in general emotional reactivity to the novel testing environment because estrogen is known to modulate the stress response system (Viau, 2002) which can be activated by exposure to a novel environment (Denenberg, 1969). However, the lack of treatment effect on the general activity level did not support this explanation. A second possibility is that long-term estrogen treatment specifically reduced anxiety associated with social interaction (McCarthy et al., 1997). While this hypothesis remains to be tested, a reduction in social anxiety could affect social recognition memory in two ways: behaviorally or physiologically. At the behavioral level, the reduced social anxiety could allow a more prolonged display of social interest in investigating novel conspecifics, which consequently increases the memory encoding time. At the physiological level, the reduced social anxiety may be accompanied by a difference in the circulating stress hormone concentration, which in turn can affect cellular mechanisms that support memory encoding and consolidation (de Kloet et al., 1999; McEwen and Sapolsky, 1995).

Regardless of the precise mechanisms via which a change in social anxiety can affect the subsequent memory formation for novel conspecifics, we found that 24-h habituation of social investigation was positively correlated with the baseline frequency of social investigation. Furthermore, when the estrogen effect was partialled out, the correlation remained significant. Individual differences in the baseline social investigation can account for approximately 40% of the variance in 24-h habituation after the removal of the estrogen effect. This finding suggests that the level of baseline social investigation can have a rather large impact on habituation scores, a commonly used index for social recognition memory.

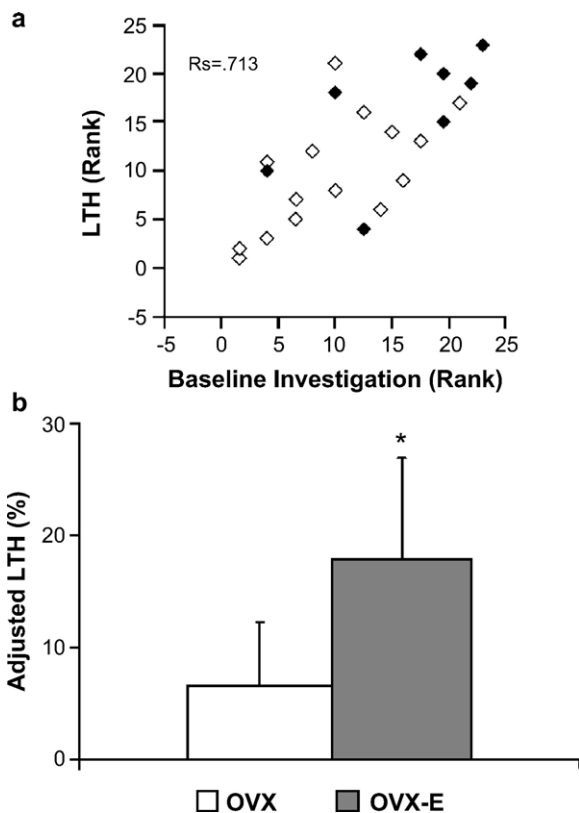


Fig. 3. (a) Individual differences in baseline social investigation were associated with individual differences in long-term social recognition memory (LTH). Filled squares: OVX-E, open squares: OVX. (b) Adjusted LTH scores. OVX-E mice continued to display significant LTH.

Thus, more sophisticated statistical analysis, such as partial correlations or ANCOVAs are needed for the analysis of habituation data. This manuscript provides one example of such analysis (see next section).

This finding also contrasts with previously reported enhancements of social recognition that were not accompanied by changes in baseline social investigation (Choleris et al., 2003; Ferguson et al., 2000; Tang et al., 2003), thus suggesting that estrogen's effect on social recognition may involve neural mechanisms that differ from those underlying estrogen receptor knockout (ERKO) (Choleris et al., 2003; Ferguson et al., 2000) or early life stimulation effects (Tang et al., 2003). On the other hand, similar to our finding, oxytocin (OT) specifically affects the acquisition of social recognition but not the retrieval (Ferguson et al., 2001). In humans, a PET study also revealed a similar estrogen effect on the acquisition of memory (Maki and Resnick, 2000).

#### *Estrogen replacement increased 24-h habituation in social investigation*

Given the above discussed estrogen effect on baseline social investigation, it is particularly important to point out that habituation scores used in this study, as well as our previous studies (Reeb and Tang, in press; Tang and Reeb, 2004; Tang et al., 2003), were defined as the difference between two sessions, *normalized* by the baseline measure. This effectively sets every individual's baseline measure to 100%, thus "equalizing" baseline performance. Thus, the habituation score is expressed as the percent reduction of social investigation relative to the initial level of social investigation. Using this habituation measure, we found that 24-h habituation in social investigation was absent among the OVX but present among the OVX-E mice, suggesting that estrogen replacement may be responsible for the persistence of memory for a novel conspecific 24 h later.

One may ask whether this 24-h habituation reflects differences in memory-related mechanisms or whether it is only an epiphenomenon of the estrogen effect on initial social investigation. First, ANCOVA results provided a direct answer to this question. After removing the effect of baseline frequency of social investigation, the adjusted 24-h habituation scores remained significantly greater than zero, consistent with the presence of a memory for a novel conspecific encountered 24 h earlier. Second, we found no estrogen effect on habituation of social investigation over a very short time interval (<30 min). Thus, the effect of estrogen replacement on OVX mice was selective for long-term but not short-term habituation. This dissociation is consistent with the interpretation that the observed estrogen effect on 24-h habituation reflects an effect on the transfer of a short-term memory to a long-term memory. Third, other alternative explanations, such as differences in sensory and motor function and in social fatigue, were ruled out by the similar levels of dishabituation observed after the 24-h habituation measure. Finally, even for the 40% of variance

in 24-h habituation accounted for by the baseline frequency of social investigation, the underlying mechanisms mediating this correlation may involve cellular mechanisms operating at the stage of the initial memory encoding. Together, these findings suggest that estrogen replacement not only affects levels of social interest during the initial social encounter but affect the subsequent memory process as well. In addition, the fact that this estrogen effect was associated with a serum concentration of ~30 pg/ml (unpublished data), that is slightly below the range of 35–75 pg/ml reported in mice during proestrous (Grasso and Reichert, 1996; Nelson et al., 1992), suggests that our observed effect of estrogen replacement on social recognition performance is likely to be relevant to normal social function.

#### *Possible indirect effects mediated by the neuropeptide oxytocin*

One possible specific mediating mechanism for this estrogen effect on social memory may involve the neuropeptide OT, which is known to play a role in social recognition in a dose-dependent manner (Carter and Keverne, 2002; Ferguson et al., 2002; Winslow and Insel, 2004). While OT knockout (OTKO) mice failed to show habituation to familiar conspecifics (Ferguson et al., 2000), the ability to habituate could be restored by a single injection of OT (Ferguson et al., 2001). Interestingly, estrogen can increase the excitability of OT neurons in the paraventricular nucleus of the hypothalamus (Akaishi and Sakuma, 1985; Negoro and Akaishi, 1981) and can increase plasma OT in OVX rats (Yamaguchi et al., 1979). Given this estrogen modulation of OT activity, it is not surprising that, similar to OTKO mice with OT replacement, the OVX mice with estrogen replacement showed a relative enhancement in social memory. These effects parallel the recently reported similarity between social memory in ERKO and OTKO mice. Thus, our results provide support for the theoretical model proposed by Choleris et al. (2004) in which estrogen modulates the OT regulation of social memory via estrogen receptors.

#### *Estrogen modulation of HPA function and non-specific effects of long-term estrogen replacement*

We found that delayed corticosterone stress response was reduced in OVX-E mice. This result indicates that long-term estrogen replacement affects HPA function in OVX mice, similar to what was found in both rats and humans (Cucinelli et al., 2002; Dayas et al., 2000; Ferrini et al., 1999; Redei et al., 1994). Given the well known effects of corticosterone on synaptic plasticity (de Kloet et al., 1999; Pavlides and McEwen, 1999; Pavlides et al., 1993, 1994, 1996), it is likely that the reduced corticosterone allows for greater synaptic plasticity that is necessary for the consolidation of social memory. On the other hand, it is also

possible that by reducing corticosterone concentration, estrogen rendered the social interactions less stressful, for example, making the mice less anxious about approaching a strange mouse, therefore causing an increase in basal frequency of social interaction. This increased investigation may allow for longer acquisition time. Therefore, the initial encoding and the subsequent consolidation processes could both be modified by long-term estrogen replacement.

The wide distribution of corticosterone receptors within the brain (McEwen et al., 1968) suggests that the effects of long-term estrogen replacement via the output of HPA axis is likely to be widespread thus non-specific. Given this nonspecific effect, it is inconceivable that long-term estrogen replacement affects only the basal frequency of social investigation without affecting memory related cellular and molecular processes. More broadly, it is well established that estrogen modulates other cognitive functions, particularly spatial memory (Frick and Berger-Sweeney, 2001; Frick et al., 2002; Li et al., 2004; McEwen, 2002; Williams, 1998). Along with these studies, the emerging pattern from human studies also supports the conclusion that estrogen replacement is not selective for a specific area of function and extends well beyond the domain of memory (Hogervorst et al., 2000).

### Summary

We studied the long-term effect of estrogen replacement on social investigation and social recognition memory in OVX mice. We found that this estrogen replacement (1) increased the frequency of social investigation during the initial encounter with a novel conspecific; (2) permitted the memory for the novel conspecific to persist for at least 24 h after the initial encounter; (3) reduced corticosterone stress response. These findings suggest that estrogen replacement can affect social behavior and that these effects may involve changes in HPA function.

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